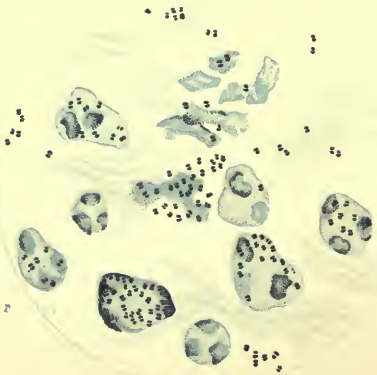
PLATE I.

FIG. 1.

Cover-glass preparation of pericardial exudate, showing bacillus pyocyaneus stained blue, and the bacillus tuberculosis stained red. (Ernst.)

FIG. 2.



Urethral discharge from a case of gonorrhœa, showing gonococci enclosed in pus corpuscles, and lying free in the discharge. Stained with methylene blue. (C. E. Simon.)

Lea's Series of Pocket Text-Books.

BACTERIOLOGY.

A MANUAL FOR STUDENTS AND PRACTITIONERS.

BY

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PREFACE.

In preparing this volume it has been the author's aim to furnish the student and general practitioner with a book on Bacteriology from which all unnecessary scientific discussions have been excluded.

All the matter has been carefully gone over; and in its finished form this book presents Bacteriology from a standpoint which enables the beginner to gain a full and comprehensive view not only of this subject itself, but also of its practical relation to medicine.

The book is systematically arranged on the basis of the course of lectures and laboratory exercises given by the writer for several years past; and is also a composite of what to his mind are the most valuable features of the larger works, the practical side of Bacteriology being especially emphasized.

Each chapter is intended to represent one lecture, although in some instances two chapters are embraced because of the brevity of the subjects contained.

The limited scope of this work has made it impossible to give credit in the text to the references used; but the author wishes to acknowledge his obligations to the works of Abbott, Park, McFarland, Sternberg, Levy and Klemperer, Vaughan and Novy, and Newman, of which free use has been made in the preparation of the text.

F. C. Z.

224 Central Park Av.,
Chicago, 1903.

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BACTERIOLOGY.

PART I.

CHAPTER I.

MORPHOLOGY OF BACTERIA.

Description: Bacteria are minute unicellular vegetable organisms which resemble an ordinary tissue-cell. They belong to the *fission-fungi*, a subdivision of the *Thallophytæ*, a class of the *Cryptogamia*. They differ from lichens and algæ in that they contain no chlorophyll and live only on organic matter.

The fission-fungi are divided into:

SCHIZOMYCETES—*bacteria*.

HYPHOMYCETES—*moulds*.

SACCHAROMYCETES—*yeasts*.

All bacteria possess a limiting *cell-wall* or *capsule*, which encloses a homogeneous or granular *cell-protoplasm* and a *nucleus*.

This **protoplasm** consists principally of an albuminous substance known as *mycoprotein*. The chemical formula of this proteid substance is $C_{25}H_{42}N_6O_9$.

Water and salts also enter largely into the chemical composition of bacteria. One group, the *Beggiatoa*, a higher species of plant, contains in addition sulphur granules. When grown on culture-media, the composition of bacteria is subject to variations depending upon the kind of media used.

The anilin dyes, which are used almost exclusively for staining bacteria, stain the organism uniformly so that the *nucleus* is obscured; but when special nuclear stains are used,

it is possible to distinguish the nucleus with a high-power lens ($\frac{1}{12}$ oil immersion). The extreme minuteness of bacteria is more or less of an obstacle to making studies of their real appearance.

Some bacteria (*Bacillus Megaterium*) contain also very deeply staining *granules*. These granules are of two kinds: *metachromatic* granules, which are believed to be the result of a degenerative process; and *polar* granules, which are still a matter of speculation. It was discovered recently that the protoplasm of many bacteria also contains fat droplets. This finding may possibly be of service in differentiating certain organisms.

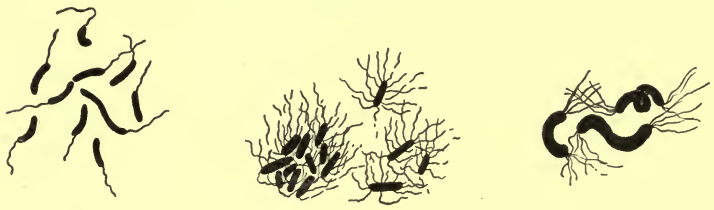
Cell-membrane: The cell-membrane is usually quite thin, but it may be of such thickness as to resemble a distinct capsule or envelope of mucus. Such a capsule is seen to best advantage enclosing the pneumococcus when the germ is examined in the sputum of an individual suffering from lobar pneumonia. The capsule is seen rarely when the organism is grown artificially. This is true of all pathogenic bacteria. Special reagents and stains must be used for the demonstration of this capsule. The methods will be described later, in the chapter on Microscopic Examination of Bacteria.

Flagella and Motility: Many varieties of bacteria have very delicate projections of the cell-membrane, called flagella, which appear to be organs of locomotion. So far as their appearance is concerned, they correspond to the cilia of an epithelial cell. Some bacteria have only one or more terminal flagella (Fig. 1), while others have both terminal and lateral flagella. The number varies from one terminal flagellum—*monotrichia* (*Vibrio cholerœ*), to two or more at each end—*lophotrichia* (*Bacterium syncyaneum*). Organisms with both terminal and lateral flagella belong to the class *peritrichia* (*Bacillus typhosus*). These flagella, with one exception (*Micrococcus agilis* of Ali-Cohn), are seen only on the rod-shaped or spiral bacteria. The most active bacteria usually have the greatest number of flagella, although there are a few bacteria which are well supplied with flagella and yet are devoid of motility; and others with few flagella are actively motile. It has been suggested that the flagella, in addition

to being organs of locomotion, aid to increase the food-supply of the germ by stimulating the flow of the current of the nutrient fluids past the bacterium.

The so-called *Brownian movement* is observed frequently in bacteria, and especially in the cocci. It is simply a molecular movement or rearrangement of the protoplasmic granules without changing the position of the organism in the least. The spirilla frequently exhibit *a rotary movement*, which may be very rapid. The movement due to the currents present in

FIG. 1.



a *b* *c*

a. Spiral forms with a flagellum at only one end. *b*. Bacillus of typhoid fever with flagella given off from all sides. *c*. Large spirals from stagnant water with wisps of flagella at their ends (*Spirillum undula*). (Abbott.)

all fluids must not be confused with actual motility. Frequently it is mistaken for such, especially when the organism is examined in the hanging drop.

Size: Bacteria vary greatly in size. The micromillimeter is the standard of bacteriologic measurement. It is equal to about $\frac{1}{25000}$ of an inch. The cocci are the smallest bacteria, and the twisted, spiral, or chain organisms the largest. The cocci vary in size from 0.1 μ to 2.8 μ; the bacilli range from 1 $\mu \times 0.2\ \mu$ to 5 $\mu \times 1.5\ \mu$. The anthrax bacillus is the largest of the bacillus group, and the bacillus of mouse septicæmia the smallest. Some of the spirilla are as many as 40 μ in length (spirillum of relapsing fever). It is almost impossible to form any definite conception of the size of bacteria. In order to determine their measurements special microscopic attachments are necessary. The *weight* can be arrived at by a lengthy mathematical calculation. Naegeli estimated the weight of an average organism at $\frac{1}{1000000000}$ of a milligram.

This is by no means a visionary calculation, but the actual weight.

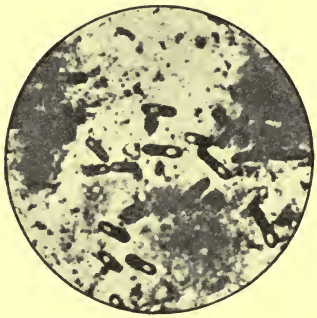
Reproduction: Bacteria multiply in two ways, by *fission* or *binary division*, and by *sporulation*.

When all the conditions necessary for growth are present, **fission** progresses with astonishing rapidity. When the bacterium is ready to divide, it is seen first to increase slightly in size, and then it is divided into two nearly equal parts by a gradual constriction in the middle. If the separation is incomplete, chains of varying length are formed. The rapidity with which this phenomenon occurs is entirely dependent upon conditions influencing the growth of the organism. If these conditions are favorable, millions of bacteria will develop from a single organism in the course of twenty-four hours. It has been estimated that if each organism reproduces itself by binary division once every hour, the result in twenty-four hours will be 16,777,200 individual germs, or 281,500,000,000 in forty-eight hours.

Reproduction by **sporulation** resembles the seeding process of the higher plants. It is seen mostly in the rod-shaped bacteria, especially when they are no longer purely vegetative or when conditions for rapid multiplication are unfavorable. Fraenkel says that sporulation is an indication of the vital perfection of an organism, and not a sign of deficient nutrition. These spores are oval or spherical, very refractive little bodies which develop within the organism itself, and are termed *endospores*. They usually develop singly, and are situated either at the end or in the centre of the germ (Figs. 2, 3, 4). When they are of unusually large size the shape of the parent cell is changed correspondingly. Thus *spindles*, *clostridia*, and *drum-sticks* may result. The spore is set free by a degeneration of the parent germ. Spores do not stain well with the ordinary methods of staining, and they are also very resistant to drying, heat, light, and chemicals. These properties are due to a very thick and almost impenetrable membrane by which they are enclosed. This peculiar resistance to extraneous influences is of great importance in surgery and also in the cultivation of bacteria. It will be considered more fully later.

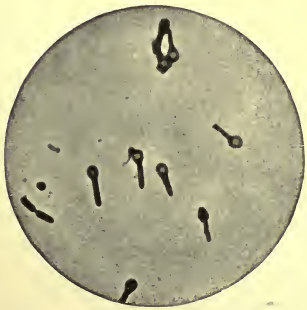
Reproduction by *arthrospores* is recognized by many prominent bacteriologists. It is seen especially in the coccus group. Various definitions of this method of reproduction have been given. According to Hueppe (and he is probably correct),

FIG. 2.



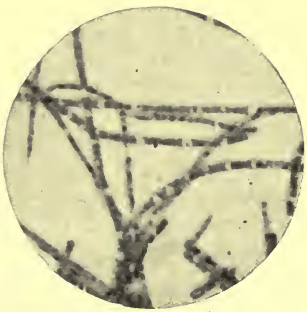
Unstained spores in slightly distended bacilli. (The spores are the light spots in heavily stained bacilli.) (Park.)

FIG. 3.



Spores in distended ends of bacilli. (Park.)

FIG. 4.



Unstained spores in centre of bacilli arranged in chains. (Park.)

they are larger and more resistant cells which take charge of the perpetuation of the species in the guise of a resting-stage or spore. Apparently the spore is evolved from the entire germ, or represents a transformation of the germ into a spore.

Another equally plausible explanation is that it is a sprout from one end of the cell, or a constriction; but this definition is not so satisfactory as that of Hueppe.

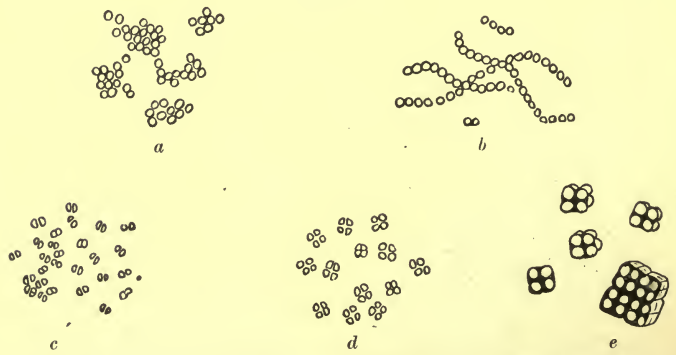
Whenever spore-formation is referred to, the endogenous spore (*endospore*) is meant unless the other variety is mentioned specifically.

CLASSIFICATION: It is rather difficult to classify bacteria properly. In order to include all the varieties and species, several classifications must be made.

First, as to their **shape**, we have three principal divisions or groups: *micrococci, bacilli,* and *spirilla.*

The **micrococci** (Fig. 5) are spherical or slightly oval in

FIG. 5.



a. Staphylococci. *b.* Streptococci. *c.* Diplococci. *d.* Tetrads. *e.* Sarcinæ. (Abbott.)

shape, non-motile, and do not form spores. They grow by binary division. This group is subdivided further into the following varieties:

Diplococcus: two micrococci remaining attached to each other, or an imperfect division. They may be absolutely spherical or the contiguous surfaces may be slightly flattened or concave, the "biscuit" coccus or "semmelkokken."

Tetrad: a group of four cocci, the result of division in two directions.

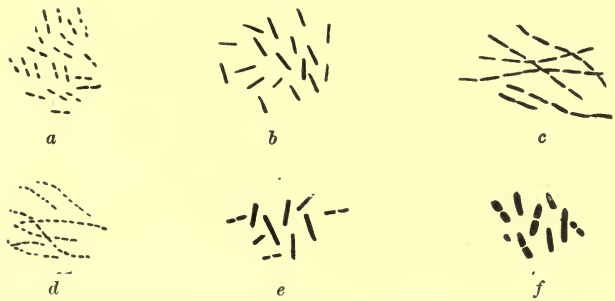
Sarcina: a packet or cube of eight cocci, the result of divi-

sion in three directions. This form resembles in appearance a bale of cotton or a dice.

Staphylococcus: the most common form, in which the cocci occur in irregular groups of varying numbers and without definite arrangement. The name is derived from the Greek *σταφυλή*, and is given to this form because of resemblance to a bunch of grapes.

Streptococcus: chains of cocci. When division occurs in only one direction, with adhesion or attachment of the individual members, chains of varying length are formed. Some authors distinguish a streptococcus longus and a streptococcus brevis,—that is, long chain and short chain; and a few, a

FIG. 6.



a. Bacilli in pairs. *b.* Single bacilli. *c* and *d.* Bacilli in threads. *e* and *f.* Bacilli of variable morphology. (Abbott.)

streptococcus conglomeratus. When the chain is composed of diplococci, it is called a streptodiplococcus.

Ascococcus and *leuconostoc* are two very unusual groupings of cocci. In the former the cocci are associated in globular or lobulated masses held together by a firm, gelatinous, intracellular substance. In the second variety the cocci grow in chains or masses, and are surrounded or enclosed by a very thick and tough gelatinous capsule.

The **bacilli** (Fig. 6) are rod-shaped or filamentous bacteria, motile or non-motile, flagellated or not, reproducing themselves both by fission and sporulation. They are not subdivided into groups, but exhibit considerable variation of

shape. Some are quite short and thick; others long and slender; some very large and some very small. They may be so short as to resemble a coccus, hence the term oval coccus. Some have rounded ends; others pointed, squared, or slightly concave ends. They may be spindle-shaped, rod-shaped, club-shaped, or a clostridium shape. Their arrangement is in some instances characteristic. They may be seen to lie singly or in pairs, in parallel rows or in chains of varying length, sometimes interlacing freely. Very long, slender, and indistinctly articulated filamentous bacilli are known as *leptothrix*; when these filaments present pseudobranchings, they are termed *cladothrix*.

The **spirilla** (Fig. 7) are curved or twisted rods of varying length, endowed with motility and a peculiar rotary movement, flagellated and reproducing themselves by both fission

FIG. 7.

a and *d*. Spirilla in short segments and longer threads—the so-called comma forms and spirals. *b*. The forms known as spirochæta. *c*. The thick spirals sometimes known as vibrios. (Abbott.)

and sporulation. They may be very rigid or exceedingly flexible. The short, slightly bent rods resemble a comma so closely that they frequently are referred to as "comma" bacilli (cholera), or as a *vibrio* because of their vibratory motion. The extremely long and flexible forms are called *spirochæta* (relapsing fever). A *spiromonas* is a ribbon-shaped spirillum. When sulphur granules are found in the protoplasm of the organism, it is called an *ophidomonas*.

Several **higher forms** of bacteria also are recognized. They approach the plant in structure and method of growth. Among these is the *streptothrix*, the only form which is encountered in animal pathology. The *Streptothrix actinomyces* (ray fungus) (Fig. 8) is the type of this class. The tubercle

bacillus and the diphtheria bacillus are included by some authorities in this class. The streptothrix presents true dichotomous branchings and forms very finely tangled

Fig. 8.

Actinomyces. × 250.

masses. In the course of its growth many stages of the germ are seen. Occasionally the filaments break up and resemble chains of bacilli or cocci, or the free ends of the filaments form club-shaped masses, which may be an evidence either of degeneration or sporulation.

Depending upon their **environment** and **habits,** bacteria are divided into *saprophytes* and *parasites.*

Saprophytes feed only on dead organic matter, and usually are not disease-producing bacteria, unless by absorption of the poisonous products formed by them from the breaking-down proteids.

Parasites always feed on living organic matter.

An organism may, however, be both parasitic and saprophytic, but a saprophytic existence precludes parasitism.

According to the **results of their vital activity,** bacteria are *pathogenic* and *non-pathogenic.*

A **pathogenic** organism is one which is capable of producing disease.

A **non-pathogenic** organism does not of itself produce disease.

Pure saprophytes are always non-pathogenic germs; whereas parasites are usually pathogenic.

The terms **obligative** and **facultative** are used to express the absence or presence of the ability of accommodation to surroundings. For example, organisms which may be either saprophytic or parasitic are said to be facultative (typhoid and cholera bacilli). Obligative bacteria are those which must be either one or the other; as, for instance, the lepra bacillus, which is a strict or obligative parasite.

According to the **products of their metabolism,** bacteria may be classified as:

AËROGENIC—*gas-producers.*
ZYMOGENIC—*fermentative bacteria.*
SAPROGENIC—*putrefactive bacteria.*
CHROMOGENIC—*color-producers.*
PHOTOGENIC—*phosphorescent bacteria.*

Migula recently proposed a classification of bacteria which is based on their **morphology.** Although this classification is technically correct, yet it is hardly wise to adopt it at this time. It is too radical a departure from the classification now in use; and, furthermore, with the more advanced study of bacteria and our increasing knowledge of the subject it can safely be assumed that still further changes in the classification will become necessary. For the present, therefore, we would recommend the usual nomenclature.

Migula's classification is as follows:

FAMILIES.

I. Cells globose in a free state, not elongating in any direction before division into one, two, or three planes	1. *Coccaceæ.*
II. Cells cylindrical, longer or shorter, and dividing in only one plane, and elongating to twice the normal length before division:	
1. Cells straight, rod-shaped, without sheath, non-motile or motile by means of flagella.	2. *Bacteriaceæ.*
2. Cells crooked, without sheath	3. *Spirillaceæ.*
3. Cells enclosed in a sheath	4. *Chlamydobacteriaceæ.*
4. Cells destitute of a sheath, united into threads, motile by means of an undulating membrane	5. *Beggiatoaceæ.*

GENERA.

1. *Coccaceæ.*

Cells without organs of motion:	
a. Division in one plane	1. *Streptococcus.*
b. Division in two planes	2. *Micrococcus.*
c. Division in three planes	3. *Sarcina.*
Cells with organs of motion:	
a. Division in two planes	4. *Planococcus.*
b. Division in three planes	5. *Planosarcina.*

2. *Bacteriaceæ.*

Cells without organs of motion . . . . . 1. *Bacterium.*
Cells with organs of motion (flagella):
a. Flagella distributed over the whole body . . 2. *Bacillus.*
b. Flagella polar . . . . . 3. *Pseudomonas.*

3. *Spirillaceæ.*

Cells rigid, not snake-like or flexuous:
a. Cells without organs of motion . . . . . 1. *Spirosoma.*
b. Cells with organs of motion (flagella):
1. Cells with one, very rarely two or three polar flagella . . . . . 2. *Microspira.*
2. Cells with polar flagella-tufts . . . . . 3. *Spirillum.*
Cells flexuous . . . . . 4. *Spirochæta.*

4. *Chlamydobacteriaceæ.*

Cell contents without granules of sulphur:
a. Cell-threads unbranched:
I. Cell-division always in only one plane . 1. *Streptothrix.*
II. Cell-division in three planes previous to the formation of conidia:
1. Cells surrounded by a very delicate, scarcely visible sheath (marine) . . 2. *Phragmidiothrix.*
2. Sheath clearly visible (in fresh water) 3. *Crenothrix.*
b. Cell-threads branched . . . . . 4. *Cladothrix.*
Cell contents containing sulphur granules . . . . 5. *Thiothrix.*

5. *Beggiatoaceæ.*

Only one species known (*Beggiatoa Trev.*), which is scarcely separable from Oscillana.

CHAPTER II.

BIOLOGY OF BACTERIA.

Distribution: When beginning the study of bacteriology the student is rather inclined to scoff at the statement that bacteria can be found everywhere. This skepticism frequently breeds carelessness on his part, necessitating much extra work. It is well, therefore, to begin one's study with the firm conviction that, no matter how careful he is, it is still possible for contamination to occur. Bacteriologic technique is tedious and time is precious, hence the student will do well to "make haste slowly". The cultivation at this time of habits of carefulness, thoroughness, and cleanliness will save him much worry and many sleepless nights in later life, when he may have ample opportunity to observe and become cognizant of the ever-present bacteria.

Bacteria are found in the air, in water, in the ground, and in all kinds of food and drink. The surface of the body invites the lodgement of both pathogenic and non-pathogenic bacteria, and some varieties even penetrate the protecting surface epithelium. This is of importance to the surgeon, and enforces the oft-repeated admonition of the bacteriologist, that the hands of the operator should receive most careful attention prior to an operation in order to assure even a modicum of safety and freedom from infection of fresh wounds. The various cavities which lead into and out of the body also contain a diversified flora. Under normal conditions the body-juices and -tissues are entirely free from bacteria. By means of cultures it can readily be demonstrated that the walls and floors of rooms, and especially hospital wards, are never free from bacteria. Fortunately most of them are not pathogenic organisms. It is well for us all constantly to bear in mind this universality of bacteria. It will account for the

otherwise inexplicable contamination of cultures in the laboratory.

Conditions influencing the growth of bacteria: It is evident from the foregoing that certain conditions are necessary for the development of bacteria and the manifestation of their presence. We will consider these requirements separately.

Oxygen: Most bacteria require oxygen. Some will develop only when there is not even a trace of oxygen present; while others can, in a measure, accommodate themselves to surrounding conditions (facultative). In accordance with their affinity for oxygen, bacteria are divided into: *aërobes*, those which require oxygen; and *anaërobes*, those which do not require oxygen; further, there are the facultative and obligative aërobes and anaërobes. The following examples will serve to illustrate:

OBLIGATIVE AËROBE—*Bacillus subtilis.*

OBLIGATIVE ANAËROBE—*Bacillus tetani.*

FACULTATIVE ANAËROBE—*Bacillus typhosus.*

Light: Most bacteria are not influenced by ordinary light; but the direct rays of the sun or reflected light either kill bacteria or retard their development. The same is true of the electric arc light. A blue light materially interferes with the life-processes of bacteria. The virulence of pathogenic bacteria is reduced if they are grown in the light. Some of the color-producing bacteria, however, will not produce their pigment unless the culture is exposed to the light. Some cultures, on the other hand, must be kept in the dark.

Electricity: The electric current checks bacterial development. The effect of the Roentgen ray on bacteria is still a matter of speculation; and further study and experimentation are necessary before any positive statements can be made as to its influence on bacterial activity. The Roentgen ray and direct sunlight are being used in the treatment of pulmonary tuberculosis, but the results are still *sub judicæ*. The use of the Roentgen ray in cancer is attended apparently by very good results; but it is still an open question whether or not this disease is of bacterial origin.

Water: All bacteria require a certain amount of moisture.

The amount needed varies considerably, and a few bacteria possess wonderful accommodative powers in this respect. They can grow on bread which contains only a trace of moisture. In laboratory work it will be found that bacteria develop most rapidly in liquid culture-media. Solid culture-media must contain at least 80 per cent. of water. Many bacteria will grow in ordinary water provided it contains some organic matter, even if it is only a trace.

Nutriment: Inasmuch as only a few bacteria contain chlorophyll, it is absolutely necessary that their food contain organic matter. In the case of a few bacteria only a very slight amount of organic matter is required, but as a rule a great deal is necessary. Carbon and nitrogen must be constituents of all culture-media, although a few organisms can obtain these elements from the ammonium salts. When characteristic growths are wished for, the carbon and nitrogen must be supplied. By cultivating bacteria on different media, it will be seen that many bacteria exhibit a decided preference for a certain medium. This is an important feature in the differentiation of species. It is possible, however, by frequent transplantation to accustom an organism to a certain medium, but this is done at the loss of its characteristic appearance and growth. Bacteria which grow very poorly or not at all on an ordinary culture-medium, will develop luxuriantly on media to which a little glycerin or glucose has been added.

Reaction: With a few exceptions the culture-medium should be either neutral or faintly alkaline. An excessively alkaline or an acid medium inhibits bacterial development. A very few germs require either a decidedly alkaline or a slightly acid medium. Moulds thrive best in an acid medium.

Movement: It is a well-known fact that a rapidly flowing body of water will purify itself. The same is true of bacteria in culture. A slight to-and-fro movement does not interfere with their development, but a violent shaking either hinders or prevents their growth. This is an important factor in the purification of rivers when sewers empty directly into them. By creating a rapid current the growth of the contained germs is checked and the water again becomes pure.

Temperature: It is impossible to establish an exact average temperature limit. As a rule bacteria will not develop in a temperature lower than 10° C., or higher than 40° C. Exceptional germs will grow very feebly, or possibly only retain their vitality, in a temperature as low as 6° C. and as high as 70° C. A temperature of 10° C. will inhibit the growth of many bacteria, but will not kill them. Some germs safely withstand freezing. Ravenel exposed anthrax spores, diphtheria and typhoid bacilli, and *Bacillus prodigiosus* to liquid air (—312° F.) for from three hours to thirty minutes, and when the spores and germs were transplanted to bouillon they grew with their customary rapidity. A temperature over 60° to 70° C. is fatal to most bacteria. Spores are more resistant, but are killed in boiling water in a few minutes. They will withstand dry heat (150° C.) for hours. The non-pathogenic bacteria are better able to accommodate themselves to temperature extremes than the pathogenic germs, all of which develop best at the body temperature.

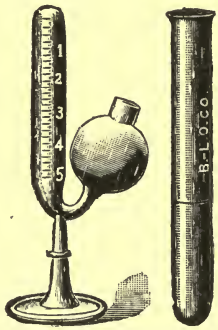
Association: The influence of one species of bacteria upon the growth of another, when associated with it in culture, is a matter of considerable interest as well as importance. Such association will at times increase or diminish the virulence of pathogenic germs. The toxin of the streptococcus is much more virulent when obtained from a combined culture of *Streptococcus pyogenes* and *Bacillus prodigiosus*. On the other hand, the virulence of anthrax is diminished when the germ is associated in culture with the *Bacillus prodigiosus*. Sanarelli's bacillus of yellow fever grows better when associated with certain moulds.

Products of bacterial activity: In both the animal and the vegetable world the result of metabolism is certain waste products and substances which are necessary to support life. Some of these waste products are highly injurious. The products of bacterial activity are many, some of them inert, and others more or less toxic.

Pigment: Those bacteria which produce pigment are said to be chromogenic. The pigment may be of any shade. The most beautiful colors are produced, and attempts are now being made to utilize these pigments commercially. These

pigments are the result of bacterial action on the albumins and peptones. Some pigments are soluble in water and others are not. Therefore we may see either the entire medium colored or only a portion of it; the bacteria themselves are free from pigment. It has already been mentioned that some germs produce pigment only in the presence of light and others only in its absence. Oxygen appears to be necessary for color-production. Some bacteria produce more than one pigment. The *Bacillus pyocyaneus* produces a blue and a green pigment; one is soluble and the other is insoluble. High temperatures check color-production. The reaction of the media modifies the shade of the pigment.

FIG. 9.



Fermentation-tube on left side, ordinary tube on right side.

Phosphorescence: Some bacteria exhibit strong phosphorescence in culture. They are designated as photogenic bacteria. Most of these varieties are found in sea-water. Sufficient illumination may be caused by a single culture to enable one to tell the time by the watch in a dark room. Sea-water gelatin is the best culture-medium. The *Bacillus phosphorescens* is the type of this group.

Gases: Certain bacteria produce gases as the result of decomposition and fermentation. These gases are not detected unless searched for by special methods. The most common gases are CO_2, CH_4, H_2S, NH_4. Gas-production is determined by the use of the fermentation-tube (Fig. 9). The tube

is filled with sterilized glucose-bouillon and inoculated. Any gases which may form will accumulate at the top of the arm of the tube. The usual precautions are taken to prevent escape of the gas, so that an accurate reading may be made. To determine the presence of CO_2, add sodium hydroxide and shake; the two will combine and the difference between the original amount of gas and the amount now present is the amount of CO_2. Hydrogen gas is detected as follows: Allow the gas in the long arm to diffuse into the bulb, mix with air, remove the plug, and apply a match. An explosion indicates hydrogen or marsh gas. In peptone-bouillon considerable H_2S is produced, which is detected easily by the odor. The presence of gases may also be determined by adding a little glucose or dextrose to nutrient agar. If gas is formed, the agar will be split into chunks.

Odors: In addition to the odors caused by the gases mentioned above, a number of very characteristic odors are perceptible in cultures. Many of them are unpleasant.

Aromatics: The most important aromatic produced is *indol*, as it is one of the means of differentiating the typhoid from the colon bacillus. Other aromatics are skatol, phenol, and tyrosin.

Liquefaction of gelatin: Many pathogenic and non-pathogenic bacteria liquefy gelatin by the production of digestant substances, such as trypsin. Anything that interferes with the development of the germ prevents liquefaction. For instance, the addition of carbolic acid or glycerin in excess. When the bacteria are removed from the culture by filtration the filtrate continues to liquefy. The liquefaction of gelatin is a very valuable aid in the differentiation of bacteria, as many of them liquefy gelatin in a constant and characteristic manner.

Fermentation: Bacteria produce certain metabolic products called ferments, and the process by which these ferments are formed is known as fermentation. Many of our food-products are the result of fermentation caused by bacteria. The manufacture of wine, cheese, butter, and indigo is largely dependent on such fermentation engendered by the bacteria which these substances harbor. Some bacteria evolve proteolytic

ferments, diastatic ferments, inverting ferments, and rennet ferments. Other varieties cause lactic, acetic, and butyric acid fermentations. The well-known alcoholic fermentation is not due to bacteria, as was supposed at one time, but to one of the yeast fungi. Fermentation always occurs in carbohydrates.

Putrefaction: This is analogous to the fermentation of the nitrogens. The albumins are converted into peptones, which finally are split up into acids, bases, and salts. Putrefying substances give off a very foul odor. Putrefaction occurs only when the supply of oxygen is deficient for proper combustion in the tissues. Ptomaines, indol, skatol, cadaverin, phosphoretted hydrogen, ammonium sulphide, and valerianic acid are putrefactive products. Ice-cream and cheese-poisoning are due to a poisonous substance known as *tyrotoxicon*, which is produced by the putrefaction of the milk proteids before the cream or cheese is made. Other examples are botulismus, or meat-poisoning, and fish-poisoning.

Acids and alkalies: *Acids* are formed by bacterial action on the sugar contained in the media. *Butyric*, *acetic*, and *lactic* acids are examples. Ethyl alcohol, aldehydes, and acetone are produced in less quantity. As the formation of the acid continues, the growth of the bacterium is checked and finally inhibited completely. By adding a little blue litmus solution to sterile milk, acid-production is manifested by the changing of the blue color to red. Rosolic acid changes the red to orange. When *alkalies* are formed, they usually combine with the acids to form salts, and it is more difficult to detect them than the acids. The beef-extract used in the preparation of culture-media contains a slight amount of grape-sugar.

Reduction of nitrates: The nitrifying bacteria form nitrites from nitrates, nitrogen, and ammonia. Others form nitrates directly from nitrogen and ammonia; and still another group, which is found in the soil, oxidizes ammonia into nitrites and these into nitrates. This nitrification is of considerable importance to the horticulturist. Some bacteria assimilate nitrogen and combine it so as to furnish nourishment for animals and vegetables. Sterile ground is fertilized in this

way. The farmer turns under with the plow a few crops of clover, which decompose and liberate nitrogen. This free nitrogen is acted upon by the nitrifying bacteria, especially the nitromonas, an organism isolated and described by Winogradsky. Most of the nitrifying bacteria are non-pathogenic saprophytes.

Enzymes: Bacteria produce certain substances known as enzymes, which, according to some authorities, play a very important part in the production of immunity. They have a decided bacteriolytic action, and are perhaps responsible for the phenomenon of agglutination. The limited course run by every infectious disease is supposed to be due to the bacteriolytic action of these enzymes. They are called nucleases. For instance, typhoid nuclease would be referred to as typhase, for short, etc.

Peptonization of milk: Milk may be said never to be free from bacteria, and it is nearly always impossible to detect their presence by simple inspection, as the appearance of the milk may not be changed. Some bacteria digest the casein without altering the appearance of the milk. Others produce coagulation; others, gelatinization. The milk may be transformed into a watery fluid. Ptomaines may be formed, which give rise to poisoning, *e. g.*, that due to tyrotoxicon.

CHAPTER III.

CULTIVATION OF BACTERIA—PREPARATION OF MEDIA.

In order that bacteria may be studied properly, it is necessary that we have some means at our command by which we can observe the growth and development of the individual organism and note its cultural characteristics. For this purpose they are grown on **media** having a standard composition and one which is most suitable for observation.

A great variety of different media has been proposed; but it is necessary to mention only those which are the most useful and which may be used for general work in any laboratory. In the preparation of a medium, the aim must be to approximate the body-juices as nearly as possible. The best media are those which can easily be liquefied and solidified, since these permit of the most accurate observation. All media must contain at least 80 per cent. of water. The *reaction* should be neutral or slightly alkaline. Some bacteria require a special culture-medium; and this will be described later when such organisms are considered.

Bouillon or **Beef-tea**: This is the most easily prepared and the most useful of all the media used in the laboratory. It also forms the basis of nutrient gelatin and agar-agar. It can be prepared from chopped beef or from the extract, which is more convenient and answers all practical purposes:

Beef-extract (Liebig),	2 grams;
Dried peptone (powdered) (Witte),	10 "
Sodium chloride (table salt),	5 "
Distilled water,	1000 c.c.

Mix the beef-extract with a little water until it is thoroughly dissolved. Add the peptone gradually, avoiding

clumping. Then stir in the salt and add the rest of the water. Boil for fifteen minutes; test the reaction with litmus-paper (it is always slightly acid because of the acid in the meat-extract), and alkalinize with a saturated solution of sodium bicarbonate or sodium hydrate, adding the solution drop by drop until the bouillon is neutral or faintly alkaline. The mixture should be stirred constantly. As soon as the mixture is cold it is ready to be filtered. It should never be filtered while hot, as it will be cloudy because of the precipitated meat-salts. Fold the filter-paper and moisten it with hot water before using. Pour the bouillon on the paper slowly so as not to break the filter. If the filtrate is cloudy, it should be filtered again until it is perfectly clear. If the solution is too alkaline, it will be cloudy. The alkalinity can be reduced by the addition of a little hydrochloric acid; but it is better not to do this, as too much manipulation is very apt to interfere with the usefulness of the medium. Enough water should be added to replace that lost by evaporation.

The liquid is then filled into sterile test-tubes, about 10 c.c. in each tube, and each tube plugged with a cotton stopper. The tubes, previously placed in wire test-tube baskets (Fig. 16), are sterilized in the steam sterilizer for fifteen minutes on each of three successive days. This sterilization must be carried out carefully, in order to prevent contamination from any bacteria which may have been contained in the bouillon. When completed, the bouillon should be of a light straw color.

This method is subject to such changes and modifications as may appear necessary or desirable. The proportions of the various ingredients may be changed, but none of these can be omitted or displaced. The beef-stock is prepared from the meat itself, as follows: place 500 grams of finely chopped lean beef in 1000 c.c. of clean water, and allow this to stand on ice for twenty-four hours. The liquor is then decanted and the beef thoroughly expressed through a clean white cloth, and sufficient water added to make 1000 c.c. Then the peptone and salt are added, and the bouillon is finished as already described.

Some objection is raised to the use of sodium solutions as described for alkalinizing the bouillon, as it is so easy to add too much.

The reaction may also be taken by Schultze's method. He uses a 0.33 per cent. alcoholic solution of phenolphthalein and sodium hydroxide, which is added with a burette until a faint red color appears. This indicates a faintly alkaline reaction.

The first method is certainly less complicated, more easily performed, and when carefully carried out gives the same results. The sodium bicarbonate must be added slowly, a little at a time, and the mixture constantly stirred so that the soda will be thoroughly distributed.

The final product may be modified by the addition of from 1 to 3 per cent. of glucose, lactose, or saccharose. This is known as *sugar-bouillon*. When it is desired to have a medium free from sugar, the grape-sugar contained in all meat-extracts is removed from the bouillon by inoculating it with the colon bacillus, which destroys the sugar by fermentation. After twelve hours the bouillon is filtered and the resulting clear fluid is free from sugar. This bouillon can also be used for making accurate fermentation-tests, as the correct percentage of sugar can be added.

Nutrient gelatin: Gelatin has the same nutrient value as beef-tea, but possesses the additional advantage of being solid. It cannot, however, be placed in the incubator, as its melting-point is 25° C. It is made from the beef-tea stock with the addition of 10 per cent. of gelatin:

Beef-extract,	2 grams;
Peptone,	10 "
Salt,	5 "
Gelatin (gold label),	100 "
Water,	1000 c.c.

The various ingredients are dissolved as in the case of the bouillon. The gelatin is broken up finely and added. The mixture is then boiled until the gelatin is completely dissolved. It is well to stir the solution constantly, as the gela-

tin is very liable to burn. If the gelatin is soaked in warm water before it is added to the bouillon, it will dissolve much more rapidly when boiled. Neutralize and cool to 60° C. Take the whites of two eggs and beat them up thoroughly with a little water; add this to the gelatin mixture and stir well. Boil for ten minutes, stirring constantly. The whites of the eggs clear the solution by embracing most of the impurities in their coagulum.

In order to hasten the filtration the solution is filtered while it is hot. The portion that is not poured on the filter at once should be kept hot over a low flame until ready to be used. Gelatin may be filtered either through filter-paper, a thick layer of cotton, or a double layer of close-woven cheese-cloth. A very rapid way is to place the filter-paper in the funnel, and then spread over the top of the funnel a layer of cheese-cloth. As the solution is poured on the cheese-cloth this catches the coarser particles of coagulated albumin and prevents clogging of the filter-paper. The gelatin should be clear. Excessive alkalinity clouds the medium. Prolonged boiling prevents solidification.

After filtration the gelatin is decanted into test-tubes, 10 c.c. in each tube, and sterilized for fifteen minutes on each of three successive days in the steam sterilizer. After the last sterilization the tube should be placed in the upright position, so that the solidified medium will have a flat, horizontal surface. In warm weather it is necessary to add more than 10 per cent. of gelatin in order to keep the medium solid. Gelatin can be modified by the addition of glucose, glycerin, or blood-serum.

Agar-agar: This is a Ceylonese sea-weed having a very high melting-point, and is suitable for cultures which must be subjected to the incubator temperature for a long time. Unlike gelatin, it cannot be liquefied repeatedly without spoiling it. Agar-agar medium is the hardest to prepare. Its preparation is extremely tedious, and unless the greatest care is exercised it is very apt to spoil. It requires constant watching. Its formula is the same as that for beef-tea, plus 1 per cent. of agar-agar:

Beef-extract,	2 grams;
Peptone,	10 "
Salt,	5 "
Agar-agar,	10 "
Water,	1000 c.c.

The agar is chopped very fine and placed in hot water to hasten its solution. The other ingredients are dissolved as already described in the preparation of the bouillon and gelatin. The mixture is boiled until the agar is thoroughly dissolved. This may occur in half an hour or in two hours, and during all this time the mixture is stirred steadily, as agar burns very rapidly. After it is all in solution, neutralize, cool, clarify with the whites of one or two eggs, and boil again until the coagula are formed.

Agar filters very slowly because it solidifies so rapidly. To obviate this, the filter and the filtering fluid should be kept as hot as possible. A hot-water funnel may be used for this purpose; or the filtering-stand is placed in the steam sterilizer, over a low flame, which keeps the water hot without causing it to boil. Only a little agar should be filtered at a time, the remainder being kept hot. The first filtration may be made through a finely woven cheese-cloth, after which filter-paper should be used. The filtrate should be perfectly clear and transparent. If all the steps in the process of preparing the agar have been carried out carefully, the filtration ought to be completed in about half an hour. Usually, however, it requires several hours, because students do not boil the solution sufficiently in the first place, so that the agar is not thoroughly dissolved. After filtration the medium is transferred to sterilized test-tubes, about 10 c.c. in each tube, and sterilized in the steam sterilizer for one hour on that day and for half an hour on the next. Agar is solidified in the inclined position, so as to have a large slanting surface for inoculation. The water which usually collects on the surface of solid media must not be removed. It prevents the medium from drying too rapidly, and also favorably influences the growth of the bacteria.

Glycerin-agar-agar: Some species of bacteria will not grow

on agar unless from 3 to 5 per cent. of glycerin is added (tubercle bacillus). The glycerin is added just before the medium is put into the tubes.

Sugar-agar: From 1 to 5 per cent. of sugar is added to the agar in solution. This medium is used to demonstrate fermentation or gas-production.

Blood-agar: The surface of the medium is smeared with a drop of blood taken from the finger-tip or ear, or from the vein of an animal. This medium is used for cultivating the influenza bacillus.

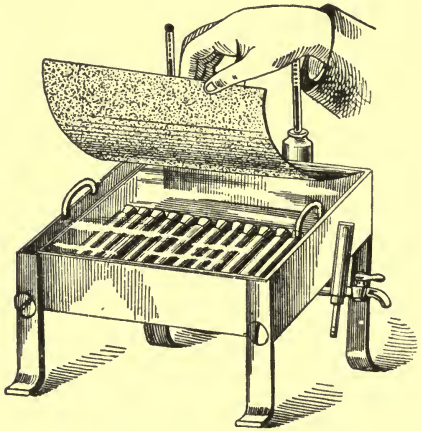
Blood-serum: This is made directly from the blood. Quite a number of organisms will grow on blood-serum only, hence it is well always to have a supply of this medium on hand; a few tubes will suffice, as it dries up very rapidly. The blood may be obtained from an abattoir. After the animal begins to bleed a coagulum is soon formed on the hair around the wound. Then the blood is allowed to flow into sterilized flasks, which are well plugged with cotton as soon as filled. The blood-coagulum acts as a mechanical barrier to contamination of the blood by the bacteria which always are found on the hair and skin. For this reason no blood is taken until this coagulum has formed. As many flasks may be filled as desired. After a firm coagulum has formed in the flasks they are placed on ice for forty-eight hours. The clear supernatant fluid is then removed with a sterile pipette and transferred to sterile tubes. Frequent handling is not advisable, as it increases the possibility of infection.

The method of sterilizing the tubes containing the blood-serum varies. If a liquid medium is wanted, they are exposed to a temperature of 60° C. on each of five consecutive days. If a solid medium is wanted, they are exposed to a temperature just below the boiling-point (100° C.) for one hour on each of two successive days. The medium is solidified in the slanting position. The color of the finished product is usually grayish and opaque. If it contains many red blood-corpuscles, it is of a reddish color. The serum may be sterilized either in Koch's blood-serum sterilizer (Fig. 10) or in the ordinary steam-chest; but the latter should not be closed tightly, because if the temperature is raised too rapidly the

serum will contain air bubbles. Blood-serum can be preserved in bottles or flasks by adding an excess of chloroform. The chloroform is evaporated subsequently, when the medium is again sterilized.

Loeffler's blood-serum: This is a mixture of blood-serum and bouillon. 1 part of a 1 per cent. glucose-bouillon is added to 3 parts of liquid blood-serum. After the mixture is transferred to test-tubes it is sterilized and solidified as

FIG. 10.



Chamber for sterilizing and solidifying blood-serum. (Koch.)

described above. This medium is used especially for making cultures of the diphtheria bacillus.

Alkaline blood-serum: This also is used for the diphtheria bacillus. It consists of 100 c.c. of blood-serum and from 1 to 1.5 c.c. of a 10 per cent. solution of sodium hydrate.

Milk: After perfectly fresh milk has been skimmed, it is placed in test-tubes and sterilized. To determine acid-production, sufficient blue litmus is added to color the milk. The litmus solution should always be freshly prepared, as it spoils very rapidly.

Dunham's solution: This is used to detect the aromatics

produced by certain bacteria. The indol reaction is demonstrated with this medium. It is made as follows:

Sodium chloride,	0.5 gram;
Peptone,	1 "
Water,	100 c.c.

Mix and boil thoroughly; filter; fill into tubes and sterilize. The solution is clear and colorless.

The *indol reaction* is determined as follows: Inoculate a tube of Dunham's solution with the *Bacillus coli communis* (or any other germ producing aromatics) and place it in the incubator for twelve hours. Then add 1 c.c. of a 0.01 per cent. solution of KNO and 10 drops of chemically pure H_2SO_4. A pink or faint red color, gradually turning to purple, indicates indol.

Potato: This is a very serviceable and easily prepared culture-medium, especially for the cultivation of moulds. The potato is cut into slices and cylinders. Only sound potatoes should be used. First the potato is washed well in water and scrubbed with a small hand-brush, after which it is placed in a 1 : 1000 mercuric chloride solution for an hour; then it is washed in sterile water, and the skin scraped off with a sterile knife. Large potatoes are cut into slices about one-quarter of an inch in thickness, and these slices are placed in sterile Petri dishes.

The cylinders are cut with an apple-corer or a cork-borer. They are made about two inches long and five-eighths of an inch in thickness. The ends are squared. Each cylinder is cut into two equal parts by an oblique incision which will give a large inclined surface. The cylinders are placed in test-tubes with the slanting surface up. Special potato-tubes can be used, or an ordinary wide and short tube, in the bottom of which a little pledget of cotton has been placed. The special tubes have a small chamber in the bottom formed by a constriction of the tube. These tubes can be made in the laboratory by heating a tube and constricting it with a wire. Plug the tube with cotton. Sterilize both the tubes and Petri dishes in the steam sterilizer for one hour, and the

next day for half an hour. To prevent the potatoes from turning dark, Abbott advises that the cut cylinders be allowed to stand in running water for twenty-four hours. Before sterilizing, it is well to place a few drops of sterile water in each tube to keep the potatoes moist.

Potato-juice: This is made as follows: Add 100 grams of grated potato to 300 c.c. of water and place on ice over night. Express the juice through a cloth and cook for one hour on a water-bath. Filter and add 4 per cent. of glycerin. Transfer to tubes and sterilize the same as bouillon. This is said to be an excellent medium for the tubercle bacillus.

The **phosphorescent** bacteria grow best in a medium containing 2 or 3 per cent. of sodium chloride. The addition of 3 or 4 per cent. of potassium nitrate will demonstrate the reducing power of the **nitrifying** bacteria.

Urine can be used as a culture-medium, especially for the *gonococcus* and *the Micrococcus ureæ*.

Fresh eggs, raw or boiled, *whites of eggs, the yelk, bread paste, hydrocele fluid, ascitic fluid, aqueous humor,* and many other substances and preparations, have been used as culture-media.

CHAPTER IV.

STERILIZATION AND DISINFECTION.

Sterilization of Culture-media and Laboratory Apparatus.

BECAUSE of the presence of bacteria in the air and in rooms, and on articles of furniture, etc., a very important part of bacteriologic technique consists in destroying these micro-organisms by sterilization and disinfection. In this way the contamination of the *culture-media* is prevented and germs can be studied in pure culture, free from all other organisms.

Sterilization is accomplished either by *heat, filtration,* or the *action of chemicals.* Usually the term sterilization is intended to imply destruction of bacteria by heat. Disinfection means the destruction of bacteria by the use of chemicals. Any substance which is capable of killing bacteria is called a *germicide.* One which inhibits the development of bacteria is called an *antiseptic.* An object is said to be *sterile* when it is entirely free from bacteria and their spores. An object is *septic* when it contains actively growing bacteria or their poisonous products. *Aseptic* is synonymous with sterile.

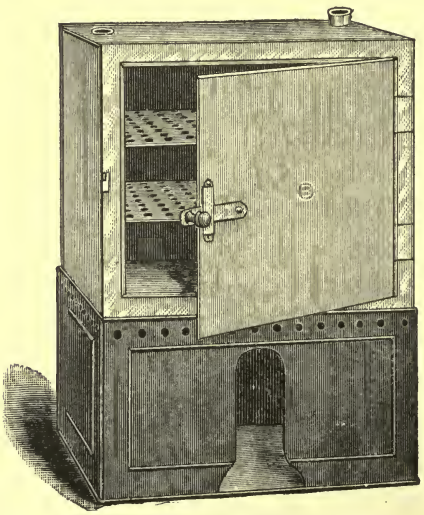
All bacteria have a *thermal death-point,* and the method of sterilizing and time of exposure are regulated accordingly. Culture-media, fluids, and anything that can be subjected to it, are sterilized by some form of heat.

STERILIZATION BY HEAT: This is accomplished by *fire; dry heat* or *hot air; live steam; superheated steam,* or *steam under pressure;* and *boiling.*

Fire: This form of sterilization is absolutely certain in its results, because it completely destroys all infected matter. Naturally that would limit its use considerably. In the laboratory the scissors, knives, forceps, and inoculating needles are sterilized by passing them through the flame of a Bunsen

burner or alcohol lamp a few times, or by holding them in the flame for a few seconds. The platinum inoculating needle is held in the flame until it is incandescent. The glass handle should also be sterilized for at least half its length. Every time this needle is picked up and before it is laid down again it should be sterilized. This is very important, because it not only insures its being sterile, but it also protects the bacteriologist from infection. Steel instruments should not be held in the flame for any length of time, as it affects the temper of the metal. They can be passed through the flame a few times.

FIG. 11.



Hot-air chamber. (Leitz.)

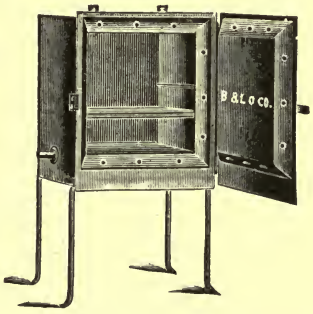
Dry heat: This also has a very limited application; a very high temperature is required, 150° C., and an exposure of at least one hour. This will kill all known bacteria and their spores. Its application is limited to the sterilization of glassware used in the laboratory. Articles made of rubber, wood, or crockery cannot be sterilized by dry heat.

The *tubes*, *dishes*, and *flasks* are well washed and scrubbed

in clean water. The tubes and flasks are plugged with cotton (raw cotton will do), which permits of the entrance of air but is an effectual barrier to the entrance of bacteria. These articles are then placed in the dry heat sterilizer for one hour. After sterilization the cotton plug can be covered with a small rubber cap as an additional safeguard against infection.

The *hot-air chamber* (Figs. 11 and 12) is a single or double-walled sheet-iron or copper chest having a door on one side and several removable shelves on the inside. The top of the chest is perforated by two holes, in one of which is placed a thermometer to indicate the temperature of the inside of the chest. The other opening is plugged with cotton. A large

FIG. 12.



Dry heat sterilizer.

Bunsen burner is placed under this chest, which rests on an iron frame. In order to distribute the heat evenly, a piece of wire gauze is placed over the burner.

The articles to be sterilized are placed on the shelves within the chamber, but not until the temperature has reached 150° C., so that they will remain in the chest exposed to that temperature for one hour. Frequently the mistake is made of counting the time from when the tubes, etc., are placed in the chamber while it is still cold.

Steam: All culture-media, woollen and cotton fabrics, wood, and crockery must be sterilized by steam. Steam is very penetrating, and is, therefore, a most effective sterilizing agent.

The Arnold *steam sterilizer* (Fig. 13), or one patterned after this, (Fig. 14) is used for this purpose. It is simple in construction, easily used, and inexpensive. An exposure of one hour is sufficient to destroy bacteria, but bacteria which are in the resting-spore stage may resist the action of steam for hours.

As such a prolonged steaming would spoil the culture-medium, this should not be exposed to its action for such a long

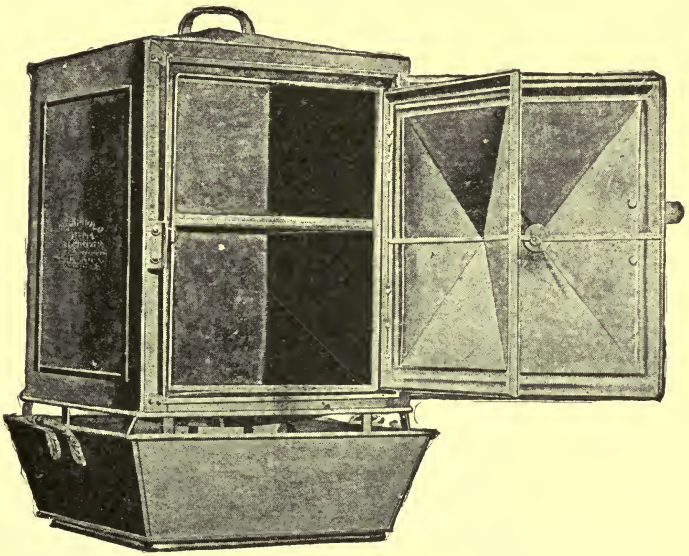
FIG. 13.

Arnold steam sterilizer.

time; and for that reason the *intermittent* or *fractional* method of sterilizing is used. The culture-medium is exposed to the action of the steam for fifteen minutes on each of three successive days. The first sterilization will kill all the fully developed bacteria. Any spores which may have survived this sterilization will develop into bacteria in the course of the succeeding twenty-four hours, and these are killed by the next

sterilization. After the third sterilization the medium can safely be said to be absolutely sterile. If the cotton plugs are not removed, the medium will remain sterile forever, but unfortunately it will, in the course of time, dry up. If the tube is hermetically sealed, the medium will remain intact. Any period of sterilization should not be continued too long, as it may spoil the medium by preventing its solidification ; and

FIG. 14.



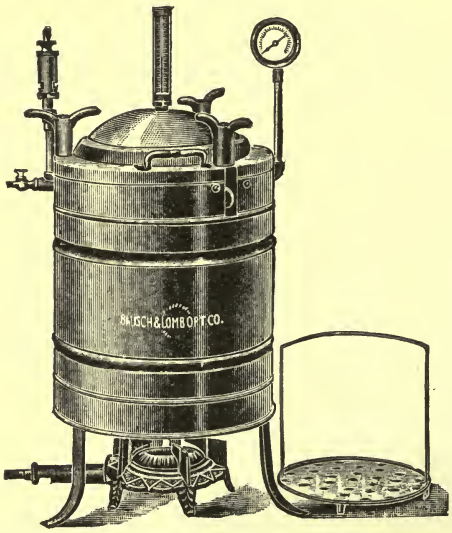
Boston Board of Health steam sterilizer.

undue prolongation of the first period may also inhibit the development of the spores, which will thus escape the effect of the later sterilizations and develop afterward. Agar-agar is sterilized only twice, because it cannot withstand repeated liquefying.

Sterilization by steam may also be effected by exposing the article to be sterilized to the action of *streaming* or *live steam* for one hour, or for fifteen minutes to the action of steam

under a pressure of two or three atmospheres, which is sufficient to destroy the spores. This is done by superheated steam in an autoclave. The medium is placed in the autoclave, the top is screwed down firmly, and the escape valve left open until the steam has displaced the hot air. The valve is then closed and steam is generated for fifteen minutes or longer if desired (Fig. 15). Cooling must be allowed to take place gradually, as otherwise the cotton plugs will be

FIG. 15.



Autoclave for sterilization with live steam under pressure.

forced into the tubes or flasks by the atmospheric pressure. The principal objection to the autoclave is that the reaction of the medium is altered by the chemical changes brought about by the excessively high temperature. The advantages are rapid and absolute sterilization.

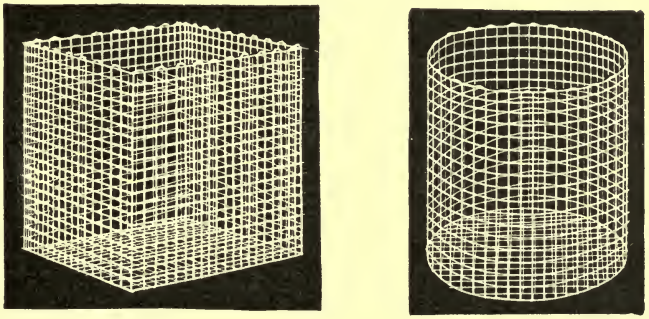
FILTRATION: Liquids can also be freed from bacteria by filtration. They are filtered through *unglazed porcelain, gravel, sand, powdered glass, charcoal,* or *crushed stone.* Porcelain is

the best. Unstable toxins and antitoxins, which are destroyed easily by heat, are filtered through porcelain.

The objection to this method of sterilization is that it is effective for a time only. All these filters become clogged with bacteria in the course of a very short time, and then the bacteria are carried through *en masse*.

Before a new filter is used, it should be sterilized by dry heat. Filters should be cleansed thoroughly at least once a week. Porcelain filters are scrubbed and then heated in the flame of a blowpipe or Bunsen burner until all the contained organic matter is consumed. As the organic matter

FIG. 16.



Wire test-tube basket (see p. 37).

is charred the filter turns black, but it gradually regains its white color as the organic matter is consumed. Sand, powdered glass, etc., are replaced with new material as often as is necessary.

PASTEURIZATION: This is partial sterilization at a comparatively low temperature with an exposure of two or three hours. The temperature should be high enough to destroy not only the saprophytic bacteria, but also the pathogenic bacteria, especially the tubercle bacillus and the *Bacillus typhosus*. Pasteurization is used principally for the sterilization of milk.

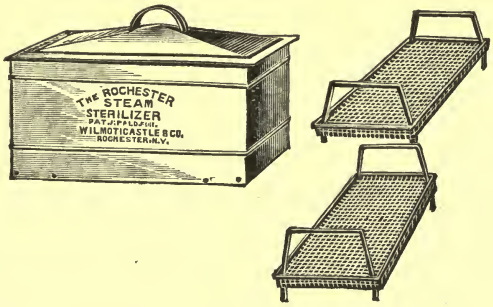
The milk, contained in a bottle, is placed in hot water at a

temperature of about 75° C. for two hours. This does not alter the composition of the milk, and it will keep on ice for several days.

Disinfection of Instruments and Materials Used in the Operating-room.

The same care that is used in the laboratory must be observed in the operating-room. Everything should be absolutely sterile. Any instrument, towel, sponge, or ligature

FIG. 17.



Portable steam sterilizer for instruments.

that falls to the floor or comes in contact with any unsterilized object should be removed at once and resterilized before it is used again.

Instruments may be sterilized by dry heat; but it is preferable to subject them to steam or to boil them in a 1 per cent. solution of sodium bicarbonate, which prevents rusting and does not dull the edge of sharp instruments. They can either be placed directly in the water or be wrapped in towels or pieces of gauze. As soon as they are removed from the sterilizer (Fig. 17) they are immersed in sterilized or distilled water or in a 5 per cent. carbolic acid solution. Mercuric chloride is unsuited for the disinfection of instruments because it dulls the edges of cutting instruments, and also deposits mercury on their surfaces.

Catgut can be sterilized by boiling. One of the best methods in use at the present time is the *cumol method.* The catgut is dried first in the hot-air chamber or on a sand-bath, and then is boiled in cumol at a temperature of 168° C. for one hour, which kills not only bacteria, but their spores as well. The cumol is evaporated or poured off and the catgut dried at a temperature of 100° C. for two hours. It is then transferred with sterile forceps to sterile test-tubes, and these are plugged with cotton.

Catgut may also be soaked in a 4 per cent. *formalin solution* for twenty-four hours; boiled in water for fifteen minutes, and preserved in 95 per cent. alcohol to which are added 0.1 per cent. of mercuric chloride and 5 per cent. of glycerin. Formaldehyde catgut is stronger and less brittle than the old-fashioned chromicized catgut. It is easily tied and the knot does not slip. *Pyoktanin* catgut is boiled in a solution of pyoktanin.

Ligatures of silk and silkworm-gut are boiled in water or placed in sterile test-tubes and sterilized in the steam sterilizer by the fractional method for one-half hour each time.

Dressings are packed loosely in a canvas bag or gauze wrapper, and sterilized in the steam sterilizer by the fractional method. The autoclave may also be used.

Sterile water and **sterile salt solution** should always be on hand in large quantities in every modern operating-room. They are, of course, sterilized by boiling.

Drainage-tubes, hand-brushes, etc., are kept in a disinfectant solution. A 5 per cent. carbolic acid solution answers very well.

Site of operation: It is advisable to begin preparing the site of operation the day before operating. If the part is covered with hair, it should be shaved, then thoroughly scrubbed with green soap and water, shaved again, scrubbed again, washed with alcohol followed by ether, sterile water, or bichloride. The part is then covered with a protective dressing. Recently an artificial skin has been devised which is recommended very highly by some operators. It consists of a large sterilized piece of dental rubber, coated on one side with an adhesive substance. It is spread evenly with the

hand over the site of operation, the warmth of the hand softening the gum sufficiently to make the rubber adhere firmly to the skin. It is treated just as the skin would be ordinarily, and when the wound is sutured it is included in the sutures. When the stitches are removed, it is pulled off like a porous plaster. The advantage claimed for it is that it can be sterilized properly, which the skin cannot, and thus insure absolute cleanliness of the wound and field of operation.

Hands of operator: The disinfection of the hands of the operator and his assistants is a very important matter, and many methods are in vogue aiming to procure absolute asepsis of the hands. It is theoretically impossible to do this, because of the many crevices in the surface epithelium and under the finger-nails, which afford secure lodgement to all kinds of bacteria. Cultures of pathogenic bacteria have been obtained from the skin and from under the nails after the most thorough attempt at disinfection.

The method most employed at the present time is that recommended by Welch. After having trimmed and cleansed the nails the hands are washed and scrubbed with soap and water, the water of as high a temperature as can be borne, for at least ten minutes, and then rinsed in clean water. They are then immersed in a warm saturated solution of potassium permanganate for five minutes, washed in oxalic acid to remove the stain of the permanganate, and then in warm water or salt solution, after which they are soaked for two minutes in a 1 : 500 mercuric chloride solution. They may then be considered sterile. The hand-brush must be sterile, and should be kept in a 1 : 1000 biniodide of mercury solution.

Another method is to scrub the hands with green soap and water and then immerse them for two minutes in a solution of biniodide of mercury in methylated spirit, after which they are rinsed in a 1 : 2000 biniodide of mercury solution. The objection to the mercury salts is that they crack the skin. Many surgeons cannot tolerate them at all, and for that reason the permanganate is preferred. Every surgeon has a method which he believes the best. Personal experience will govern the choice of a method.

Some surgeons favor the use of sterilized *rubber gloves.* The objection seems to be that they obtund the sense of touch; but they are to be recommended when operating on septic or suppurative conditions. Like the artificial skin, they can be rendered absolutely sterile. A German operator has recently advocated covering the hands with a very thin coat of a specially prepared varnish.

Infected wounds are washed thoroughly with sterile water or salt solution, dusted with some antiseptic powder, and covered with an antiseptic dressing. The antiseptic powder may be omitted. Solutions of bichloride and carbolic acid are not only irritating, but they also form chemical compounds (albuminates) with the tissues and discharges, which constitute a protective barrier for the organisms beneath and render the antiseptic value of the solution *nil.* Strong solutions interfere with the healing of the wounded tissues.

Cleanliness and sterilization have in a large measure displaced antiseptic solutions in the treatment of fresh and infected wounds. When there is much pus in an open wound, hydrogen peroxide may be used with benefit. It liberates oxygen, which in its nascent state possesses great germicidal power. When dressing a wound, everything that comes in contact with it must be absolutely sterile, including the hands of the dresser.

CHAPTER V.

ANTISEPTICS AND DISINFECTANTS.

It is impossible in the limited space at our disposal to enter into a detailed discussion of the antiseptic value of all the known antiseptics. We will, however, consider the most important and most commonly used.

Mercuric chloride in a solution as weak as 1 : 30,000 restrains the development of anthrax spores; 1 : 1000 kills them in a few minutes. The addition of 5 parts of hydrochloric or tartaric acid to 1 part of the mercuric salt will prevent precipitation of the mercury by the albumins of the tissues, which lessens its germicidal value considerably. The bacteria are embraced in the albuminous coagulum and thus escape the action of the bichloride. A 1 : 1000 solution kills the tubercle bacillus in one minute. Growth of the pus cocci is restrained by a 1 : 30,000 solution; 1 : 1000 kills them in from five to ten minutes. Sternberg advocates its use as a general disinfectant in 1 : 1000 or 1 : 500 solution for spore-containing material, and in 1 : 5000 or 1 : 2000 for non-sporulating pathogenic bacteria.

Potassium permanganate, in 5 per cent. solution, kills anthrax spores in twenty-four hours. The dilute solutions used for irrigating purposes and urethral injections are absolutely worthless so far as their antiseptic action is concerned. They are usually administered hot, and to the heat must be ascribed their much vaunted value as germicides. Potassium permanganate is decomposed easily by wound secretions.

Silver nitrate destroys anthrax spores in twenty-four hours in a 1 : 10,000 solution. Behring says it is superior to mercuric chloride. It is very irritating, and combines with chlorides and albumins to form insoluble silver salts which have no germicidal value. The various other silver salts (organic) now on the market do not combine with the albumins, and

are less irritating than the nitrate, but the clinical reports are so contradictory that it is impossible to determine their antiseptic value with any degree of positiveness. Hardly any two men favor the same compound.

Calcium hydroxide, or slaked lime, in 3 per cent. solution, kills the typhoid bacillus in six hours; 6 per cent., in two hours. By adding 2 per cent. of milk of lime containing 20 per cent. of calcium hydroxide to typhoid stools the bacillus is killed in one hour. It is not effective against the tubercle bacillus nor against anthrax spores. It is used very widely as a disinfectant of typhoid dejecta, and is far superior to either bichloride or carbolic acid for this purpose. An excess of lime should be used in order to insure perfect results. The chlorinated lime from which the milk of lime is made should contain not less than 25 per cent. of chlorine. It should always be freshly prepared, as it decomposes rapidly.

Boric acid is practically worthless as a disinfectant. A saturated solution fails to kill pus cocci in two hours. It is a very weak antiseptic. A 5 per cent. solution failed to destroy anthrax spores in five days (Koch). It is used very widely as a dusting-powder on wounds.

Alcohol (absolute) kills the tubercle bacillus after five minutes' exposure. Alcohol in 40 per cent. solution kills the pus cocci in two hours.

Pyoktanin: Many of the anilin dyes are germicides, especially blue pyoktanin or methyl-violet. The pus cocci and anthrax bacilli are killed in thirty seconds by a 1 : 1000 solution; the typhoid bacillus in thirty minutes. Malachite-green possesses even greater germicidal value than pyoktanin. The objection to these dyes is that they stain and discolor the tissues.

Chlorine: All the haloid elements are active germicidal agents. Chlorine combines readily with hydrogen and liberates nascent oxygen. It is most active in the presence of moisture. A moist atmosphere, containing the gas in the proportion of 1 : 2500, kills the anthrax bacillus in twenty-four hours. In the proportion of 1 : 200 it kills the tubercle bacillus in an hour.

Hydrogen peroxide: The solutions on the market are ex-

tremely variable in strength and the results of their use uncertain. They all deteriorate very rapidly. Peroxide is used principally for cleansing suppurating wounds, as it possesses the power of liberating nascent oxygen, which oxidizes the purulent secretions.

Iodoform is very mildly antiseptic and possesses slight germicidal power. Its odor was for a long time accepted as an evidence of its antiseptic power. Its action is due entirely to the liberation of its iodine.

Carbolic acid: This is the best known and most widely used disinfectant. It is used in strengths of from 1 to 5 per cent. Like mercury, it forms insoluble albumin compounds which interfere with its penetrating power. By combining carbolic acid with an equal amount of hydrochloric acid its germicidal power is increased considerably. Anthrax spores are destroyed in three hours if exposed to a 5 per cent. solution at the body temperature. The tubercle bacillus is killed in thirty seconds. Creolin, lysol, trikresol, and similar preparations, possess the same germicidal power as carbolic acid.

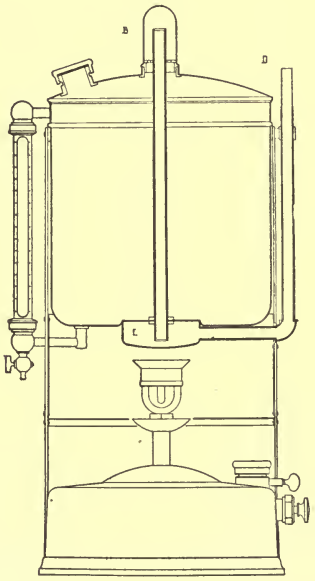
Sulphur is used very extensively for the disinfection of sick-rooms after contagious diseases. It is used commonly in the form of a sulphur candle, which is placed on a tin pan, floated in a basin of water, and lighted. The room should be closed tightly, so as to confine all the vapor. When powdered sulphur is used, it is placed on a bed of sand or ashes in an iron pot surrounded by water before ignition. The action of the fumes is much greater if the air in the room contains an excess of moisture. This is obtained by evaporating a gallon of water in the room just before igniting the sulphur. Liquid sulphur dioxide may be used. It vaporizes at the room temperature.

The antiseptic and germicidal value of sulphur has been much overestimated, and it is not used so extensively as it was some years ago, it having been displaced by a much more active agent, viz.:

Formaldehyde: One of the most active disinfectants and germicides is formaldehyde. It is obtained in the market under the trade name of formalin or formalose, a 40 per cent. aqueous solution of the gas formaldehyde. The gas is ex-

tremely penetrating, and is very irritating to the mucous membranes. This limits its use to the disinfection of inanimate objects. A 15 per cent. solution at 150° C. kills anthrax spores in one and a half hours. Used as a liquid, it does not possess any advantages over carbolic acid and similar preparations. When *vaporized*, it is vastly superior to all other agents. Robinson found that it will penetrate a mattress

FIG. 18.



Formaldehyde apparatus.

and kill test-tube cultures placed within it. The gas is generated rapidly and continuously by any of the different styles of formalin generators on the market (Fig. 18). Formalin pastilles can now be procured which contain the product in such quantity as to permit of its inhalation. It is also used in the form of a spray; or sheets saturated with formaldehyde solution are hung up in the tightly closed room for

twelve hours, after which the doors and windows are thrown wide open and the room thoroughly aired. The number of sheets required will depend on the size of the room. In a room 10 × 10 feet two sheets will suffice. All crevices, keyholes, etc., should be packed with cotton, so that none of the vapor will escape.

Novy's and the "Central" formaldehyde generator, and Schering's lamp, are exceedingly simple in construction and inexpensive. Others, like Trillat's autoclave, are complicated and expensive. Either the apparatus is placed in the room, or the vapor is sent in through the keyhole by means of a supply-tube. The temperature of the room should be about 21° C., and it should contain sufficient moisture.

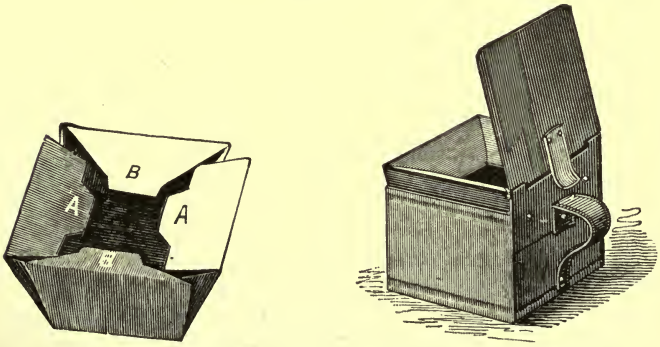
Sulphate of copper is an excellent and at the same time a very cheap disinfectant. It is not irritating and has no odor. It is especially valuable for the disinfection of typhoid stools. A pound of the sulphate is dissolved in $2\frac{1}{2}$ gallons of water, and a pint of this solution is kept constantly in the vessel which receives the discharges from both bowels and bladder. The poison is destroyed in fifteen minutes if the infected material is mixed thoroughly with the solution.

CHAPTER VI.

PRACTICAL DIRECTIONS FOR DISINFECTION.

Excreta: It has already been pointed out that mercuric chloride and carbolic acid when mixed with typhoid or diarrhœal stools, for the purpose of disinfection, fall far short of producing the desired result, and as the excreta are the most common means for conveying infection in typhoid, it is manifest that any disinfection to be of value must be absolute. Excretions and discharges from the nose, mouth, lungs, or conjunctivæ should be received in old rags or paper napkins and burnt. Tubercular patients should be instructed to use a

FIG. 19.



Pasteboard boxes for receiving sputa of tubercular patients.

spit-cup, which is disinfected easily by boiling or by the addition of chemicals. A small earthenware vessel or the pasteboard box (Fig. 19) recommended by nearly all boards of health are very practical. These boxes are placed in iron frames and are removed once a day and burnt. They are so inexpensive as to be within the reach of everybody. The

metal or earthenware spit-cups are filled partly with a 0.4 per cent. solution of chloride of lime or a combination of carbolic and hydrochloric acids.

The stools and urine of patients sick with an infectious disease, especially typhoid, intestinal tuberculosis, and cholera, should be received in a vessel containing sulphate of copper solution, or a 5 per cent. solution of chlorinated lime, or 20 per cent. milk of lime. The excreta are mixed thoroughly with the disinfecting solution and allowed to stand for an hour or two before final disposition is made of them. The urine should be regarded with the same suspicion as the stool, and should be disinfected immediately after its passage.

Sick-room and hospital wards: The custom of placing in a sick-room vessels containing disinfecting solutions, with the expectation of disinfecting the air in the room, is ridiculous. It is impossible to disinfect a room while the patient is occupying it. It is important, however, that the ventilation be perfect, so that there is a constant supply of fresh air to take the place of the vitiated air. There is nothing more disagreeable, and at the same time harmful, than to enter a sick-room and be greeted by a heavy, foul-smelling atmosphere. It undermines the patient's vitality and makes him unfit to cope with the disease. Besides, fresh air is a good purifier. If it is desired to disinfect the room while occupied, the walls, floors, and ceiling should be well washed with a 1 : 1000 mercuric chloride solution or a 2 per cent. carbolic acid solution. The walls may be rubbed down first with fresh bread. For reasons of cleanliness, sick-rooms should contain as little furniture as possible, and no drapings, curtains, or anything to which the contagium can cling.

After the room has been vacated it is fumigated, with all its contents, with sulphur dioxide for twelve hours. At least 3 pounds of sulphur should be used for each 1000 feet of cubic air-space. Then all the surfaces are washed with bichloride or carbolic acid solutions, followed by plenty of hot water and soap and thorough ventilation. Whenever possible, disinfection should be done by means of formalin vapor. This will also disinfect the furniture and any articles which were in the room during its occupancy by the patient.

It is much more efficient than any other method, and just as cheap.

Clothing, Bedding, etc.: All bedding, clothing, linen, nurse's outer wearing apparel, and anything that may have come in contact with the patient in any way, should be disinfected. If of little value, it should be burnt. Otherwise soak in a 1 : 2000 bichloride or a 2 per cent. carbolic acid solution for four hours, then boil thoroughly for one hour; hang the washing outdoors where there is plenty of air circulating, and leave it there for a day. This will further cleanse the clothing and dispel any odor of carbolic acid which would otherwise cling to it. Sunlight is also a disinfectant. It is cheaper to burn a straw mattress than to attempt to disinfect it. Clothing, etc., can also be sterilized by steam if the proper apparatus is at hand.

Patient: The body of the patient can be washed with a very mild solution of bichloride. After each evacuation of the bowels the nates are cleansed with a cloth or piece of gauze wet with bichloride. The body of the convalescent is treated in the same way. After the exanthematous fevers the entire body is washed first in a hot bichloride solution and then anointed with vaselin, plain or carbolated, olive oil, benzoinated lard, or any other unguent. This will prevent the scales from being distributed broadcast and lodging on the clothing of the nurse, doctor, or members of the family, to be carried to others. Such patients should also be isolated until there is no longer any danger of infection.

The dead: The body of a person dead of an infectious disease should be wrapped in a sheet thoroughly saturated with either a 0.4 per cent. chloride of lime solution, 1 : 500 bichloride or a 5 per cent. carbolic acid solution. The body should not be touched by any one, and an early burial is advisable, and should be insisted upon if possible. Although cremation is really the best disposition of such bodies, it is not absolutely necessary. Esmarch has shown that when a body is placed in the soil all the pathogenic bacteria die, probably because of the lack of oxygen or because decomposition and putrefaction are inimical to their development.

Utensils: All the eating utensils, combs, brushes, etc., used

by the patient should be kept separate from those used by the family, as infection doubtless is carried in this way. They should be kept in the patient's room. Such prophylaxis constitutes no small part of the treatment of an infectious disease, and the medical attendant should be very specific in his instructions in that regard. He must insist on their being carried out. When the patient is nursed by the family, many of the minor details of thorough disinfection and prevention of a spreading of the disease are apt to be overlooked, or more often disregarded because of their apparent insignificance in the eyes of the family.

CHAPTER VII.

CULTURES AND THEIR STUDY.

A **culture** is an artificial growth of bacteria on one or another specially prepared medium. Any number of different kinds of bacteria may be contained in a culture. If the growth contains only one variety, it is called a *pure culture.* The objects in view in the preparation of culture-media are the separation of bacteria and the making of a pure culture, so that the morphology and biology of each variety of organism may be studied accurately. The disease caused by any one germ may be reproduced experimentally in the laboratory by the injection of a pure culture of the germ in question. Until Koch suggested the use of solid culture-media and the making of plate cultures it was impossible to isolate bacteria and make pure cultures of them. Microscopic examination alone is frequently insufficient for a positive identification of a germ, as many species have the same morphologic characteristics. Hence it is a matter of necessity to cultivate these germs artificially, so that their cultural differences may be observed.

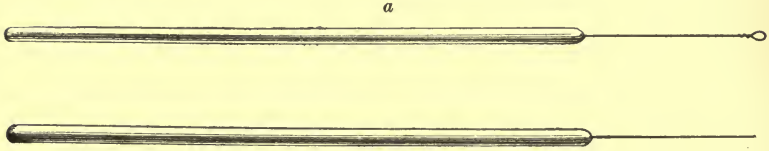
Bacteria are isolated in **pure culture** by the *Koch plate* method; *Petri dish* method; or *Esmarch roll* culture.

The **preliminary steps** for making a pure culture by any of these methods are as follows: Label three tubes of gelatin or agar-agar 1, 2, 3; liquefy the medium over a flame or in water kept at a temperature of about 40° C. Sterilize the platinum needle (Fig. 20) and inoculate tube No. 1 with a loopful of the material to be examined. Replace the plug and shake the tube gently, being careful not to wet the cotton plug. Inoculate tube No. 2 in the same manner with a few loopfuls obtained from tube No. 1; tube No. 3 is inoculated from tube No. 2. This gives us three different dilutions. Another method for obtaining a pure culture is to inoculate

three beef-tea tubes, from each one of which a tube of liquefied gelatin is inoculated. This complicates the work considerably, and errors are also more likely to occur because of the frequent transfer of the material.

When one tube is to be inoculated from another, tube No. 1 is held between the thumb and first finger of the left hand with the closed end directed toward the back of the hand. Tube No. 2 is held in the same manner between the first and second fingers. The inoculating needle is held in the right hand. The glass handle of the needle should be at least six

FIG. 20.



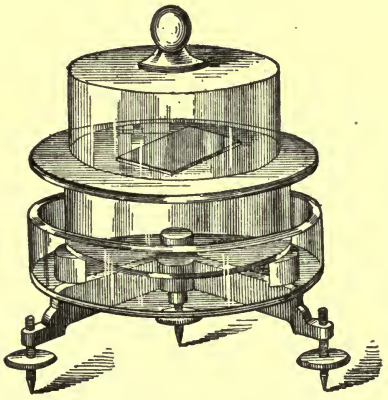
(*a*) Looped and (*b*) straight platinum wires in glass handles.

inches long, and the platinum wire three inches long and of medium thickness. A little loop is made in the free end of the wire. Before inoculating the tubes, the cotton plugs are twisted so that they can be removed easily. The tubes should be held very obliquely, as the air of the laboratory is always laden with germs. When ready to inoculate, sterilize the needle, and with the ring and little fingers of the right hand remove the cotton plug of tube No. 1, take out a loopful of material and immediately replace the plug. Now remove the plug from tube No. 2 as before, pass in the platinum needle, shake gently, withdraw the needle, and replace the plug. Sterilize the needle. The cotton plug is always held between the fingers. It must not be laid on the table even for an instant, because of the possibility of contamination. Holding the plug between the fingers mentioned leaves the hand practically free to perform the inoculation unhampered and without delay, and the plug is safe from contamination. With a little practice, this method can be carried out with considerable accuracy and rapidity. Inoculations should not be

made in a draft or near an open window. They should be done as quickly as possible.

Koch plate culture: This method requires considerable apparatus, and on the whole is not so satisfactory as the two

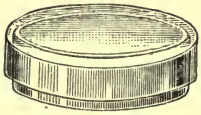
FIG. 21.



Levelling-tripod with glass chamber for plates.

other methods. The cultures are made on clean smooth glass plates, which are placed on a levelling apparatus under a sterile glass chamber (Fig. 21). The technique is the same as for the Petri dish culture.

FIG. 22.

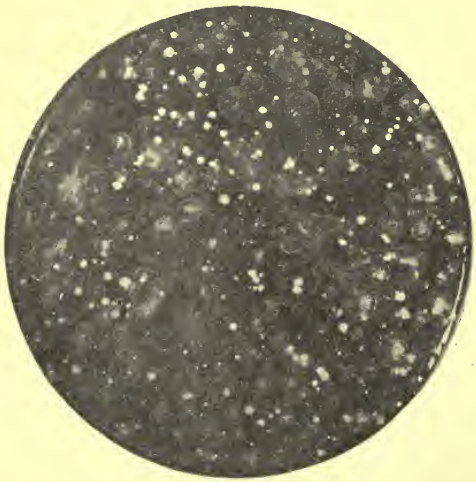


Petri dish.

Petri dish culture: This is the method commonly used. It is easily done and gives the most satisfactory results in every way. Petri dishes (Fig. 22) are glass dishes, about four inches in diameter and about $\frac{1}{2}$ inch in depth, provided

with an accurately fitting lid. These dishes are washed and sterilized by dry heat for one hour. The covers should not be removed until the dishes are ready to be used. Take tube No. 1, burn off the projecting end of the cotton plug in the flame of an alcohol lamp or Bunsen burner; then with sterilized forceps push the plug down into the tube for about an inch. Hold the end of the tube containing the plug in the flame for a few minutes to sterilize it. An assistant now

FIG. 23.



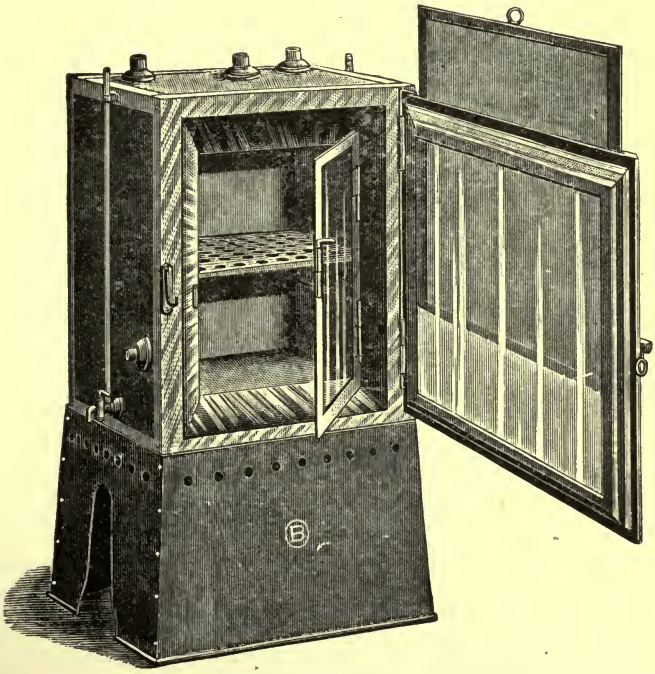
Colonies in Petri dish.

raises the lid of the Petri dish directly over the dish about one inch (just high enough to admit the end of the tube), the cotton plug is removed with sterile forceps, and the contents of the tube (which are still liquid) poured into the dish. The cover is replaced immediately and the dish moved gently to and fro so that the medium will be spread evenly over the bottom of the dish. The film of medium in the dish should be sufficiently thin to be transparent, so as not to hinder microscopic examination of the colonies of bacteria (Fig. 23).

This process is repeated with each of the two other tubes, and the dishes labelled 1, 2, 3, respectively. In dish No. 3 the colonies will be much fewer in number than in either No. 2 or No. 1, and the dilutions may be used for comparison.

Cultures of *pathogenic bacteria* must be kept at a temperature approximating that of the body. To attain this temperature, a special warming-oven or *incubator* is used.

FIG. 24.

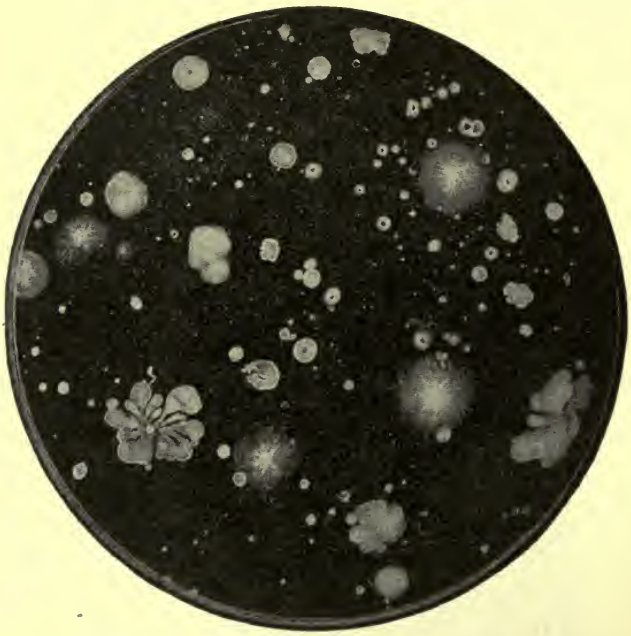


Laboratory incubator.

The incubator (Fig. 24) resembles the hot-air oven. The space between the walls is filled with water, the amount of which is registered by a water-gauge placed on one side of the box. The roof is perforated by three holes, one of

which is plugged lightly with cotton so as to allow of plenty of circulation of the air. One of the holes is meant to contain a gas-regulating apparatus, which passes into the water in the jacket. Any good regulator will answer the purpose

FIG. 25.

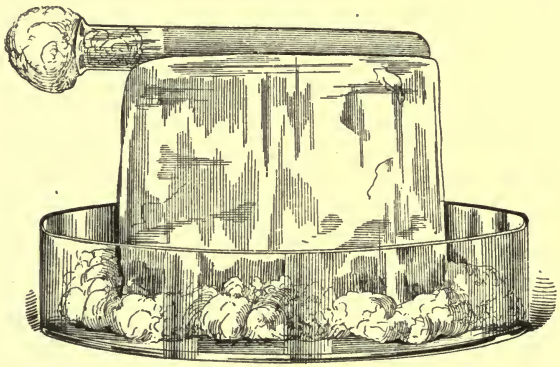


Showing certain macroscopic characteristics of colonies. (Abbott.)

of regulating the temperature in the incubator. A thermometer passes through the third hole into the inside of the incubator. Incubating ovens are easily devised in case of necessity. The vest pocket or watch pocket, or the axilla, or the pocket of the night dress, will serve very nicely as an incubator for cultures contained in small metal boxes or any container which is not easily broken. For laboratory work, however, an incubator is a necessity.

The Petri dishes are placed in the incubator for twenty-four hours, when the surface of the medium is seen to be studded with fine dots, the number depending on the degree of dilution. These dots or specks are called *colonies*. Colonies also develop, but more slowly, in the body of the medium. Koch found that when bacteria are transplanted on a large flat surface (Fig. 25) they invariably exhibit a tendency to isolate themselves and form colonies which are characteristic of each species of bacteria. The study of these colonies is the first

FIG. 26.



Demonstrating Booker's method of rolling Esmarch tubes on a block of ice. (Abbott.)

step in the differentiation of bacteria. Each colony represents a pure culture of the germ, and from it the species is propagated for further study. The colonies are examined first with the naked eye and then with a low power ($\frac{2}{3}$) of the microscope, the Petri dish being placed upside down on the stage of the microscope. An accurate note is made of the appearance of the colony, its color, contour, size, etc. The description should be accompanied by a sketch or photograph.

Esmarch roll culture (Fig. 26): In this method the wall of the test-tube takes the place of the plate or Petri dish. Roll cultures are made easily, and possess the advantage that

frequent handling and exposure of the medium are not required. Tubes used for this purpose should contain not more than 5 c.c. of culture-medium. The medium is liquefied and inoculated as in the preceding method. The cotton plug is burnt, pushed down even with the mouth of the tube, and covered with a rubber cap. The tube is then held under cold running water or laid on a block of ice and twirled rapidly between the fingers, so that the medium will solidify on the wall of the tube in a thin film. The colonies form on the sides of the tube, and are studied in the same way as those in the Petri dish. When the tube is twirled, care should be taken not to wet the end of the cotton plug, so that it will not adhere to the tube when the gelatin or agar solidifies.

Tube cultures: After the colonies have fully developed, *tubes are inoculated* from them. Any kind of medium may be used, but it is customary to inoculate a set consisting of a tube each of beef-tea, gelatin, agar, potato, blood-serum, and glycerin-agar. If the suspected material is believed to contain bacteria which will grow only on special media, such media must be used; as, for instance, in the case of the tubercle bacillus, typhoid bacillus, diphtheria bacillus, etc.

The tubes and needle are held as before, the platinum wire being perfectly straight and without a loop at the end. The cover of the Petri dish is raised slightly, and with the sterile needle a very small amount of the colony is removed and transferred to the test-tube. The wire must not come in contact with anything except the colony and the medium to be inoculated.

For **bouillon** inoculation, or any other liquid medium, the needle is passed into the medium and the adhering culture shaken off.

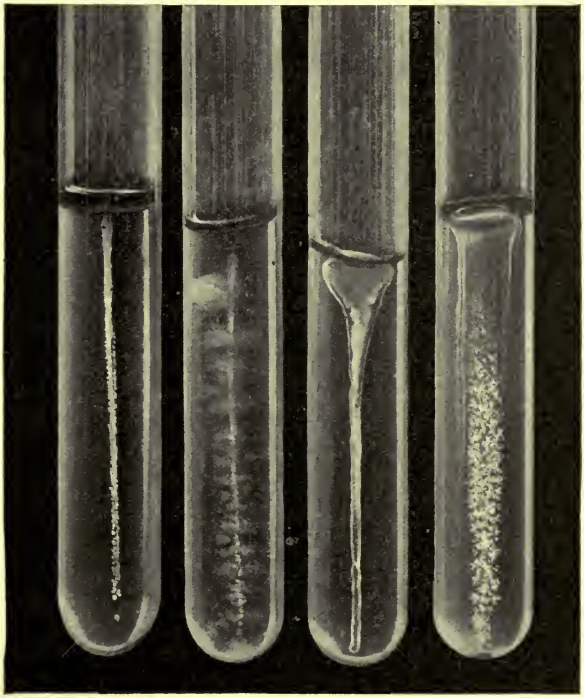
In **gelatin** the so-called *stab culture* (Fig. 27) is made. The wire is passed vertically into the centre of the medium for about half its length and is then slowly withdrawn.

Stab cultures are made principally for the purpose of studying liquefaction. The growth in or on gelatin is varied, and often quite characteristic. Thus, it may be limited to the surface of the medium; or to the stab; or it may appear both

on the surface and along the stab. Some species liquefy the gelatin and others do not. Some bacteria liquefy the medium in a manner which is characteristic of the germ.

Gelatin cultures may also be *embedded* and *sectioned:* The

FIG. 27.



Series of stab cultures in gelatin, showing modes of growth of different species of bacteria.

tube is warmed a little so as to loosen the gelatin (without melting it), which then is transferred to and fixed in Mueller's fluid. It is hardened in alcohol, embedded in celloidin, sectioned, and stained like tissue preparations. Winkler suggests filling a cavity in a block of paraffin with sterile gelatin.

After it has solidified it is inoculated. When the growth has developed sufficiently, the entire block is sectioned under alcohol and the sections are stained with a weak carbol-fuchsin solution.

On media having a slanting surface, such as **agar**, potato, or blood-serum, a *stroke* or *smear culture* is made. For a stroke, the wire is passed in a straight line across the centre of the surface; for a smear, the needle is rubbed gently over the entire surface. In neither case does the needle penetrate the medium.

The comportment of the germ toward **oxygen** is noted also. A heavy surface growth denotes aërobic tendencies. A heavy growth along the line of inoculation and a slight surface growth denote anaërobic tendencies.

These tubes are **incubated** and a record of the growth made from time to time. Gelatin tubes and dish cultures cannot be placed in the incubator because of the low melting-point of the gelatin. For this reason agar is used mostly in the preparation of the dish cultures.

FIG. 28.

Zoöglœa of bacilli. (Abbott.)

The membranous pellicle formed by bacteria on the surface of **liquid media** is called a *mycoderma*. When the growth forms principally in the mass or body of the medium, gelatinous-looking masses are formed, the *zoöglœa* (Fig. 28).

Klatsch preparation: Occasionally it is desirable to make a microscopic specimen of an entire colony. Take a clean cover-glass, warm it slightly, and lay it carefully on the colony. A little pressure will remove the air bubbles under the cover-glass without destroying the contour of the colony. The cover-glass is then lifted up carefully, when the colony

will be found to adhere to it. This is called a Klatsch or adhesion preparation. The preparation is dried, fixed, and stained like any ordinary microscopic specimen, as will be described later.

Animal inoculation: Pure cultures may also be obtained by animal inoculation. The suspected material is mixed with a small quantity of sterile water and injected into the abdominal cavity, subcutaneous tissue, or ear vein of a mouse or guinea-pig. This method is restricted almost entirely to obtaining pure cultures of the tubercle or anthrax bacillus.

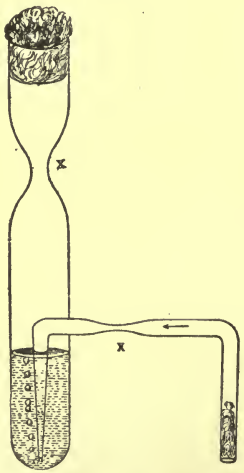
All these various cultures can be preserved for **museum specimens** by fixing them in formalin, which is applied in the form of a spray or dilute solution; or the tubes are placed in a tightly closed jar containing a little dilute formalin solution. After ten days they are removed and the ends of the tubes are sealed in a flame; or are stoppered with a cork or wooden plug and dipped in paraffin. Cultures at different stages of development and in all kinds of media may be thus preserved and used for class-room instruction. They make a very attractive and instructive museum exhibit.

CHAPTER VIII.

CULTIVATION OF ANAËROBIC BACTERIA.

PASTEUR, as early as 1861, demonstrated that certain species of bacteria will grow only in the absence of oxygen. Anaërobic bacteria grow best in a medium containing from 1 to 2 per cent. of sugar. Various methods have been devised for the cultivation of these organisms. Some of them are

FIG. 29.



Liborious tube for anaërobic cultures: x = places sealed.

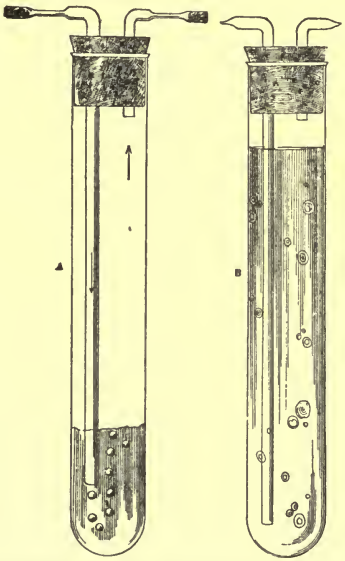
simple and others very complicated, requiring considerable apparatus. A tube containing at least 20 c.c. of medium is inoculated while the medium is liquid. The anaërobic bacteria develop in the body of the medium.

Hesse covers the surface of the inoculated medium with sterile olive oil, more effectively to exclude oxygen.

Smith fills the tube with sterile gelatin, thus burying the culture. He uses an ordinary fermentation-tube for this purpose. A test-tube answers as well.

Liborius expels the air from the tube and medium by boiling. The liquid medium is inoculated and then hardened in ice water. The tube (Fig. 29) is sealed with the blowpipe.

FIG. 30.



Fraenkel's method for the cultivation of anaërobic bacteria.

Esmarch makes a roll culture, and then fills the tube with sterile gelatin.

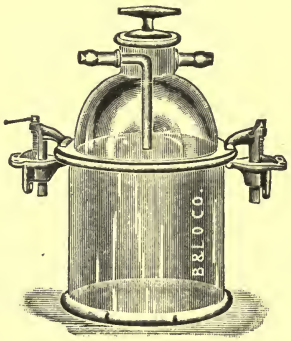
Buchner absorbs the oxygen with *pyrogallic acid.* 1 gram of pyrogallic acid and 10 c.c. of a 6 per cent. solution of potassium hydroxide are placed in a large dissolving tube. The tube containing the culture is stood inside of the large tube, which then is corked and sealed with paraffin.

Roux plants a strict aërobe (*Bacillus subtilis*) on the top of the anaërobic culture. The medium is boiled first, then quickly cooled and inoculated. The top is covered with sterile gelatin inoculated with the aërobe. The aërobe absorbs the oxygen and the anaërobe develops in the bottom of the tube where there is no oxygen.

Gruber exhausts the air in the tube with an air-pump.

Hueppe inoculates eggs in the shell through a very small hole made with a hot needle. The opening is sealed with collodion after inoculation.

FIG. 31.



Jar for anaërobic cultures.

Fraenkel uses an ordinary test-tube stoppered with a rubber stopper perforated by two holes, through which two glass tubes are passed (Fig. 30). One tube is short, and the other passes through the medium to the bottom of the test-tube. Both glass tubes are bent at right angles above the stopper and drawn out to a very small calibre, so that they can be sealed easily. Hydrogen is passed into the tube through the long glass tube and escapes through the short tube. After five or ten minutes the projecting free ends of the glass tubes are sealed at the places previously prepared for that purpose, and the entire rubber stopper is covered with paraffin to prevent entrance of oxygen and leakage of hydrogen.

Novy uses a large glass-stoppered jar (Fig. 31) constructed on the same plan as Fraenkel's tube.

Sternberg prepares three Esmarch roll cultures. The cotton plug is pushed down into the tube for a short distance. The end of the tube is closed with a soft-rubber stopper carrying two glass tubes. This stopper is pushed below the level of the tube half an inch, and the space is filled with melted sealing-wax. The test-tube is inverted and hydrogen gas passed in through one of the tubes. The gas diffuses through the cotton plug into that portion of the tube containing the culture. After a few minutes the outlet tube is sealed and then the inlet tube. It is not necessary to sterilize the rubber stopper, as the cotton plug is interposed between it and the culture.

Ravenel places the culture in an air-tight chamber partially filled with pyrogallic acid and a 10 per cent. solution of caustic potash. The air is exhausted with an air-pump and the chamber is then hermetically sealed. The chamber contains an upright shelf-stand on which Petri dishes are placed. Any oxygen left in the chamber, or which enters, is absorbed by the pyrogallic acid.

Koch covers the surface of a glass plate containing the culture with a mica plate, thus excluding oxygen.

Botkin uses a large bell-jar, which is stood in a dish containing liquid paraffin. Two rubber tubes are carried through the paraffin into the jar. Hydrogen gas is then sent into the jar through one of the tubes, and when only pure hydrogen escapes from the other the tubes are withdrawn, the solidifying paraffin closing the holes so that no oxygen can enter the jar. It is advisable to put a little pyrogallic acid into the jar in case of leakage.

Roux suggests still another method. The medium is liquefied and inoculated. While still liquid it is drawn into a long small-calibred piece of glass tubing, the ends of which have been slightly drawn. When the tube is full the ends are sealed. When the colonies have developed, the tube is broken with a file or diamond and the culture transplanted.

Any of these methods is subject to such modification as may be desired by the individual investigator.

CHAPTER IX.

MICROSCOPIC EXAMINATION OF BACTERIA.

MOTILITY: The first step in the microscopic examination of bacteria is to determine whether the organism is motile. This is done by means of the *hanging drop*. The living bacteria can be observed for days at a time, if necessary, in their characteristic grouping, and their multiplication and formation of spores watched.

A cover-glass is cleansed thoroughly and a drop of sterile water or bouillon placed in its centre. With a sterile platinum loop some of the culture is transferred to this drop. The cover-glass is then inverted over a glass slide having a round excavation in its centre (Fig. 32), a concave slide. In

FIG. 32.

Hanging drop.

order to increase the size of this little chamber, and also to exclude the air from the drop, the edge of the depression is rimmed with vaseline, the cover-glass being dropped on the vaseline. The drop may be examined with a powerful hand lens or with a high-power lens ($\frac{1}{6}$ or $\frac{1}{8}$) of the microscope. An oil-immersion lens cannot be used unless it is focussed simply on the cover-glass without changing the field. Any attempt at changing the field is apt to spoil the drop. The lens must be brought down very slowly, so as not to break the cover-glass. The iris-diaphragm is closed almost completely, as the bacteria are very refractile.

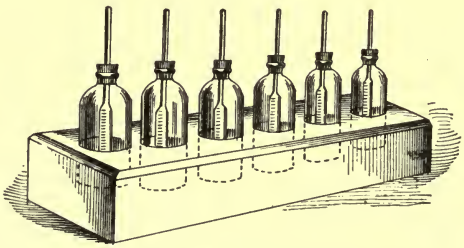
The bacteria are seen as minute shining bodies that either remain stationary or scamper across the field with either a

sinuous, undulating, or rotary movement. It is impossible to see the flagella.

When the examination has been completed, the slide and cover-glass are dropped into a 5 per cent. carbolic acid solution and left there for one hour. Disinfection by heat is dangerous, because the drop is very apt to sputter and thus be the means of carrying infection.

PREPARATION OF STAINS: The **anilin dyes** are used almost exclusively for the staining of bacteria. Those manufactured by Gruebler are the most reliable, and should always be specified when dry stains are ordered. The most widely used stains are gentian-violet, methylene-blue, fuchsin, vesuvin, and malachite-green. So-called *stock solutions* of these stains should always be on hand. From these, small quantities of stain are prepared for immediate use, as they deteriorate very rapidly. They are kept in small bottles supplied with stoppers and pipettes (Fig. 33). Vesuvin and malachite-green are

FIG. 33.



Rack of bottles for staining solutions.

kept in 1 per cent. aqueous solution. Of all the other stains alcoholic stock solutions are made. Aqueous stock solutions can be prepared when needed. The stock solutions are made by adding 1 part of the powdered dye to 4 parts of absolute alcohol. A glass-stoppered bottle is filled to one-quarter of its capacity with the dye, and sufficient absolute alcohol (or water for aqueous solutions) added to fill the bottle. An excess of the dye may be used to insure saturation; the excess remains undissolved in the bottom of the

bottle. The stains are prepared from these stock solutions by adding 5 c.c. of the solution to 95 c.c. of distilled water; or the stock solution is added to the water drop by drop until a good color is produced. The first method is preferable, as it is more exact.

Loeffler's **alkaline methylene-blue** is made as follows:

Saturated alcoholic solution of methylene-blue,	30 c.c.;
Caustic potash (1 : 10,000),	100 "

This stain is always used for staining the diphtheria bacillus.

The **anilin-water stains** are prepared as follows: 5 c.c. of anilin oil are shaken thoroughly with 100 c.c. of distilled water. This solution is filtered through filter-paper until it is perfectly clear. To 100 c.c. of the filtrate add 10 c.c. of absolute alcohol and 11 c.c. of the concentrated alcoholic solution of the stain. The anilin-water stains are used where a very strong stain is required. They are decomposed easily, however, and should be prepared in small quantities only. The anilin water may be kept on hand, so that the stain can be prepared rapidly when needed.

STAINING SLIDES AND COVER-GLASSES: Bacteria may be examined on a slide or a cover-glass. Cover-glasses, because of their size, can be placed in the stain, but slides are handled more easily. They should be absolutely clean and free from fatty matter. First immerse them in a mineral acid or a mixture of sulphuric acid and potassium bichromate; then in successive washings of water, alcohol, and ether. They are kept in ether until ready to be used. If desired, they may be kept in alcohol, when the ether washing is omitted. For use, they are wiped dry with a piece of cheese-cloth and passed a few times through the flame of an alcohol lamp or Bunsen burner.

For convenience we will consider the preparation of a **slide** (the cover-glass is prepared in the same way):

Spread the material on the slide as thinly as possible. Students usually make the mistake of spreading it too thick for fear that there is not sufficient on the slide. The thinner

the preparation, the better the result. If the material is too thick or viscid, a drop of distilled water is placed on the slide and the material gently spread in this. The film must be *dried in the air*, and not in the flame. Drying in the flame is more rapid, but it always spoils the specimen. If the film is thin, it will dry rapidly. After it is thoroughly dry, it is fixed in the flame. It should not be held in the flame, but passed through it three or four times. This fixes the bacteria on the slide by coagulating the albumin in them. The next step is to *stain the specimen.*

Several different kinds of *forceps* have been devised to hold the slide or cover-glass, but the wire forceps (Stewart) are the best. They hold the glass securely and prevent soiling of the fingers or clothing by the stain. With the ordinary bladed forceps the stain is drawn up between the blades by capillary attraction, and then it is impossible to keep it from staining the fingers.

The film is covered well with the stain, which is allowed to act for a few minutes. To facilitate staining, the stain may be warmed slightly. Decant the stain and wash the slide in water. Stain again if necessary. Dry in the air, first removing the excess of water with a blotter, and mount in Canada balsam. The specimen is ready to be examined. A $\frac{1}{12}$ inch oil-immersion lens should always be used if possible.

TUBERCLE BACILLUS: Special stains and staining-methods are necessary for the tubercle bacillus. Many different methods have been devised. The *principle* of them all is to use a stain which stains so intensely that it cannot be removed from the bacillus with either mineral acids or alcohol. The tubercle bacillus is extremely resistant to decolorizing agents. The film is prepared in the same manner as for the ordinary bacteria. If the slide is made from sputum, the little white or yellowish nodules should be selected, as they are most apt to contain the tubercle bacillus. By shaking the sputum with a 5 per cent. solution of carbolic acid little albuminous masses are formed. One of these may be selected and spread on the slide as thinly as possible; or the mass is opened and only its centre placed on the slide. The utmost care is necessary in the handling of tubercular matter or any matter

which is suspected of being tubercular. Nothing but the needle should come in contact with it, and as soon as there is no further use for it it should be covered with a 5 per cent. carbolic acid solution or milk of lime. The inoculating needle should be sterilized immediately after use, and likewise anything else that may have come in contact with the suspected material. The film is dried in the air and fixed in the flame before staining:

Ehrlich-Weigert method: Float the cover-glass, film side down, in a watch-crystal containing anilin methylene-violet; or immerse the cover-glass film side up. Heat until the stain begins to steam; then set it aside for two or three minutes. Remove the specimen from the stain and decolorize in 1 part of nitric acid and 3 of distilled water. Hold the cover-glass with the forceps and move it gently to and fro for a few seconds. Wash in 60 per cent. alcohol and then in water. If a contrast-stain is wanted, cover the film for a few minutes with a saturated solution of vesuvin. Wash, dry, and mount in Canada balsam. The tubercle bacillus is stained violet or purple, and all the other organisms, as well as the mucus, are stained a light brown.

Friedlaender method: The stain used in this method is known as Ziehl's solution. It consists of:

Fuchsin,	1 gram;
Alcohol,	10 c.c.

Dissolve, and add 100 c.c. of a 5 per cent. solution of carbolic acid.

Cover the smear with this stain and heat gently until steam is given off. Decolorize with:

Nitric acid,	5 c.c.;
Alcohol (80 per cent.),	95 "

and counter-stain with methylene-blue. Wash in distilled water, dry, and mount in Canada balsam. The tubercle bacillus is stained a bright red, everything else blue.

Gabbett method: This is an exceedingly simple method,

the contrast-stain being added to the decolorizing agent. The smear is stained with Ziehl's solution as before, after which it is immersed for a few minutes in Gabbett's solution:

Methylene-blue,	2 grams;
Sulphuric acid (25 per cent. aqueous solution),	100 c.c.

Wash in water, dry, and mount in balsam. The tubercle bacillus is stained a bright red, everything else is stained blue. The objection to this method is that it is impossible to determine, without removing the specimen from the stain and washing, just when decolorization is complete. The acid and the methylene-blue may be used separately.

Ziehl-Neelson method: The cover-glass is floated on the carbol-fuchsin solution, film side down, for from three to five minutes, or until it commences to steam; or the stain is heated first and then poured over the film and allowed to act for a few minutes. Decolorize in 25 per cent. nitric acid or sulphuric acid until the film is a very light brown or yellow. Wash in 60 per cent. alcohol for a few minutes, then water; mount in balsam after drying. A contrast-stain may be used if desired.

Ehrlich method: Float or immerse the cover-glass in anilin-water gentian-violet and place it in the incubator for twenty-four hours. Wash in water, and alternately in 33 per cent. nitric acid and 60 per cent. alcohol until the color has almost entirely disappeared. After a final washing in the alcohol, wash in water and counter-stain with vesuvin or Bismarck-brown. Methylene-blue may also be used. Wash in water, dry, and mount in balsam. The tubercle bacilli are stained a dark blue, and the other bacteria and the tissue-cells are colored brown.

Dorset method: Dorset found that Soudan III. has a selective action on the tubercle bacillus because of the large proportion of fat (40 per cent.) contained in the bacillus. Immerse the film for ten minutes in a cold saturated 80 per cent. alcoholic solution of Soudan III. Remove the excess of stain with successive washings of 70 per cent. alcohol.

Wash in water, dry, and mount. The tubercle bacilli are stained a very brilliant red.

Some experience is necessary before the tubercle bacillus can be stained satisfactorily. Care must be taken that the stain has acted sufficiently, and that the decolorizing agent is not such in fact. It is impossible to overstain. Decolorization with the dilute mineral acids is achieved in thirty seconds. The film should always contain a sufficiency of stain. The stain must be replaced if evaporation occurs. A 3 per cent. alcoholic solution of hydrochloric acid is also a good decolorizer. Spirit of nitrous ether may be used. It is of agreeable odor, and does not stain the hands, nor is it irritating to the mucous membranes. When staining for tubercle bacilli in sputum or other tubercular material, it should be borne in mind that it is not always possible to find the bacillus in the first specimen. Oftentimes as many as a dozen must be made before it can be found. A negative result should never be accepted as such until a large number of slides have been examined. Neither should the bacillus be confused with other organisms which have a strong resemblance to it.

Gram's method is used for the differentiation of various bacteria, especially the GONOCOCCUS. It is, therefore, very frequently referred to as the gonococcus stain. A thin film is prepared, dried in the air, and fixed in the flame. It is stained for a few minutes with anilin-water gentian-violet. The result will be better if the stain is warmed slightly. Pour off the stain and immerse the specimen in Gram's solution. It has the following formula:

Iodine crystals,	1 gram;
Potassium iodide,	2 grams;
Distilled water,	300 "

Stain until the specimen turns a dark brown. Wash in 95 per cent. alcohol until color ceases to be given off and the section is of a grayish color. Vesuvin or eosin may be used as a counter-stain. Wash in water, dry, and mount in Canada balsam. By the action of Gram's solution on the bacteria a compound is formed by the bacterial mycoprotein and the

iodine which is insoluble in alcohol. Some bacteria are stained by Gram's method and some are not. Those that are not stained are sometimes said to be decolorized by the stain. Those that stain have a purplish or blue-brown color.

The following organisms **do not stain** by Gram's method:

Micrococcus of gonorrhœa, or gonococcus.
Bacillus of typhoid fever, or Bacillus typhosus.
Bacillus coli communis.
Spirillum of Asiatic cholera, or Comma bacillus.
Bacillus of influenza.
Bacillus of bubonic plague.
Bacillus Mallei (glanders).
Bacillus of malignant œdema.
Bacillus of Friedlaender (pneumobacillus).
Spirillum of relapsing fever.
Diplococcus intracellularis meningitidis.
Bacillus proteus vulgaris.
Bacillus pyocyaneus.

Among the bacteria that **are stained** are the:

Pneumococcus (Diplococcus pneumoniæ).
Staphylococcus pyogenes.
Streptococcus pyogenes.
Bacillus of diphtheria.
Bacillus tuberculosis.
Bacillus of leprosy.
Bacillus anthracis.
Bacillus of tetanus.
Bacillus aërogenes capsulatus.
Streptothrix actinomyces.

SPORES: Mention has been made of the peculiar resistance exhibited by spores to extraneous influences. It is also very difficult to stain them. The methods generally employed are the same as those for staining the tubercle bacillus, but the film must be exposed to the action of the stain for a longer time.

McFarland recommends the following method: Place the preparation in a test-tube half filled with a carbol-fuchsin solution and boil it for at least fifteen minutes. Decolorize with 3 per cent. hydrochloric or a 2–5 per cent. acetic acid

solution. Wash, counter-stain, and mount. Another method is first to immerse the film for two minutes in chloroform and then in 5 per cent. chromic acid solution for 1 to 2 minutes. When stained after the method of staining the tubercle bacillus the result is not very satisfactory. The decolorizing agent should not be so strong; a 3 per cent. solution of the acid is sufficient.

Ajezky first places the film, before fixing, in a hot 0.5 per cent. hydrochloric acid solution for three to four minutes, heating it gently. Decolorize with 4–5 per cent. sulphuric acid. Counter-stain with methylene-blue or malachite-green and mount.

Neisser's method: Float the preparation on an anilin-water fuchsin solution for one hour, heating it constantly to near the boiling-point. Wash in water, decolorize with a 25 per cent. solution of hydrochloric acid, counter-stain, and mount.

Fiocca's method: To 20 c.c. of a 10 per cent. ammonia solution add from 10 to 20 drops of an alkaline solution of any of the anilin dyes. Heat until steam is given off, then place the film in the hot staining solution for from 5 to 15 minutes. Decolorize in a 25 per cent. solution of nitric or sulphuric acid; wash in water, counter-stain with malachite-green, vesuvin, or safranin, and mount.

All these methods stain the spore red, and the parent germ takes on the color of the contrast-stain. None of these methods is entirely satisfactory, and spore-staining still remains a discouraging procedure. Many attempts must be made before even a measure of success is attained.

CAPSULES: When bacteria are grown artificially on culture-media, capsules usually do not appear. For the demonstration of the capsules the fresh, germ-containing material must be used. The sputum from a case of lobar pneumonia is most suitable.

Johne's method: The film is stained in a warm 2 per cent. solution of gentian-violet for a few minutes and decolorized in a 2 per cent. solution of acetic acid. After washing in water the specimen is mounted in water. Canada balsam shrinks the capsule. For permanent specimens the cover-

glass is rimmed with Tarrant's cement or any other good cover-glass cement.[1]

Welch's method: From a pipette, drop glacial acetic acid on the film, allowing it to remain for a few seconds. Pour off the acid (do not wash or wipe it off) and stain with anilin-water gentian-violet. Wash and restain until all the glacial acetic acid has been removed. Then wash in water containing 1 or 2 per cent. of sodium chloride. Examine in salt solution.

FLAGELLA: It is more difficult to stain flagella than either the tubercle bacillus or spores; but it is possible to stain and demonstrate them.

The method of **Loeffler** is the best. He uses three solutions, Nos. 1, 2, and 3:

Solution No. 1.

20 per cent. solution of tannic acid, 10 grams;
Cold saturated aqueous solution of ferrous sulphate, 5 "
Aqueous or alcoholic solution of fuchsin or methyl-violet, 1 gram.

Solution No. 2.

A 1 per cent. solution of caustic soda.

Solution No. 3.

An aqueous solution of sulphuric acid of such strength that 1 c.c. will exactly neutralize an equal quantity of caustic soda solution.

In order to have the flagella show well, the film should be spread as thinly as possible. Mix a small quantity of the culture with a drop of sterile water, and from this mixture take a small portion and spread it on a clean slide or cover-glass. Dry in the air and fix in the flame. Cover the film with solution No. 1 (the mordant) and heat until it begins to steam. Then wash in distilled water followed by absolute

[1] A *modification* of Johne's method is to wash the film for one minute in a 1 per cent. solution of acetic acid before the stain is applied.

alcohol until the glass is clean. Dry and stain with anilin-water fuchsin having a neutral reaction. Wash in water, dry, and mount in Canada balsam. The stain is neutralized with solution No. 2. If the organism produces alkalies, solution No. 3 is added in the proportion of 1 drop to 1 c.c. in 16 c.c. of the mordant. If the organism, on the other hand, produces acids, solution No. 2 is added in the same way.

Loeffler has determined the exact quantity to be added to each 16 c.c. of mordant solution for staining the flagella of the following:

Cholera spirillum, ½–1 drop of solution No. 3.
Bacillus typhosus, 1 c.c. of solution No. 2.
Bacillus subtilis, 20–30 drops of solution No. 2.
Bacillus of malignant œdema, 36–37 drops of solution No. 2.

Van Ermengen's method, though complicated, yields very satisfactory results. The film is placed in a fixing solution consisting of 1 part of a 2 per cent. solution of osmic acid and 2 parts of a 10 to 25 per cent. solution of tannin, for one hour at the room temperature. It is then thoroughly washed in distilled water, and the film transferred for a few seconds to a 5 per cent. solution of nitrate of silver; then into the following for a few seconds:

Gallic acid,	5 grams;
Tannin,	3 "
Fused potassium acetate,	10 "
Distilled water,	350 c.c.

Return the film to the silver solution, allowing it to remain there until it has turned black; wash well in water, dry, and mount.

Pittfield uses a method which is both a mordant and a stain. The solution is prepared as follows:

Saturated aqueous solution of alum,	10 c.c.;
Saturated alcoholic solution of gentian-violet,	1 "

Mix well and filter. Add:

Tannic acid,	1 gram ;
Distilled water,	10 c.c.

The cover-glass is spread with a very thin film, dried, and fixed. Cover with the mixed solution and heat almost to boiling. Wash in water until the glass is clean, dry, and mount in Canada balsam.

Muir has modified this method by using two solutions, a mordant and a stain :

Mordant.

Tannic acid (10 per cent. aqueous solution, filtered),	10 c.c. ;
Corrosive sublimate (saturated aqueous solution),	5 "
Alum (saturated aqueous solution),	5 "
Carbol-fuchsin,	5 "

Mix well, and after the sediment has settled the supernatant clear fluid is pipetted into a clean glass-stoppered bottle.

Stain.

Alum (saturated aqueous solution),	10 c.c. ;
Gentian-violet (saturated aqueous solution),	2 "

This stain may be substituted for the carbol-fuchsin in the mordant. Cover the film with the mordant solution and heat until steam arises. Wash in running water for two minutes. Dry carefully over a flame and stain, heating as before. Wash in water, dry, and mount in a drop of xylol balsam.

L. Smith stains the flagella with night-blue. The method is simple. He advises, however, that only young cultures be used. The cover-glass must be clean and free from fatty matter. Mix 1 gram of potassium alum with 40 c.c. of distilled water and place in the incubator over night. Dissolve 5 grams of night-blue in 20 c.c. of absolute alcohol and mix with the first solution. Filter until the filtrate comes through

clear. Stain for five or ten minutes; wash in water, dry in the air, and mount in Canada balsam.

STAINING BACTERIA IN TISSUE: The tissue is removed, fixed, and hardened according to the rules laid down in text-books on histology, and embedded in celloidin or paraffin. The cut sections are stained with Loeffler's alkaline methylene-blue, and then differentiated with a 1 per cent. solution of hydrochloric acid for a few seconds, dehydrated in absolute alcohol, cleared in xylol, and mounted in balsam.

Pfeiffer stains the sections for one-half hour in dilute carbol-fuchsin, and then transfers them to absolute alcohol slightly acidulated with acetic acid. As soon as the section takes on a reddish-violet color it is transferred to xylol, cleared, and mounted in balsam.

A very simple method is to stain with the ordinary aqueous staining solutions for from five to eight minutes, and then decolorize in a 1 per cent. acetic acid solution for a few seconds. This removes the stain from the tissues, but leaves the color in the bacteria. Any contrast-stain may be used, after which the specimen is dehydrated in absolute alcohol, cleared in xylol, and mounted. Gram's stain is used also for staining bacteria in tissue. The specimen is stained first in Ehrlich's anilin-water gentian-violet for from five to thirty minutes, washed in water, immersed in Gram's solution for a few minutes, washed in 95 per cent. alcohol until thoroughly decolorized, dehydrated in absolute alcohol, cleared in xylol, and mounted.

CHAPTER X.

EXPERIMENTS UPON ANIMALS.

THE **inoculation** of animals with the germs of various infectious diseases has been the means of enabling us to observe accurately and to record the progressive growth and development of pathogenic bacteria in the living body and the conditions resulting therefrom. Animal experimentation has been of inestimable value in medicine, especially in physiology, materia medica, therapeutics, and bacteriology. In bacteriology it is an aid in the differentiation and detection of bacteria as well as in the determination of their pathogenicity.

White mice, guinea-pigs, rabbits, and monkeys are the **animals** usually used for this purpose. They are of a size that will not interfere with the work; they can be kept in the laboratory; and they are also very susceptible to the various infectious diseases.

We must, however, remember that some animals possess a natural immunity or resistance to certain diseases, and cannot be used for work connected with such diseases.

The animals should be kept in a clean wire cage in a light, well-ventilated room. They should have plenty of good food and fresh water, and every care taken of them. The experiments should be performed with the same care as an operation—for such the experiments really are—on the human being. Careless and negligent handling of these animals is not only unpardonable, but also unnecessary.

The inoculations may be **made** directly into a *vein* (intravenous injection); into the *subcutaneous tissues;* or into the *peritoneal cavity.*

It is preferable to use a **syringe** that can be sterilized easily. Koch's syringe is used more than any other. It contains no piston, the contents being forced out with a rubber bulb; and when this bulb is removed, it can be sterilized by

dry heat. The objection to most syringes used for this purpose is that the packing is made of rubber or leather, and cannot be sterilized by heat of any kind. The syringes and all other apparatus used in the performance of these animal experiments should be sterilized thoroughly both before and after using.

Inoculations are made also with the **platinum needle,** which is passed in through a small opening made in the skin.

No matter where nor how the inoculation is to be made, the **first steps** are to remove all hair from the site of inoculation with scissors and a razor; and then to disinfect the skin in order to avoid any possible contamination or infection with pus cocci. Some bacteriologists advise snipping off a small piece of skin with curved scissors, of a size sufficient not to draw blood, but to remove the surface epithelium, thus leaving a denuded spot through which the needle can be introduced. This absolutely precludes the possibility of contaminating the injection.

Fluids in large quantities are injected into the circulation or into the peritoneal cavity by forcing them through a slender canula with a syringe or rubber bulb.

When injections are made into the **peritoneal cavity,** care must be taken not to injure any of the abdominal organs. The intestines usually slip out of the way of the advancing needle or canula, and are not so apt to be injured as the liver or stomach. The injection is made in the same manner as is any hypodermic injection and under the same antiseptic precautions.

Intravenous inoculation is done usually on rabbits because the ear vein of the rabbit is conspicuous and within easy reach. Small animals are unsuited for this form of inoculation. After thoroughly cleansing the ear the veins are distended momentarily by compressing them at the base of the ear. The needle is introduced at the root of the ear, pointing in the direction of the current of blood, and the injection made slowly. The puncture is sealed with collodion.

For **subcutaneous injections,** mice are used. The inoculation is made at the root of the tail, where a little pocket is made for the reception of the material.

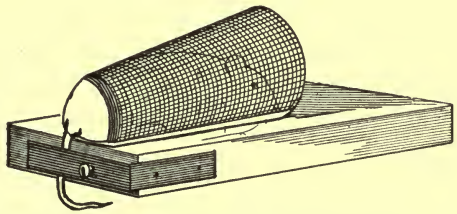
If it is desired to make an inoculation directly into the *lymphatics*, some organ with a very poor blood-supply should be chosen. The choice is the testicle, the injection being made deeply into the organ.

Pasteur inoculated rabbits with hydrophobia virus by injecting an emulsion of the spinal cord of an animal sick with hydrophobia *beneath the dura mater* through a trephine-opening made a short distance back of the eyes.

For the purpose of studying the local effects of various bacteria, inoculations are made into the *anterior chamber* of the eye. An incision is made through the cornea with a cataract knife and a liquid culture injected, or solid material is introduced with the platinum needle or forceps.

Special considerations: Injections may be made without the use of an *anæsthetic*. Painful operations are made under

FIG. 34.

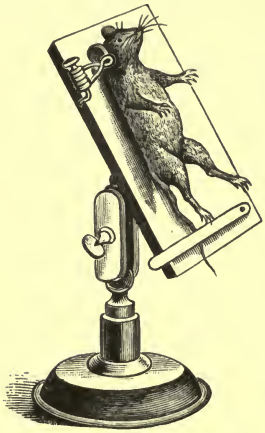


Mouse-holder, with mouse in proper position.

ether anæsthesia. Chloroform is apparently not well borne by animals. When the inoculation is to be made, the animal is wrapped securely in towels, leaving the site of inoculation exposed. An assistant holds the animal and keeps it from struggling. Special holders (Figs. 34, 35, 36) have been devised for the smaller animals. They are simple in construction, convenient, and inexpensive.

Young animals are preferable to old ones. Intravenous injections are the most fatal, and only a small dose of the culture or suspected material should be injected. Peritoneal injection produces results more rapidly than subcutaneous injection. Virulent cultures should always be used. They

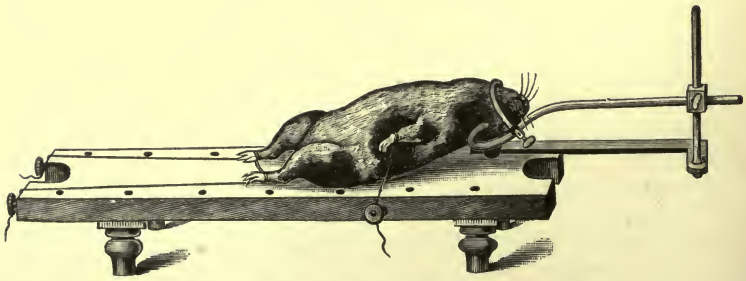
FIG. 35.



Kitasato's mouse-holder.

are grown on such media as have a tendency to increase their virulence. The amount of material introduced should be in proportion to the size of the animal.

FIG. 36.



Holder for larger animals.

After inoculation the animal should be watched constantly and a note made of any change in its condition. The temperature is taken at regular intervals per rectum, with a spe-

cial rectal thermometer kept for that purpose. The normal temperature-standard of the animal should be ascertained before the experiment is begun. The animal should receive the same care as is given to a patient after operation. Blood for experimental purposes is taken from any vein; or from the carotid artery of the smaller animals.

The animal itself may be used as **culture-medium and incubator** combined. A collodion capsule containing the inoculated bouillon is suspended in the peritoneal cavity of the animal. The body-juices pass through the walls of this capsule, supplying the culture with ideal nourishment, and the germs are not subject to destruction by phagocytosis. The animal remains free from infection. Such cultures grow rapidly and luxuriantly.

Autopsy: Many important data can be collected from autopsies held on animals that succumb to inoculation. The animal is washed thoroughly with a 1 : 1000 bichloride solution and laid on its back on a small autopsy board. In the sides of this board are a number of small nails, to which the legs of the animal can be tied. The scalpel is held in the flame for a few minutes, and the thorax and abdomen are opened after the surface of the skin has been seared with the heated knife. Every precaution should be taken to avoid infection and contamination. Bouillon cultures are made from the body-juices and from the internal organs. The surface is seared, incised with a sterile knife, and a particle of the organ removed with the platinum loop.

When the animal is killed for the purpose of studying the results of the infection, smear-preparations are made of the heart's blood or blood obtained from any vessel. Pieces of tissue of suitable size are fixed and hardened in 95 per cent. alcohol.

At the completion of the autopsy both the animal and the board are placed in a 1 : 1000 bichloride solution for one hour and then destroyed by burning. All the instruments, etc., used in the conduction of the autopsy are sterilized by live steam. The bacteriologist should take every precaution to protect himself from infection. All wounds of any kind on the hands should be protected with collodion; or rubber gloves worn.

CHAPTER XI.

POISONOUS PRODUCTS OF BACTERIA.

All the symptoms manifested as a result of the presence of bacteria in the body are due to the elaboration by these bacteria of substances, the chemical composition of which resembles that of the vegetable alkaloids. Some of these substances are formed by bacteria outside of the body and are ingested with food, as poisoning by decomposed meat, fish, cheese, or ice cream. In most cases, however, the poison is elaborated by the bacteria after they have gained entrance into the body, as in the infectious fevers. The severity of the reaction depends upon the kind and the quantity of poison elaborated.

Ptomaines: A ptomaine is an organic chemical compound, basic in character, formed by the action of bacteria on nitrogenous matter (Vaughan and Novy). A ptomaine is crystallizable. It may be either inert or very poisonous. Brieger calls the non-poisonous substances ptomaines, and the poisonous ones, toxins. When a ptomaine is injected into an animal in large quantity or for a long period of time, symptoms of intoxication are produced. Some ptomaines contain oxygen and some do not. They all contain nitrogen. Ptomaines are typical vegetable alkaloids.

Ptomaines have also been called *cadaveric alkaloids*, because they are the result of putrefaction. The kind of ptomaine produced depends largely on the individual germ, the temperature, amount of oxygen present, virulence or activity of the germ, and the quality of the nutritive medium. Ptomaines are regarded as cleavage-products, temporary forms of matter, as they are changed from an organic to an inorganic state by the action of the bacteria. Although ptomaines may be formed by pathogenic bacteria, they are usually the result of the activity of the non-pathogenic saprophytes.

Leucomaines: A leucomaine is an animal alkaloid, a basic substance, resulting from the tissue metabolism of the normal body. They are derived principally from the nucleins of the nuclei and the proteids of the cell-protoplasm. According to some, they play a very important rôle in the antitoxin theories. All the various poisonous substances, elaborated and excreted in the urine and saliva, etc., as well as the venoms of certain snakes, etc., belong to this group. At one time they were regarded as being due to bacterial activity.

Toxalbumin: A toxalbumin is a non-basic bacterial substance partaking of the chemical composition of an albumin. Toxalbumins are not volatile and are *extremely* poisonous. The poisons produced by the typhoid bacillus, spirillum of cholera, Staphylococcus pyogenes, bacillus of diphtheria, and the tetanus bacillus, belong to this group. Many bacteriologists have abandoned this term entirely because of the as yet undetermined nature of these substances. They usually are considered together with the toxins. Ricin and abrin, which are obtained from the castor oil bean and the jequirity bean, respectively, are toxalbumins of vegetable origin. They are extremely sensitive to the action of light, heat, and chemicals.

Toxins: A toxin is a synthetic product elaborated by bacteria when growing in nutrient media or in the body. It is not crystallizable. It is extremely poisonous, even in minute doses. The chemistry of the toxins is not definitely known, as it has so far been impossible to isolate a toxin chemically because of its instability. It has been suggested that the toxins are closely allied to the ferments, because when injected into an animal considerable time elapses before death occurs. Further, because only a very small quantity is sufficient to cause death. Snake-venom, abrin, and ricin resemble the bacterial toxins in their action.

Toxins are of two kinds: First, those which are probably *bacterial excretions*. Second, those which are present only in the *body* of the germ. The first named are obtained by filtration from an actively growing bouillon culture. The toxins of different germs differ very greatly in their poisonous qualities. For instance, the poison of the tetanus bacillus is extremely virile. A mouse weighing 15 grams can be

killed by the almost inconceivable amount of 0.00000005 of a gram of the toxin. The toxins of other organisms are not nearly so poisonous. The severe constitutional symptoms accompanying typhoid, diphtheria, tetanus, pneumonia, etc., are sufficient evidence to prove that the germs causing these diseases have not merely a local action, but that they also elaborate some substance which is absorbed and disseminated throughout the body, and to which the general reaction is due. These substances are certainly poisonous, or toxic, and therefore are referred to as toxins. The intensity of the general reaction would depend naturally on the amount of toxin absorbed and its intensity.

That the *body* of the germ also contains a toxin can be demonstrated by the injection of dead cultures, which is followed by a typical reaction.

Buchner calls all these toxins *bacterial proteins*. Those which cause fever he terms pyogenic; those which cause inflammation, phlogogenic. Koch's old tuberculin and mallein are proteins.

CHAPTER XII.

INFECTION.

By **infection** we understand the entrance of bacteria into the body and their multiplication there. Theoretically that definition is correct; but something more than the entrance and multiplication of bacteria is necessary to constitute an infection. Their presence and multiplication must be manifested by *symptoms*. A person may swallow the typhoid bacillus without contracting the disease. Theoretically that constitutes an infection, but that is not the case practically, because the germ does not manifest its presence by any disturbance of the health of the individual.

Some *injury* must be done the body by the germ before there can be said to be an infection. The surface of the skin is known to harbor bacteria at all times, but unless searched for they remain unrecognized. They multiply there, and yet there is no evidence of infection. When these same germs enter a wound and produce substances which react on the body, an infection is said to have occurred. It is impossible to give a definition of infection that can be embraced in one sentence.

Infection may *occur* not only with the vegetable organisms (*i. e.*, bacteria, moulds, and yeasts), but also with the animal parasites, such as the *Amœba coli* and the malarial hematozoön.

An **infectious disease** is one caused by micro-organisms, and which is liable to be communicated to others. The *non-pathogenic bacteria* are incapable of producing an infectious disease. Some organisms are infectious for animals but not for man, and *vice versa*. In the condition known as *saprœmia* the infection is due to *saprophytes* which, while not entering the blood themselves (*i. e.*, remaining in the focus of infection), yet produce substances that *are* absorbed by the body and enter into the blood and lymph-channels.

By *mixed infection* is meant the presence of more than one

organism at the same time. This is seen frequently in tuberculosis, pneumonia, wound infections, etc. A *secondary infection* is one occurring in the course of another infection, such as the streptococcus infections which occur in the course of pulmonary tuberculosis. A *terminal infection* is one occurring in an individual suffering from some chronic organic disease and which ends fatally.

When the poison is generated within the body itself as the result of faulty metabolism or inadequate elimination of waste products and their subsequent decomposition, a form of poisoning occurs known as *autointoxication*, or *autoinfection*. This form of poisoning should not be confused with poisoning by alcohol, which also is defined as an intoxication.

An infectious disease is said to be *contagious* when the infection is conveyed by fomites, as, for instance, the scales in scarlet fever. All contagious diseases are infectious diseases as well; but all infectious diseases are not contagious. Typhoid fever is a typical infectious disease. The two terms are really synonymous, and yet there is a difference in the method of conveyance of the disease.

In an infectious disease the germ is the infecting medium, and is carried from the sick to the well; whereas in a contagious disease some medium, such as the scales in the exanthematous diseases, apparently carries the infection. A more thorough study of the contagious diseases and the finding of the exciting germ-cause may obviate the necessity of a division into infectious and contagious diseases.

The term *miasmatic* had reference to a disease due to a "miasm,"—that is, noxious matter carried in the air. Malaria was looked upon as the type of this class. The term is no longer used since the method of infection has been determined.

Although certain germs bear a causal relationship to disease, it is evident that the development of the disease, or the manifestation of an infection, is dependent on many conditions which influence the infection either one way or the other. All the conditions favorable to the development and growth of an organism must exist before disease will result. A whole community may be exposed to the infection in its most

virulent form and yet escape the disease. On the other hand, certain individuals will succumb rapidly even when the infective agent is attenuated. Again, the severity of the disease depends not only on the individual susceptibility, but also upon the vitality of the germ, the source of the infection, and the time and point of entrance of the infective agent.

The accidental presence of a germ in the body of an individual during disease cannot be accepted as evidence of its etiologic relationship with that particular disease. Koch has laid down some requirements, known as *Koch's law*, with which the organism must comply before it can be considered as the specific cause of a disease:

1. That the organism should always be present in the body of the animal having the disease, and that its presence must explain the changes met with in that disease.
2. That it must be possible to isolate and make a pure culture of the organism outside of the body.
3. That the inoculation of an animal with a pure culture of this organism will result in the production of the typical disease from which the germ was obtained.
4. That the typical organism be found in the tissues of the animal thus inoculated.

A number of organisms have been accepted as *specific* that do not meet all of these requirements, but the evidence in their favor is so overwhelming that the specificity cannot be doubted. The tubercle bacillus is an example. It has not, according to many observers, as yet met the last two requirements in man.

Bacteria are *phlogistic*, producing simply a local inflammatory reaction (staphylococcus); *toxic*, a local growth (*i. e.*, without invading the body themselves), with rapid and extensive dissemination of the toxin (tetanus and diphtheria); *septic*, invading (themselves) the body-fluids (anthrax).

A disease is said to be *sporadic* when only isolated cases appear; *endemic*, when the disease is always present; *epidemic*, when it is unusually prevalent and exhibiting a marked tendency to spread beyond its usual limitations; *pandemic*, when the disease is widely spread, as in several states or countries.

Conditions Modifying Infection.

THE GERM: **Virulence**: Much depends upon the activity of the germ at the time of infection. Its activity is known as its virulence. This virulence is extremely variable and subject to many influences. Not only is there a difference in the virulence of various pathogenic organisms, but the virulence of any one germ is also subject to variations.

All pathogenic bacteria have a toxin-forming and a vegetative function. When the vegetative function especially is marked, the toxin-forming function is diminished, and *vice versa*. It is apparently impossible for both functions to be present in the same proportions at the same time. This variation is dependent on the biologic characteristics. In one instance the organism will grow very luxuriantly with the production of little toxin. All its energy is spent in the direction of growth and propagation. At other times it grows very poorly, but produces enormous quantities of toxin or small quantities of an extremely powerful toxin. The toxin-forming function is carried on at the expense of the reproductive function.

In the laboratory the virulence is *attenuated* by repeated transplantation on artificial media. Those conditions which are conducive to growth and multiplication are supplied, and by artificial selection the vegetative function becomes the predominating one. If this vegetative organism is then rapidly passed from one animal to another, conditions are created which favor the development of the toxin-forming function, with the result that the organism multiplies more slowly, but elaborates enormous quantities of toxin or small quantities of a very powerful toxin.

The virulence may be *increased* by growing the bacterium in a collodion sac placed in the peritoneal cavity of an animal, so that it may become accustomed to the body-fluids.

By *associating* certain organisms the virulence may either be increased or diminished. The association of the *Streptococcus pyogenes* with the bacillus of diphtheria increases the virulence of the latter considerably. Frequently this is seen clinically in cases of diphtheria in which there is a mixed

infection with the streptococcus. The virulence of the bacillus of malignant œdema is increased when it is associated with the *Bacillus prodigiosus*, a non-pathogenic organism. Typhoid cultures are increased in virulence when associated with dead cultures of the *Bacillus coli communis*.

These mixed infections play a very important role clinically, as the course of the disease is influenced considerably thereby. A mixed infection which contains the streptococcus is a very intractable process, and much more serious than a simple infection. Occasionally one organism will inhibit the growth of another, and thus diminish its virulence. A mixed infection may convert a local into a general infection. Epidemics are in a measure due to increased virulence of the germ. The virulence is exalted by the rapid passage of the organism from one individual to another.

Number: A small number of virulent germs may produce disease as rapidly as a large number would; whereas a large number is necessary if the germs are attenuated. Infection with a small number of any organism possessed of little virulence usually does not result in disease. A number that would be pathogenic for a mouse would not be pathogenic for an elephant. With these modifications, the number of the invading bacteria is of importance, otherwise not.

AVENUE OF INFECTION: Bacteria, in order to produce their characteristic results after invasion of the body, must have entered the body through the proper channel. The avenue of entrance will modify the infection. The typhoid bacillus when injected into the subcutaneous tissues does not produce typhoid fever, but simply a local abscess. When the tubercle bacillus enters through the respiratory or intestinal tract, it produces typical tuberculosis; but when infection occurs through the skin, a local tuberculosis results (lupus), which runs an extremely slow course and does not tend to end fatally. The streptococcus injected subcutaneously produces erysipelas or extensive suppuration. When injected directly into the blood-current or lymph-channels, it produces septicæmia or "blood-poisoning". Inhalation of the pneumococcus is followed by lobar pneumonia; when it enters by any other route, it causes suppuration and

abscess-formation. The same is true of every other pathogenic organism.

THE INDIVIDUAL: The susceptibility of the individual, or his resistance to disease, materially modifies the occurrence of infection. These conditions are dependent upon:

a. **Immunity**: An individual may possess a natural or acquired resistance to a certain disease, thus making the occurrence of infection with the organism causing that disease impossible or, at least, difficult. The condition of immunity is not absolute, as extremely virulent organisms will produce disease even in an immune. Usually, however, the disease then occurs in a very modified form (see also Chapter XIII.).

b. **Vital condition**: A healthy and vigorous person will naturally resist invasion much better than one suffering from some disease, such as cancer, diabetes, or a heart-, kidney-, or liver-disease. Young individuals are more susceptible to some diseases than older persons, and old people are attacked by infectious disease which the younger individual escapes. Women are more predisposed to some infections than men, and some infections are seen much more frequently in men than in women. Environment is largely responsible for this. Women, being more confined to the house, naturally are exposed to diseases which are the result of such confinement. The lack of fresh air and exercise influences the vitality and resistance of the woman. Diseases incident to exposure, such as pneumonia, occur more often in men. Anything that has a tendency to diminish bodily resistance in any way predisposes to infection.

c. **Traumatism**: This usually predisposes to infection by creating conditions favorable to the development of bacteria. The natural resistance of the uninjured tissue to infection is diminished by the traumatism, and also the shock incident thereto. The unbroken skin never serves as an infection atrium, whereas infection is invited by an injury. These injuries include operations. Tuberculosis of the bones and joints often follows an injury of some kind even when the skin is not broken. Malignant, ulcerative endocarditis never results unless there has been some previous injury of the heart valves. This has been demonstrated both clinically

and experimentally. It is evident, then, that as soon as the natural resistance of the body is lessened infection is invited.

d. **Predisposition**: In some instances there exists a predisposition to certain diseases. Certain races and peoples have an inborn susceptibility or resistance to some of the infectious diseases. For instance, the colored races are immune to yellow fever but very susceptible to smallpox. The fair-skinned races, on the other hand, are extremely susceptible to yellow fever.

e. **Heredity**: It is believed by some that certain infectious diseases (tuberculosis, syphilis) may be inherited by the offspring, from the father or mother, or from both. Considerable evidence has been offered in support of this supposition, but when this evidence is weighed carefully it falls far short of being conclusive. For the present such a theory is untenable. The child born of weak or sickly parents cannot possess sufficient vitality to combat disease, and naturally will succumb to infection more readily than a child born of healthy parents. With regard to syphilis, there is considerable evidence at hand in support of the belief that it is possible to inherit the disease or to be born with active manifestations of the disease. Space forbids a more detailed consideration of this very interesting subject, and we must refer our readers to the numerous dissertations on the subject which can be found in the current literature.

SOURCES OF INFECTION: As has been pointed out, the surface of the body, the various mucous membranes, and all the tracts which open on the surface of the body, harbor an extensive and varied flora at all times. Nuttall has shown that the newly born animal is absolutely free from bacteria, but that before long it is the host of a large number of pathogenic and non-pathogenic bacteria. Different parts of the body harbor germs which produce diseases peculiar to that part, and thus the individual may be the source of his own infection.

Skin: On the skin we always find various species of staphylococci, especially the *Staphylococcus epidermidis albus*. The *Bacillus graveolens* is responsible for the odor of sweating feet; the *Bacillus prodigiosus*, of red sweat; but neither of these germs is pathogenic. It is very improbable, how-

ever, that infection can take place through the unbroken skin, but the most minute wound or abrasion may serve as an infection atrium. The bites of insects may furnish both the wound and the infective material. Sometimes the infection atrium is so insignificant as to be almost if not quite invisible.

Conjunctivæ: The conjunctival mucous membrane is always moist and offers a most convenient lodging-place for bacteria. McFarland found a very large variety of bacteria on this membrane, but no fixed species. Others claim that it is absolutely sterile, because it is bathed continually in a secretion which possesses natural germicidal powers. Congestion or hyperæmia increases the number of leucocytes, clogging the tissues and interfering with their nutrition. Death of tissue (necrosis) follows, and every barrier to infection is removed.

Respiratory passages: These also are lined by a moist mucous membrane, but do not, contrary to expectation, contain many germs. The mucous lining of the nose appears to be endowed with remarkable germicidal powers, so that under normal conditions few bacteria ever reach the respiratory tract proper. It has been estimated that 1500 bacteria are inhaled every hour. Infection in tuberculosis, pneumonia, diphtheria, influenza, and the exanthematous fevers occurs through the respiratory tract. It is a well-known fact that pneumonia usually follows a "cold," and that frequent attacks of bronchitis or an attack of pneumonia predispose to tuberculosis. Plague (bubonic) pneumonia is also acquired by inhalation. Bacteria may also be absorbed from the mucous membranes of the air-passages and enter the adjacent lymph-glands. This accounts for the finding of tubercular lymph-glands at the root of the lung, and in the anterior mediastinum, without the existence of pulmonary tuberculosis.

Digestive tract: In addition to many non-pathogenic organisms, the *mouth* may harbor also the germs of tuberculosis, diphtheria, pneumonia, and the staphylococcus and streptococcus. The saliva possesses slight germicidal power. The tonsil may act as the portal of infection for tuberculosis. Tuberculosis of the cervical lymph-glands probably always follows tonsillar infection. An attack of pharyngitis predisposes to diphtheria.

The *stomach*, because of its acid reaction, is not a favorable place for the development of bacteria. Sarcinæ are frequently found in the stomach, especially during attacks of gastritis; also the bacilli of lactic acid and butyric acid. The *Oppler-Boas* bacillus is found in carcinoma of the stomach. At one time it was believed to be diagnostic of this condition; but it is not constant, and is found also in all diseases of the stomach where the conditions are similar to those found in carcinoma.

The *intestinal canal* can never be said to be free from bacteria. Some varieties are constant inhabitants, especially the *Bacillus coli communis*, the *Bacillus lactis aërogenes* (in milk-drinkers), and the *Streptococcus coli gracilis*. These organisms are found more often in the large than in the small bowel. Although the colon bacillus is ordinarily a non-pathogenic organism, it may, under favorable conditions, give rise to very severe and even fatal diarrhœas. It has frequently been found in suppurative lesions of the intestinal tract and its accessory glands. It is believed to be the cause of ulceration and perforation of the intestine.

The occurrence of tuberculosis of the mesenteric and retro-peritoneal lymph-glands is evidence of the fact that bacteria can and do pass through the walls of the intestine during health without causing any lesion of the intestine. An enteritis always predisposes to infection with the *Bacillus typhosus* and the cholera spirillum. Infection of the gastro-intestinal tract follows the ingestion of infected food or drink, or the swallowing of tubercular sputum. Occasionally syphilis also is transmitted through the gastro-intestinal tract.

Genito-urinary tract: A few unimportant varieties are found in the acid secretions of the *vagina*, but this acidity is rather a protection against infection. The *uterus* is normally free from bacteria. On the *external genitals* of both man and woman we find pus cocci and the *Bacillus smegmatis*, which resembles the tubercle bacillus so closely that often it is mistaken for it. When the urine is examined for tubercle bacilli, this resemblance must not be lost sight of, as the one is significant of a serious lesion, and the other possesses no pathologic significance whatever.

Infection of the genito-urinary tract and the reproductive organs may occur through the *circulation* or by *direct contact* with either pus cocci, the gonococcus, the tubercle bacillus, or the virus of syphilis.

Infection with the syphilis virus may take place through the placenta. So too may that of typhoid, tuberculosis, anthrax, and relapsing fever. *Puerperal* sapræmia is an infection of retained portions of the placenta or fœtal membranes by the streptococcus or colon bacillus (see also page 101). Intra-uterine infection usually occurs through the placenta.

The *bladder* and accessory organs may be infected from the urethra, and the infection may spread from here to the ureters and kidneys. Infection of the *kidney* may occur also from the adjacent tissues and through the blood.

The canal of the **external ear** contains many non-pathogenic germs, especially the *Micrococcus cereus flavus*. They diminish in number near the tympanum.

All these germs **gain lodgement** in or on these **various sources of infection** from the air, food, drink, the soil, the bodies of dead animals, and the excreta of persons sick with an infectious disease; but before *infection* can occur it is necessary that the bacteria enter the tissues themselves in sufficient number, and that they multiply there, when the natural resistance of the body is diminished.

Blood-current: According to Kruse, bacteria gain entrance to the blood-current by:

1. Passive entrance through the stomata of the vessels where the pressure of the inflammatory exudate is greater than the intravascular pressure.
2. Entrance into a vessel in the bodies of leucocytes.
3. Penetration of the vessel-wall by the growth of the organism.
4. Entrance through the lymphatics either passively or within a leucocyte.

Elimination of bacteria from the blood: It has been shown that bacteria eventually accumulate in the finer capillaries, especially those of the liver, spleen, lungs, and bone-marrow. From the capillaries they are carried to the surrounding tissues, and finally to the lymph-nodes; or they are excreted

by the bile or succus entericus; or discharged by the mucous membrane of the alveoli of the lungs, the tonsils, etc. Not infrequently they are discharged through suppurating wounds, to which they are conveyed by the leucocytes. Many bacteria are broken up in the body and are never excreted. The liver, through the bile, always excretes bacteria. The *Bacillus pyocyaneus*, when injected into the blood-current, is excreted by the functionating mammary gland. Bacteria are excreted also by the sweat, and by the kidneys (tubercle and typhoid), when there is a diseased condition of the renal epithelium. In pulmonary diseases the bacteria are excreted largely by the sputum; in gastro-intestinal diseases, by the feces.

Special Phenomena of Infection.

Agglutination: Gruber found that the serum of animals immune to typhoid, cholera, etc., or that of human beings who had recovered from typhoid, when added to a small amount of a culture of the specific germ, caused the organism to lose its motility and finally sink to the bottom of the test-tube as a flocculent precipitate. Gruber called this phenomenon agglutination, and considered it a reaction of immunity. Widal showed later that it really represented a reaction of the period of infection, and that the serum of typhoids, at the end of the first or beginning of the second week of the disease, gave the serum reaction. He concluded that it represented a possible reaction of the protoplasmic substance.

The typical *Widal reaction* is the agglutination of the typhoid bacilli when mixed with typhoid blood. When a small amount of blood is added to a pure culture of typhoid bacilli, the bacilli are seen to lose their motility and to gather in clumps or bunches. Agglutination is not, however, characteristic of typhoid, but is seen in many other infectious diseases. If the test is performed accurately, the result is absolute. The test will be described fully in the chapter on Typhoid Fever.

The *agglutinating substance* is contained in all normal and pathologic fluids of the diseased animal, and is present through-

out the course of the disease. Gruber named this substance *agglutinin*. It may be formed by the action of the bacteria on the tissues; or by the resistance of the tissues to the infection. The second supposition is hardly tenable. The agglutinating action may also be obtained by the addition of chemicals to the blood. Neither the composition nor the origin of this substance has as yet been determined definitely. It is quite resistant to heat and chemicals. Emmerich and Loew believe that agglutination is the first stage of the bacteriolytic action of the enzymes produced by bacteria.

Precipitins: When typhoid cultures are mixed with the blood-serum of typhoids, a precipitate (non-bacterial) is formed which soon settles, leaving a clear supernatant fluid. The same reaction is obtained with other bacteria and their corresponding blood-sera. The precipitate is called the *precipitum*, and the substance in the blood which induces the precipitation is called *precipitin*. The "anti-sera" also belong to this class. The precipitins are very resistant to heat and chemicals. Nothing is known of their chemical composition.

Lysins: Normal blood-serum is bacteriolytic to a slight extent, but during the infection specific bacteriolysins are formed, which are bacteriolytic for the specific germ and also its congeners. The composition and nature of these bodies are likewise still a matter of dispute, but it is strongly suspected that the lysins are the same as the agglutinins, although by no means identical.

All these various substances are known as "**anti-bodies**," and are the results of the action of foreign proteid matter on certain living cells; such results are chemical substances which have a specific relation to the substance under the influence of which they are produced.

Pfeiffer's phenomenon: Pfeiffer discovered that if cholera bacteria are placed in the peritoneal cavity of a guinea-pig that has been immunized to cholera, the bacteria are dissolved by the peritoneal fluid. The bacilli become immotile, swell, and break into small granules which disappear completely. The reaction is specific not only for cholera, but also for typhoid and other organisms. This reaction is bacteriolytic, and is due to the lysins.

CHAPTER XIII.

IMMUNITY.

Immunity is an inherent insusceptibility to disease.

It is a well-known fact that certain races and individuals are not susceptible to some infectious diseases, and that others are extremely susceptible to the same diseases. Negroes, as a race, are immune to yellow fever. White persons born in a yellow fever district, or who have lived in one for many years, show a like resistance to the disease. Scarlet fever and the other exanthems, except smallpox, are very common in children, but are rarely seen in adults. One attack of smallpox rarely is followed by a second; but if so, the second attack is a very mild one. Birds and snakes are not susceptible to typhoid; mice and rats, to diphtheria; and pigs, to snake-venom.

The natural barriers to infection, such as the unbroken skin and normal mucous membranes, can hardly be considered essential factors in the production of immunity. The protection offered by immunity is solely against the bacterial causes of disease which cannot develop in the body of the immune. Or the living organism possesses the power of diminishing the virulence of the germ and overcoming its toxic effects. This might be termed tissue-endurance. The immunity usually exists against both the germ and its toxin, although not against an unlimited number or quantity of either, and especially not of the latter. A susceptibility may exist to the germ, but not to its toxin; as, for instance, man is susceptible to the action of the tubercle bacillus, but not to its product, tuberculin. Immunity is always a relative condition. Fraenkel says that "a white rat is immune to anthrax in amounts sufficiently large to kill a rabbit, but it is perhaps not immune to a quantity sufficiently large to kill an elephant."

Immunity may be: (*a*) *natural*, the immunity of certain individuals and races against certain diseases at all times; (*b*) *inherited*, the transmission of immunity from the mother to the fœtus through the placenta, or from the mother to the child through the milk; (*c*) *acquired*, immunity produced by one attack of the disease, or by inoculation, or by the introduction into the body of an artificially prepared antitoxin.

Immunity may also be: (1) *passive* or (2) *active*.

Immunity is said to be active when the *tissues* of the body possess the resisting power.

In passive immunity the resisting power resides only in the *blood*. It may be produced by injecting the blood-serum of an animal which is actively immune.

The immunizing substance always is stored up in greatest quantity and for the greatest length of time in the organ or tissue which would be most seriously affected by the disease.

NATURAL IMMUNITY: It is impossible to give an exact definition of immunity, especially natural immunity. Natural immunity is not absolute, although it is more so than the acquired form. The cause of immunity is still a matter of conjecture. The most we can do is to study the conditions which influence immunity either one way or the other. Various theories as to its nature have been suggested:

Phagocytosis: The phagocytic theory of immunity was advanced by Metschnikoff. It is based on a peculiar property possessed by the white corpuscles, especially the polymorphonuclear leucocytes, and certain fixed tissue-cells, the endothelial cells. The former are termed microphages, and the latter macrophages. These cells take up or devour inert particles, hence their name of phagocytes. In an inflammation, the leucocytes rush to the seat of the inflammation, and not only wall off the process, but also assist in disposing of the effete or foreign matter. Leucocytosis (an increased number of leucocytes) is a prominent feature in nearly all the infectious diseases—in fact, the prognosis is influenced largely by the degree of leucocytosis. Metschnikoff observed that when an animal is injected with a culture of bacteria, the leucocytes are subsequently found to contain these organisms; further,

that the cells gradually break up the germs, apparently a process of digestion. In an immune, phagocytosis is usually very active, whereas in a susceptible animal there is little or no phagocytosis.

The question has been raised whether these cells take up living bacteria or whether they simply act as scavengers by removing the dead germs. Metschnikoff successfully isolated leucocytes containing anthrax spores and transplanted them to bouillon. The spores were set free by the death of the leucocyte and developed rapidly into mature and active organisms. This observation has been confirmed by others. It has also been noted that the leucocyte does not always destroy the germ, but that this may destroy the leucocyte. The phagocytes at times exhibit a selective tendency for certain bacteria.

The leucocytes are repelled or attracted by certain substances. This property is known as *chemotaxis*. Chemotaxis is either *positive* or *negative*. If positive, the chemotactic substance attracts large numbers of leucocytes, when phagocytosis is very marked. In negative chemotaxis the leucocytes are repelled; phagocytosis is then absent. In natural immunity positive chemotaxis is very apparent. The leucocytes probably kill the bacteria by digesting them or by liberating some chemical substance which destroys the germ. This chemical substance may be nucleiic acid or nuclease. The phagocytic theory of immunity is no longer tenable in view of the extensive researches made within the last few years. Phagocytosis is now regarded as the result rather than the cause of immunity.

Body-juices: In 1872 Lewis and Cunningham noted that bacteria injected into the body disappeared completely within a few hours.

Other investigators, notably Fodor and Nuttall, in 1887–88, demonstrated that the blood exhibited decided germicidal powers for some time after its withdrawal from the body.

The same bacteriolytic action was observed in other of the tissue-fluids, such as ascitic and hydrocele fluids, aqueous humor, etc.

The germicidal power of the blood is destroyed by heating

it to 55° C.; the ordinary blood-serum culture-medium illustrates this destructive action.

Germicidal power of the blood—causes: This bacteriolytic action is especially marked in the cases of those bacteria to which the animal is naturally immune. There are exceptions to this rule, however. The rabbit is very susceptible to anthrax even though its blood is markedly germicidal for the anthrax bacillus. It is possible that this action of the blood is not so much germicidal as attenuating, thus antagonizing the toxigenic power of the bacterium and enabling the tissues to dispose of the germ.

Behring attributed this germicidal action to the *alkalinity* of the blood. Buchner, after very exhaustive studies of this subject, concluded that the germicidal properties of the blood are due entirely to its soluble constituents, which he termed *alexins*. Hankin ascribed it to the germicidal globulins, which he named *defensive proteids*. They are destroyed by heating to 55°–60° C. for one-half hour, and by diluting with from eight to ten volumes of distilled water. Vaughan and McClintock attribute the germicidal action to the *nucleins*, and believe that immunity diminishes as these substances become less soluble. They extracted nuclein from the blood-serum and demonstrated its germicidal property.

Buchner's alexins are not nucleins. The alexins are derived from the leucocytes, especially the amphophiles, which Hankin calls the *alexocytes*. Buchner found that when the leucocytes disintegrated, a germicidal substance is liberated, showing that the germicidal action of the blood is not due to phagocytosis. It is probable, therefore, that the living leucocyte secretes a substance on which the so-called phagocytic action depends. Although the exact nature and composition of these alexins are not definitely known, they in all probability have their origin in the white cell of the blood, in which also resides the germicidal power of the blood.

Plasmolysis may also be of some importance in this connection. The removal of a germ from an isotonic to a hypertonic or hypotonic medium in all probability has an injurious effect on the organism. While this does not account for the

germicidal property of the blood, yet it may, to some extent, be held to account for it.

Immunity to the toxins, according to Ehrlich, is due to the fact that some of the tissue-cells are capable of forming chemical combinations with the toxin by means of so-called molecular *side-chains;* that is, that these substances, or *side-chains*, neutralize the toxin and thus allow the "natural resistance" of the system at large to dispose of the germs. This theory will be discussed more fully later on (see page 122).

Emmerich believed that bacteria produce *enzymes* which are capable of digesting not only the germs that caused their formation (conforme), but also in some cases other germs as well (heteroforme). The immune serum contains more enzymes than do artificial cultures of bacteria. These enzymes may account also for the degeneration-forms of bacteria seen in old cultures, and for the self-limitation of infectious diseases. He calls these enzymes *nucleases*. In order to distinguish them, each enzyme is named after the organism producing it, as typhase, pyocyanase, etc. An *immune proteid*, according to Emmerich, is a combination of the enzyme with some albuminous body. He believes that the bacteriolytic action of normal blood is due to the presence of enzymes.

ACQUIRED IMMUNITY: This form of immunity is peculiar to the individual, and is extremely variable in its duration. It is acquired either accidentally or experimentally, and is not a hereditary condition.

Accidental immunity: This usually results from an attack of an infectious disease, like scarlet fever, smallpox, measles, etc. Such an attack confers an immunity which under ordinary conditions is permanent. The immunity may, however, be of only short duration, after which the individual is just as susceptible to the disease as he was before the first attack. In many of the infectious diseases the immunity is of only a few months' duration. The attack which confers the immunity need not necessarily be either a severe or a typical one. For instance, a mild attack of smallpox confers as lasting immunity as a severe attack. This is seen in vaccination.

Experimental immunity: This differs from the accidental form in that it is dependent upon purely artificial conditions.

It is this form of immunity which is responsible for the many conflicting theories of immunity.

Active form: Years ago, before it even was surmised that diseases might be caused by minute vegetable organisms, it was observed clinically that one attack of some diseases rendered the individual secure from a second. It was customary to produce artificial immunity to smallpox by *inoculation*. A mild case of the disease was selected, and the healthy individual was inoculated, through an abrasion of the skin, with some of the purulent matter obtained from a smallpox pustule. As might be expected, with the conditions prevailing at that time, the person so inoculated often was the means of exciting an epidemic by developing a very severe case of smallpox, and deaths were by no means infrequent.

This primitive method of inoculation was modified subsequently by Jenner, who noticed a peculiar affection of the cow's udder resembling smallpox in man. He also noted that the milkmaids contracted this disease, and that afterward they were immune to smallpox. He then inoculated members of his family with matter obtained from the pustules on a cow's udder, and succeeded in producing immunity to smallpox. This form of inoculation is known as *vaccination*. The virus is attenuated by its passage through the cow, and its inoculation into man produces simply a local lesion with slight constitutional symptoms, but confers an immunity equal to that following a typical attack of smallpox.

This form of inoculation is no longer limited to smallpox, but is practised also in anthrax, symptomatic anthrax, cholera, bubonic plague, and typhoid fever. The principle underlying it is the attenuation of the germ; and many vaccines, viruses, or attenuated cultures, are now being used to produce these results.

It is claimed that inoculation with *saprophytic* bacteria derived from the soil and water will give protection against pathogenic organisms. When a culture of the *Bacillus prodigiosus* is injected into a rabbit sick with anthrax, the animal will recover.

This form of immunity may be secured by treating the animal to weakened cultures of the germ; by injecting steril-

ized cultures or toxins; by inoculation with cultures of the specific germ mixed with other organisms; with dead bacteria; with germs grown on media containing an antiseptic. Very virulent organisms are attenuated by growing them at high temperatures.

Immunity secured in this way is of only short duration, but is sufficient to protect the individual against the disease for the time being.

The toxicity of the virus may also be modified by heating it; or by the addition of chemicals, such as iodine or chlorine.

A certain amount of *tolerance* (really a form of immunity) may be produced by substances other than bacteria. This is seen in many forms of *intoxication.* The fact that immunity may be conferred not only by pathogenic bacteria and their products, but also by dead bacteria, saprophytes, and inert particles, forces us to the conclusion that whatever confers the immunity is a chemical compound which is distributed widely in nature. Calmette produced immunity by successive injections of snake-venom. Ehrlich's experiments with ricin and abrin were similarly successful. Others produced immunity to poisonous eels, botulismus, arsenic, etc. The immunity produced by these substances is practically the same as bacterial immunity. The immunity is due perhaps to phagocytosis or phagolysis, and the tissue becomes enriched with antibacterial substances.

Passive form: This form of immunity is always experimental. It is produced by the administration of *antitoxins.* An antitoxin is a substance derived from immunized animals, and which, when injected into another animal, confers immunity. A special chapter will be devoted to the consideration of these highly interesting substances.

Tissue-suspensions are also a source of experimental immunity. Wassermann found that the spinal cord of a rabbit when crushed and suspended in a normal salt solution and mixed with tetanus toxin protected against tetanus even when the cord alone was injected either before or after the toxin, or into another part of the body. These findings have been confirmed by others; but it is maintained that the

nervous substance must come into direct contact with the toxin in order to be effective; further, that it excited an inflammatory reaction at the site of injection, and that this alone was responsible for the resulting immunity. The brain substance must be crushed, otherwise it is ineffective. Marie found that the gray matter of the cerebral cortex possessed the greatest immunizing power. Immunization by tissue-suspensions has been practised for some time against tetanus infection, but the results have not been very satisfactory. Extracts of the liver and the adrenals have been found to counteract the cobra poison.

Inert substances: By mixing commercial carmine with tetanus toxin in the proportion of 0.5 gram to 10 c.c., the toxicity of the toxin is reduced considerably. By heating this mixture to 60° C., its protective action is destroyed; but twenty-four hours afterward the mixture has regained its toxicity, so that the toxin is not destroyed by the carmine. This peculiar action of the carmine is probably the result of the leucocytosis produced by the inflammatory reaction of the tissues when the mixture is injected.

MODIFICATION OF IMMUNITY: Inasmuch as immunity is at all times only a relative and not an absolute condition, we will now consider briefly those conditions which modify immunity. Exaltation of immunity is synonymous with acquired immunity, which we have gone into already. The modifications that are of vital importance are those which tend to *lessen* the immunity. They are all manifested by a diminution of the resistance of the tissues to infection.

Exposure to cold: This is one of the most potent agents in the reduction of immunity. Many infectious diseases follow "taking cold," especially pneumonia, tuberculosis, and other affections of the respiratory system.

Fatigue has a decided tendency to reduce tissue resistance, and consequently the immunity.

Poor hygiene: It is a matter of every-day observation that epidemics of infectious diseases are much more common under poor hygienic conditions than where these do not prevail. Living in small, stuffy rooms to which sunlight and fresh air have little or no access, overwork, insufficient sleep, worry,

anxiety, fear and fright, and improper diet, all predispose to infection by lessening immunity.

Noxious gases: The inhalation of noxious gases also tends to diminish the condition of immunity. Since the perfection of the sewerage system, infectious diseases are not so often the result of inhalation of sewer gases. Formerly many diseases were supposed to have their origin in the inhalation of sewer gas, especially diphtheria and scarlet fever; but since their infectious nature has been established, it must be evident that these gases are only operative in so far as they lessen the bodily resistance, or immunity, to these infectious diseases. Modern sanitation has certainly been effective in reducing the number of cases of the diseases mentioned, and we must, therefore, accept the statement that noxious gases play some part in their production.

Drugs, etc.: Persons addicted to the use of drugs or alcohol are much more liable to infection than total abstainers. In such individuals the disease usually manifests itself in its worst form.

Trauma and operations: These invariably reduce immunity by creating conditions most conducive to the development of bacteria.

Other diseases: Any chronic disease reduces the vitality of the individual, so that he is extremely susceptible to infection. His immune power is all used up, and he succumbs to the terminal infections, which he would escape were he in possession of his full powers of resistance.

Mixed infections: The body appears to be unable to take care of more than one infection at a time. Its energies are directed toward one point of attack, and dissolution follows as the result of the other infection. Persons who have survived an attack of typhoid or influenza succumb rapidly to a complicating pneumonia. The two occurring together is more to be dreaded than either one alone.

THEORIES EXPLAINING ACQUIRED IMMUNITY: **Exhaustion theory of Pasteur and Klebs**: In 1880 Pasteur and Klebs suggested that immunity was due to the exhaustion in the body of some substance necessary to the development of bacteria. During the first infection the germs consumed this

particular substance, and at the time of the second infection died for want of it. This theory is no longer tenable, as we have shown that even an immune will succumb to an overwhelming infection, so that a vital substance can have nothing to do with the production of immunity.

Retention theory: Chauveau suggested that the bacteria in the course of their development elaborate some metabolic product which accumulates in the body and is retained. This product is inimical to the growth of that particular organism, and a second infection cannot, therefore, occur.

Under normal conditions the tissues and fluids of the body are constantly undergoing changes, and it is extremely improbable that any metabolic product of this kind could be retained unchanged for an indefinite number of years. Furthermore, it is also improbable that an individual who has suffered from a number of different infections could keep stored up in his body the products of the various organisms causing these infections in sufficient quantity to prevent their development at a subsequent infection.

Germicidal action of the body-juices: It has repeatedly been demonstrated that freshly drawn blood, aqueous humor, ascitic fluid, and the lymph from the dorsal lymph-sac of the frog, possess a decided germicidal power. This power is positive, but is limited as to the number of bacteria which can be destroyed by a given quantity of serum. Heat promptly destroys this power. The theory is therefore untenable.

Phagocytosis: This theory has already been fully discussed.

Antitoxins: When it was discovered that bacteria elaborated toxins, the conclusion was arrived at that immunity to these toxins must be due to an antagonistic substance which neutralizes the toxins. This substance was called antitoxin. These antitoxins are probably one of the phenomena of immunity rather than the cause of it. They will be discussed fully in the next chapter.

Ehrlich's lateral-chain theory: Ehrlich believes that the various cells of the body contain a nucleus to which are attached by means of what he terms "side-chains" certain atom groups. These side-chains are concerned in the nutri-

tive function, but appear to be capable of combining with a toxin by means of *haptophorous* bodies,—the *toxophile* chains in the cell, and the *cytophile* granules in the toxin molecule. A second group of granules in the toxin molecule, called the *toxophore* group of granules, destroys the cell if they are present in sufficient quantity. If present in only a limited quantity, they merely injure the cell, stimulating it to repair, and the cell throws out new side-chains. The frequent introduction into the body of toxins results in overstimulation of the cell, and consequently it throws out for its protection more side-chains than really are needed. This surplus or overstock of side-chains is thrown into the circulating blood-current, lymph, and other body-juices, and these constitute the antitoxin, which during an infection combines with the toxin and neutralizes it. The whole theory is based on the chemistry of the cell, and the so-called "side-chains" simply represent chemical formulæ. There is no experimental foundation to this theory, but it is the most plausible of them all, as all the changes in the body are merely chemical changes.

CHAPTER XIV.

ANTITOXINS.

THOSE substances which destroy bacteria are said to be germicidal; but those substances which neutralize the bacterial toxin are of vastly more importance. They are called **antitoxins.** An *antitoxin* is a substance which has remained in the tissues and juices of the body, and which confers immunity by antagonizing or neutralizing the product of the bacteria. The most exhaustive experiments and researches have failed to reveal the exact nature of these products, although their presence in the body has been proved by many.

It is not accepted, however, that immunity is due to the presence in the body of an antitoxin. This substance is looked upon as the result of *forced immunization.*

Various theories as to the probable nature of this substance have been advanced. All experimental evidence goes to show that in certain conditions acquired immunity depends upon the formation of an antagonistic substance. Antitoxins usually possess no germicidal power.

Buchner does not regard the antitoxin as a reactive product, but as a modified, changed, "poison-free" product of the specific bacterial cells.

Ehrlich's *side-chain* theory is another theory accounting for the production of the antitoxin, that it is the product of excessive tissue stimulation.

For a long time it was supposed that the antitoxin was the *modified toxin,* but conclusive evidence against this theory is now at hand: (1) The quantity of antitoxin is not at all in proportion to the amount of toxin required to produce the immunity. (2) The removal of large quantities of blood from the body does not lessen the degree of immunity. (3) If the immune animal is bled to death and the entire vascular system carefully washed out with sterile salt solution until every

possible bit of blood is removed, infusions of certain tissues will still be found to contain the antitoxin.

It has been claimed that a toxin can be converted into an antitoxin by means of *electricity*, but Vaughan and Novy disproved this.

The bacteria themselves are not responsible for the production of the antitoxin; they simply elaborate the toxin, which must be held responsible for the production of the antitoxin.

Emmerich and Loew believe that the bacterial *enzymes* are really the antitoxin. These enzymes are not eliminated, but accumulate in the blood, thus conferring the immunity. Experimental investigation has disproved this theory. The quantity of enzymes formed is insufficient to account for the large amount of antitoxin.

Hankin calls the antitoxic substances *defensive proteids*. He classifies them according to the method of their production and their action on either the toxin or the germ. Those found in animals that are naturally immune he has named *sozines*. The *phylaxins* are responsible for artificial immunity. The prefixes *myco-* and *toxo-* are used to indicate whether the defensive action is against the germ or its toxin. These proteids are the same as those Buchner terms alexins. They probably are retained within the bloodvessels for an indefinite length of time, as they do not dialyze. Yet Ehrlich has shown that tetanus antitoxin is excreted by the mother's milk without in the least lessening the immunity of the mother, thus making it extremely probable that there is a continuous production of the protective material.

The **chemical composition** of the antitoxic substances is not known, except that they are extremely stable. They are much more resistant than the alexins. When exposed to a temperature of 60° C. for a short time only, they remain unchanged. They resist putrefaction, artificial light, and sunlight, and can be kept for a long time. The various antiseptics usually added to the antitoxic sera do not affect them in any way, and are added simply to prevent contamination and putrefaction. The antitoxins are very resistant to carbolic acid, trikresol, chloroform, and formaldehyde. The addition of alcohol precipitates the albumins and deprives the serum

of its antitoxic power. When taken by mouth, the antitoxins are digested in the alimentary canal, because of their proteid composition, without, however, conferring immunity. It is impossible to obtain antitoxin in a pure form.

Specific action of antitoxin: The action of certain antitoxins is specific. The diphtheria antitoxin protects against the diphtheria bacillus and its toxin only. Hueppe claims that specific antitoxins also protect against other infections, although in a less degree. Antitoxins, as a rule, are effective only against the toxin and not the germ as well, unless the immunization has been produced by the use of active cultures of the germ. The action of the antitoxin may be due to a direct chemical combination with the toxin, forming an inert mixture; or indirectly by a stimulation of the activity of the tissue-cells by the antitoxin. It may also act through a combination of the antitoxin with the toxin through the medium of the combining substances or ferments in the blood.

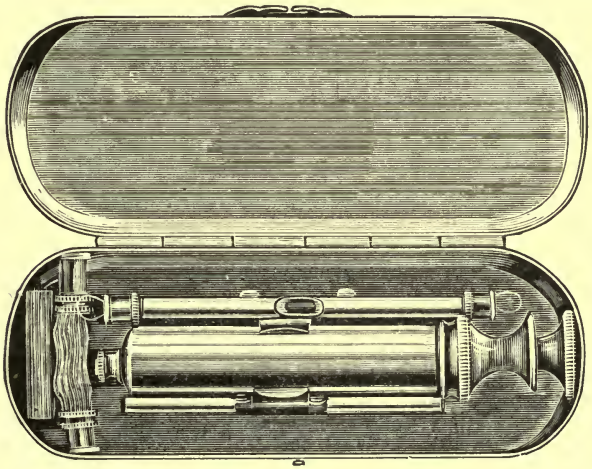
Manufacture: Antitoxin is now being manufactured on a large scale for the purpose of producing artificial immunity against certain infectious diseases, and also for use as a therapeutic agent. The method consists in immunizing large animals, principally horses, by huge quantities of toxin, and then withdrawing a certain quantity of blood when the desired degree of immunity has been attained. The preparation of the specific antitoxins will be considered in the chapters on the infectious diseases.

The exact strength of the serum must be determined before it can be used either for immunizing purposes or therapeutically. The amount of antitoxin required to protect 25,000 grams weight of guinea-pigs from the minimal fatal dose of toxin is called an immunizing unit. Usually from 600 to 1800 immunizing units are required to produce a cure. This amount is contained in 2, 4, or 6 c.c. of commercially prepared antitoxin.

Method of administration: It is useless to administer antitoxins by mouth, because, as already mentioned, they are digested in the intestinal canal. Therefore they always are administered by hypodermic injection into the subcutaneous tissues. It really does not matter where the injec-

tion is made, providing the site of injection contains much loose subcutaneous tissue and is capable of absorbing the fluid rapidly. It is also desirable to choose a site that is not exposed to pressure by the clothing or by the position of the patient, and in which the nerve-supply is not very great. If an injection is made into a part of the body which is well supplied with nerves, the injection is attended by considerable pain. This should always be avoided. The places usually chosen for injection are the

FIG. 37.



Syringe for injecting antitoxin.

flank, the tissues of the back between the shoulder-blades, or the outer aspect of the thigh. In the case of refractory patients the most convenient place is chosen. It is not necessary to inject the antitoxin in the vicinity of the lesion.

An injection of antitoxin should be given with the same *antiseptic precautions* as any ordinary injection. The skin is sterilized carefully and thoroughly, and the needle of the syringe (Fig. 37) is made aseptic by boiling for fifteen minutes. The antitoxin container is broken, the syringe filled, and the

needle carefully introduced into the subcutaneous tissues. As the injection is made the needle is withdrawn slowly and gradually, and the opening sealed with collodion. Slight manipulation of the part will facilitate absorption of the serum.

Many of the *ill-effects* following the administration of antitoxin are due to careless technique and lack of sufficient cleanliness. Localized suppuration, septicæmia, and pyæmia are quite likely to follow careless injection. The patient should be absolutely quiet while the injection is made, so that the needle of the syringe will not penetrate any further than into the subcutaneous tissues. In the case of children, it is best to wrap them in a sheet or large cloth, leaving only the site of injection exposed. The child is held firmly by an assistant to prevent its struggling.

Immunization: Immunization is the process of rendering immune. It usually implies forced immunity or the gradual production of immunity by the repeated injection of carefully graded doses of toxin. Any animal may be used for this purpose, but some large animal, like the horse, is preferable, because a larger amount of blood can be withdrawn at one time. The animal must be free from tuberculosis or glanders or any other infectious disease, and should be kept in a clean, aseptic stable, and be well cared for. Living cultures, attenuated cultures, dead cultures, filtered cultures, or the toxin, are used for this purpose.

The animal is injected first with a very small dose of the substance used, in order to ascertain its susceptibility to the poison. After it has recovered from the effects of this first injection a larger dose is given. This is repeated at intervals until the animal shows no reaction whatever to hundreds of times the original, fatal dose. This indicates a condition of tolerance, or what might be termed a toxin-habit, and results in a very high degree of immunity. The immunization must be carried on judiciously. Care must be taken not to exhaust the tolerance of the animal, as a condition of hypersensitivity may be produced to which the animal usually succumbs. There is, in short, a limit to the tolerance of the animal, and this limit must not be passed. The serum of highly immunized horses is most suitable for *therapeutic* purposes.

A lower serum standard is sufficient for *protective* purposes. Larger amounts of the antitoxic serum must be used to produce a cure than for immunization against infection.

When the horse possesses the desired tolerance, the jugular vein is laid bare under antiseptic precautions and a sterile trocar thrust into the vein. The blood is collected in sterile bottles, and after it has coagulated it is placed on ice for a few days, when the clear supernatant serum is pipetted off with sterile pipettes. This serum contains the antitoxic substance, and is known as the "antitoxin." The serum is then standardized and placed in glass containers, which are sterilized and then hermetically sealed. It is customary to add some preservative to the serum. A 0.5 per cent. solution of carbolic acid, camphor, or formalin, or a 0.4 per cent. solution of trikresol, is used for this purpose. Trikresol is the most preferable, as it is not irritating and possesses slight anæsthetic properties.

CHAPTER XV.

EXAMINATION OF AIR, WATER, AND SOIL.

Bacteria in the air: Both pathogenic and non-pathogenic bacteria are constantly present in the air in greater or less number, depending on conditions and the quality of the air. The pure air of the mountains contains very few organisms, whereas the dust-laden air of cities and towns contains very many.

The species also vary according to the temperature and the soil over which the air passes, the number of living creatures moving about in it, and their method of living, hygiene, and sanitation, the presence of decomposing material or the excreta of persons and animals affected with some disease. The air in sick-rooms and hospital wards contains more organisms than the outside air. The air of places frequented by consumptives, who expectorate promiscuously on the ground, usually is laden with tubercle bacilli. During those seasons of the year when certain infectious diseases, such as pneumonia, la grippe, etc., are epidemic, the air is more liable to contain pathogenic organisms than when such epidemics are not prevailing.

Under ordinary conditions, however, the air usually contains only saprophytic bacteria, and these are found always in the lower strata unless disseminated by winds. There are less bacteria found in the air during winter than in the summer, and very few immediately after a rainfall or snowfall. The dust which is shaken from the hides and pelts of animals is very liable to contain anthrax bacilli and their spores.

In addition to the bacteria, we find also *moulds* and *yeasts*.

The **presence** of micro-organisms in the air is **determined** easily by exposing a dish containing sterile gelatin or agar for a few minutes. Special media must be provided for those

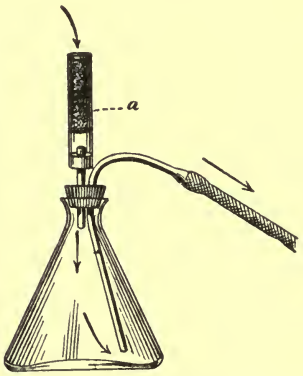
germs which do not grow on ordinary media. Large numbers of colonies of bacteria soon make their appearance.

Besides determining the actual presence of these germs, **quantitative tests** must be made. Various methods have been suggested:

Hesse draws a current of air through a glass tube 70 centimeters long and 3.5 centimeters wide, which is coated on the inside with a film of gelatin, like an Esmarch roll culture.

Petri uses small sand filters placed in a wide glass tube (Fig. 38). The sand is first sifted, then sterilized by heat,

FIG. 38.



Petri's apparatus for bacteriologic analysis of air: *a*, tube packed with sand; arrows indicate entrance and exit of air current.

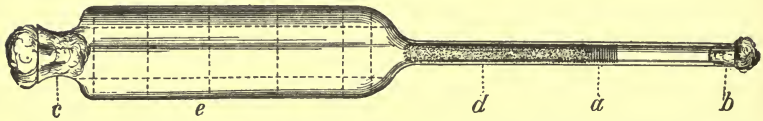
after which it is placed in the tube, supported by small wire baskets. Two such filters are placed in one tube. One end of the tube is closed with a cork, through the centre of which passes a thin glass tube. The entire apparatus is then sterilized in the hot-air sterilizer. By means of an air-pump, 100 liters of air are aspirated through the sand in from ten to twenty minutes. The sand filters are then placed in sterile dishes containing gelatin, and the colonies are counted as they develop. The objection to this method is that the sand granules are apt to be mistaken for colonies.

Tucker and Sedgwick (Fig. 39) have improved this method

by using *sugar* instead of sand. After the air has been forced in at *b* the cotton plug is replaced and through the large opening *c*, sterile gelatin is poured into the tube, which dissolves the sugar; and an Esmarch roll is made at *e*.

Uffelmann found that in the open country 1 cubic meter of air contains 250 germs; on the seacoast, 100; in the court-

FIG. 39.



The Sedgwick-Tucker aërobioscope: *a*, brass wire gauze stopper; *c* and *b*, cotton stoppers; *d*, sugar; *e*, site for Esmarch roll. (Abbott.)

yard of the University of Rostock, 450. The number was less after a rainfall, and greater on a windy day. These findings have been verified by others.

It must be remembered, however, that most of these organisms are not pathogenic. The number of bacteria found in the air is of little clinical importance unless they are pathogenic.

Examination of Water.

Water which contains even a trace of organic matter always **contains bacteria.** Bacteria will not grow in water free from organic matter, although they may remain alive in it for a considerable time. The bacteria contained in water are usually of the non-pathogenic variety. At times, however, both the *Bacillus typhosus* and the spirillum of cholera may be found in water. More bacteria are found in water after a rainfall than before, because the rain has washed them out of the air into the water. Warm water contains more bacteria than cold water; shallow water more than deep; water at rest or having only a sluggish current more than running water; unfiltered more than filtered water. Inasmuch as water plays such an important part in the human economy, the bacteria which it contains are of more than passing interest, and especially at such times when the water-borne diseases are epidemic.

Water collected for **bacteriologic examination** should be examined as soon as possible, as the contained bacteria multiply very rapidly. An examination made twenty-four hours after collection will not give the same results as an examination made within a few hours. Warm water should be examined immediately. The sample may be placed on ice, but it has been found that extreme cold is fatal to some of the germs. Ice taken from water which contains bacteria usually contains the same germs as the water. The quality of the water is not affected by the presence of large numbers of non-pathogenic germs, but a few pathogenic organisms suffice to make the water an element of danger. Lake-water contains less bacteria than river-water. Wells having a very deep supply contain very few bacteria, unless contaminated by the surface-flow. Very deep wells and springs may contain no bacteria at all. Sewer-water, of course, contains immense numbers of bacteria.

Bolton showed that two varieties of **non-pathogenic bacteria** occur in water which has been sterilized as often as six times.

FIG. 40.

Glass bulb for collecting samples of water.

The most important **pathogenic bacteria** found in water are the *typhoid* and the *cholera* organisms. These germs find their way into the water-supply from the ground-water. The feces and other excreta containing these germs are not disinfected before they are disposed of, and when they are thrown on the ground the germs make their way into the substrata of the soil, whence they are carried by the ground-water to the water-supply. These organisms retain their vitality in water for from seven to thirty days, but do not multiply. The non-pathogenic bacteria contained in water soon destroy these pathogenic bacteria. The typhoid bacillus will retain its vitality for thirty days in ice.

Samples of **water** may be **collected** in sterilized Erlenmeyer

flasks, in sterilized bottles having a ground-glass stopper, or in Sternberg bulbs (Fig. 40). If the city water-supply is to be examined, the sample may be taken directly from a faucet after the water has been running for about half an

Fig. 41.

Bottle for collecting water.

Fig. 42.

Bulb pipette.

Fig. 43.

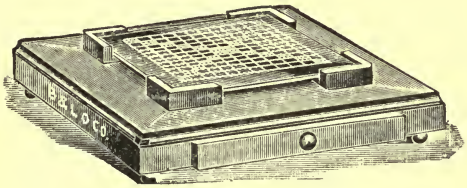
Graduated pipette.

hour. If from other sources, the container is placed at the depth at which it is desired to make the examination. The bottle is uncorked and corked under the water, and hermetically sealed before it is transported (Fig. 41). Only sterile flasks or bottles are used for collection.

Plate cultures or Esmarch rolls are made on gelatin, agar, or glycerin-agar. If the water contains many bacteria per cubic centimeter, it is necessary to dilute the water with sterile water; or use only a small quantity for the inoculation by means of pipettes (Figs. 42 and 43).

Wolfhuegel **counts** the colonies by placing the dish or plate on a large plate of glass (Fig. 44) divided into many small

FIG. 44.



Wolffhuegel's apparatus for counting colonies.

squares. The colonies in a certain number of squares are counted with the aid of a hand lens (Fig. 45), and the number of bacteria per cubic centimeter estimated accordingly.

FIG. 45.

Lens for counting colonies (Abbott.)

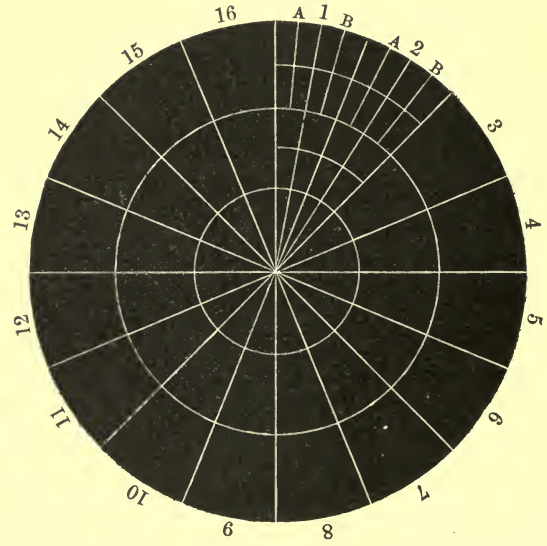
If the dilution has been sufficient, only a small number of colonies appear, and these are easily counted. Divide the result by the number of squares counted, and multiply this average by the number of square centimeters in the plate. The result is the entire number of colonies which have developed from the quantity of water used. Or Pakes's apparatus may be used: a black disk, ruled with white lines (Fig. 46), is printed on a sheet of white paper; a Petri dish, containing the colonies, with the cover removed, is then placed over the

disk and the colonies are counted as they lie between the white lines.

Ordinary hydrant-water usually contains from 2 to 50 bacteria per cubic centimeter; filtered river-water, from 50 to 200; unfiltered river-water, from 6000 to 20,000; ground-water may contain as many as 130,000.

An Esmarch roll culture may also be used, but is most serviceable when the sample of water contains but few bacteria.

FIG. 46.



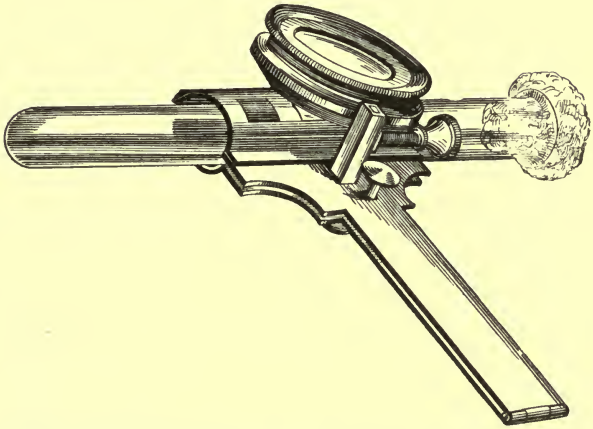
Pakes's apparatus for counting colonies (reduced one-third). (Abbott.)

The surface of the tube (Fig. 47) is divided into squares, and the colonies counted with a hand lens and estimated as in the plate culture.

Inasmuch as all water contains *liquefying* bacteria, cultures are made on both gelatin and agar. The *Bacillus coli communis* is frequently found in sewage-water and in the water into which sewage empties. It is detected readily

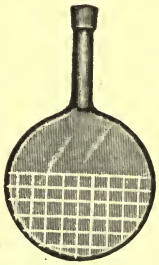
by making the fermentation-test (Fig. 48). Occasionally other fermentative bacteria are found in water.

Fig. 47.



Esmarch apparatus for counting colonies in rolled tubes. (Abbott.)

Fig. 48.



Flask for counting colonies of bacteria.

The method usually employed for isolating the **typhoid bacillus** from water is that of Pariette. He uses the following solution :

Phenol,	5 grams ;
Hydrochloric acid,	4 "
Distilled water,	100 c.c.

From 0.1–0.2–0.3 c.c. of this solution are added to 10 c.c. of bouillon in each of three test-tubes. Add from 1 to 3 c.c. of the water to be examined to each tube, and place in the incubator. The only bacteria which will develop in this medium are the typhoid and colon bacilli. These are then plated and separated (see *Bacillus typhosus*).

Abbott suggests the use of *chemical coagulants*, like alum or iron, which, by precipitating as hydroxides, drag down the bacteria. The precipitate is then examined. Or large quantities of water are passed through a Pasteur filter, and the accumulations on the filter brushed off and examined. The water may contain only a few typhoid bacilli, and it may be necessary to make numerous examinations before their presence is detected.

Examination of the Soil.

Soil to the depth of four feet always contains bacteria, especially when it contains much organic matter. The upper strata of virgin soil contain the greatest number. From the surface down there is a gradual falling off in the number, until at the depth of four feet they disappear entirely. In cultivated soil and in soil in which fertilizers have been used, bacteria are present in great numbers. In inhabited localities the upper strata contain many varieties. Sandy soil contains fewer bacteria than a clay soil. Most of the organisms found in soil are non-pathogenic. The pathogenic bacteria are of the anaërobic variety, such as the bacillus of malignant œdema and the tetanus bacillus. The nitrifying bacteria contained in soil are of value. They decompose the organic matter and convert it into suitable food for the higher plants. That, as stated before, is the reason the farmer turns under several crops of clover before sowing his grain.

Ravenel, in an exhaustive article on this subject, comes to the following conclusions:

1. Made soil, as commonly found, is rich in organic matter and excessively damp through poor drainage.

2. Made soil furnishes conditions more suited to the multiplication of bacteria than virgin soils, unless the latter are contaminated by sewage or offal.

3. Made soils contain large numbers of bacteria per gram of many different species, the deeper layers being as rich in the number and variety of organisms as the upper layers. After some years the number in the deeper layers probably becomes less. Pathogenic bacteria are more likely to be contained in made soils.

The earth may be obtained at any depth by means of Fraenkel's special **boring apparatus.** A definite amount of soil is mixed with liquefied gelatin or agar and a plate or roll culture made. The colonies are counted in the same way as in the examination of air or water.

Fluegge found about 100,000 colonies in a cubic centimeter of virgin soil. Sternberg advises washing the earth with sterile water, and after sedimentation a sterile medium is inoculated with the water. Miquel found 900,000 colonies in one gram of earth obtained from a fertilized field. One investigator examined the soil of a churchyard, and found 1,152,000 bacteria at a depth of four meters. The specimens of earth should be examined as promptly as possible, so as to avoid contamination, and also because of the rapid development of the contained organisms when in contact with oxygen and more suitable environments.

PART II.

CHAPTER I.

NON-PATHOGENIC BACTERIA.

THE study of the **non-pathogenic** bacteria is of interest largely from a commercial point of view, as many of these organisms play a very important part in the manufacture of certain articles of food.

In the bacteriologic laboratory it is convenient to begin the study of bacteria, and the methods of their culture and development, with the non-pathogenic group, because most students are inclined to underestimate the danger involved in handling bacteria and the possibility of infection. The study of the *pathogenic* bacteria is not taken up until the technique has been mastered.

The non-pathogenic bacteria are so numerous that it is impossible in a work of this size to consider them all or at any length. We shall limit the enumeration to such of these organisms which are either very common; or which, because of their resemblance to important pathogenic bacteria, are noteworthy. Furthermore, the student can easily obtain pure cultures of these germs for laboratory study. Most of the non-pathogenic bacteria are found either in water or in the air.

Bacillus subtilis: One of the most common varieties is the *Bacillus subtilis* or *hay bacillus*. It is found in hay infusions, water, soil, air, feces, and decomposing liquids. Cultures are obtained very easily, and particularly when not wanted. Most of the culture contaminations which occur in the laboratory are with the hay bacillus.

The hay bacillus is a thick rod, about three times as long as broad, with rounded ends, and resembles the anthrax

bacillus very closely. It frequently shows in its centre a large, resistant spore. It occasionally forms chains of varying length. It possesses terminal flagella; is exceedingly motile; and is a strict aërobe, growing very rapidly at the room temperature and upon all ordinary culture-media. Luxuriant growths, white in color, are formed, with liquefaction of the gelatin. It is stained readily with the anilin dyes.

The organism can be obtained in pure culture by making an infusion in water or beef-tea with finely cut hay. Boil for fifteen minutes, and then place in the incubator for forty-eight hours. The resistant spores, which have survived the boiling, develop into fully matured germs and form a thick white scum on the surface of the liquid.

Bacillus mesentericus vulgatus: This germ, known also as the *potato bacillus*, is found on potatoes, on the ground, and

FIG. 49.

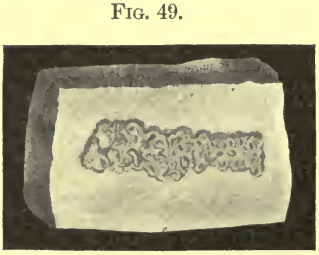


FIG. 50.

Bacillus mesentericus vulgatus.

occasionally in milk. It is a very short, thick rod, with rounded ends, frequently occurring in pairs (Fig. 50). It has terminal flagella, is excessively motile, and reproduces itself by sporulation. It stains with the anilin dyes and also by Gram's method. It is strongly aërobic, growing quite rapidly at the room temperature, especially in the presence of oxygen.

Gelatin is liquefied; *milk* is coagulated. On *potato* a heavy, wrinkled, brown or pink membrane is formed. This membrane is detached easily (Fig. 49). A culture of the potato

bacillus is obtained by exposing to the air for a few minutes a Petri dish containing a slice of sterile potato.

Bacillus prodigiosus: This germ is so short as frequently to be mistaken for a coccus (Fig. 52). It is found in the air, water, milk, on bread, potatoes, and meat, and at times in the axillæ. It is the cause of red sweat. It has flagella, but is only slightly motile. It does not sporulate, and liquefies gelatin very rapidly. It is a facultative anaërobe, and grows quite rapidly at the room temperature on all ordinary media (Fig. 51). It is stained with the anilin dyes, but not with Gram's stain.

When grown in the presence of oxygen, it develops a beautiful *deep-red* or *carmine* color, especially upon potato

FIG. 51. FIG. 52.

Bacillus prodigiosus.

and agar-agar, without discoloring the media. A trimethylamin odor is given off from all the cultures. The pigment is used commercially to some extent.

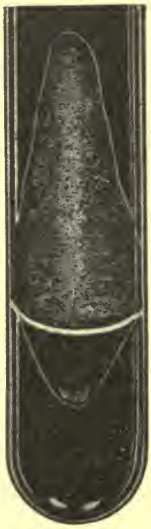
Bacillus violaceus: This is a very small, slender rod, with rounded ends, and containing a central spore. It is found in water. It possesses only a few terminal flagella, but is actively motile. It is a facultative anaërobe, growing at the room temperature on all the ordinary culture-media.

Gelatin is liquefied very rapidly without peculiar characteristics. In the presence of oxygen it produces a beautiful permanent *indigo-blue* pigment, which may be so intense as to appear black. It coagulates milk and stains with the anilin dyes. Gram's stain is not applicable.

Bacillus mycoides: Known also as *Bacillus ramosus*, the

root or "wurzel" bacillus. It is found in water and in the upper layers of the soil. It is a large, thick bacillus, with rounded ends, not flagellated and but slightly motile. It frequently, in culture, forms long chains or threads, and usually contains a central spore. It is a facultative anaërobe, with strong aërobic tendencies, growing rapidly at the room temperature with liquefaction of the gelatin. It is stained with all the anilin dyes, including Gram's.

FIG. 53.



It forms a thin whitish growth, consisting of a very dense network of fine freely interlacing threads. The growth resembles the gnarled roots of an old tree radiating from a common centre, from which it derives its name of root bacillus (Figs. 53 and 54). The growth on agar is very characteristic.

FIG. 54.

Bacillus mycoïdes.

Bacillus fluorescens liquefaciens: Found in water and putrefying liquids; occasionally in the conjunctival sac. It is a very small actively motile rod, containing no spores. It has numerous flagella. It is strongly aërobic, and grows rapidly on all ordinary media at the room temperature with liquefaction of the gelatin. It is easily stained with the ordinary dyes. It forms a *fluorescent, greenish-yellow pigment* on all media except the potato, on which the growth is

brownish. A similar organism is found in water, but it does not liquefy gelatin.

Bacillus acidi lactici: Found in sour milk. It is a short, thick rod, usually occurring in pairs; non-motile, with large shining spores. It has no flagella. It is a facultative anaërobe, and grows on all the usual media without liquefying the gelatin. When grown in milk, it breaks up the milk-sugar and *forms lactic acid* and gases, with precipitation of the casein. It stains readily with the anilin dyes. Its temperature optimum is about 20° C. On gelatin it forms a thick, dry, whitish crust. It differs from the *Bacterium* acidi

FIG. 55.

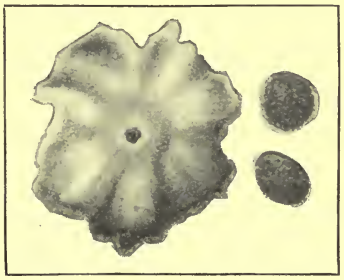
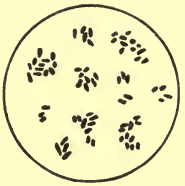


FIG. 56.



Bacterium acidi lactici.

lactici (Figs. 55 and 56) in that it does not produce alcohol in milk.

Bacillus butyricus: This organism is found also in milk, and is the cause of *butyric acid fermentation.* It is a very slender rod, of varying length, with rounded ends and a large central spore. It is flagellated and very motile. It is strongly aërobic, and grows best at the temperature of the body. Gelatin is rapidly liquefied. The casein of milk is coagulated and decomposed, with butyric acid fermentation. On gelatin it forms a very delicate, yellowish surface covering which is quite characteristic. It is stained easily with the usual dyes.

Micrococcus agilis of Ali-Cohen: This organism can be cultivated from drinking-water, and is the only flagellated, motile

coccus. It possesses a single flagellum. It is a facultative anaërobe, growing very readily on all ordinary media at the room temperature. It produces a rose-red pigment. Gelatin is slowly liquefied. The coccus stains with the anilin colors and with Gram's stain.

Micrococcus ureæ: Usually this is found only in urine which has undergone ammoniacal decomposition; but occasionally it can be cultivated from the air. It grows in varying forms, sometimes as a micrococcus, and at other times as a diplococcus or streptococcus. It is strongly aërobic, growing best at a temperature near that of the body. Gelatin is not liquefied. It *decomposes urea*, producing ammonium carbonate. It is stained easily by the anilin dyes, but not with Gram's. A *Bacterium ureæ* also is described, with properties similar to those of the micrococcus. It is probably only a variation of the latter. The tube cultures of the coccus are not characteristic. In plate culture waxy-looking colonies are formed.

Sarcina ventriculi: Several different species of sarcina have been described, of which this is the most important. It is found in the stomach of man and animals under normal conditions. During fermentative processes, and especially in dilatation of the stomach, the sarcinæ are present in excess.

It is a facultative aërobe, growing with moderate rapidity and without liquefaction of the gelatin. The growth is usually colorless, but in time becomes slightly yellowish.

Sarcina pulmonum is found in the air-passages. *Sarcina lutea*, *S. aurantica*, etc., are found in the air, and are color-producing. In every other respect they resemble *Sarcina ventriculi*.

The *Oppler-Boas bacillus* is found in the stomach of persons suffering with gastric carcinoma, and in diseases in which the conditions in the stomach are the same as in carcinoma. It is not distinctive of gastric carcinoma. It has never been cultivated.

Spirillum rubrum: This organism is of absolutely no clinical importance, but serves as an example of the spirillum class. Esmarch found it in the body of a mouse dead of septicæmia. It varies considerably in length, is very motile,

flagellated at both ends, and does not form spores (Figs. 57 and 58). It is a facultative anaërobe, growing best at the temperature of the body. It does not liquefy gelatin. In cultures it frequently forms long spirals. When grown in the presence of oxygen, the color of the growth is white. In

FIG. 57.

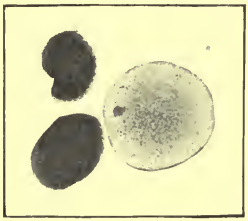


FIG. 58.

Spirillum rubrum.

the absence of oxygen a wine-red pigment is produced. It is stained readily with the anilin dyes.

Spirillum denticolum: This organism is found at the gingival junction of teeth covered with salivary calculi. It forms very long spirals, and is therefore frequently classed as a *spirochæte*. It is very slender and irregular. It has not been cultivated.

Leptothrix buccalis: Although usually this organism is classed as a non-pathogenic bacterium, it is perhaps not such in the strict sense of the word, because it is responsible for a disease,—caries of the teeth. Miller was the first to describe it accurately, and it is known also as *Miller's leptothrix*. It is a very slender, long, and much twisted organism, of extremely variable form. It has not been cultivated. Some varieties give the iodine reaction. Occasionally it is possible to demonstrate segments, but they are at no time very distinct.

Leptothrix epidermidis: This has been found on the healthy skin. It consists of thick, freely interwoven, unbranching, jointed threads. It is distinctly motile, but flagella have not yet been demonstrated. It stains with the anilin dyes and Gram's stain. It is a facultative anaërobe, growing luxuri-

antly at either the incubator or room temperature. Gelatin is liquefied rapidly. The growth on gelatin or agar is white or creamy, red on potato. It does not sporulate.

Vibrio berolinensis: This bacterium is found in water, and because of its resemblance to the spirillum of Asiatic cholera is of importance. It is not pathogenic for man, but guinea-pigs succumb rapidly when inoculated with pure cultures. Its morphology is exactly like that of the cholera germ, but in culture the difference between the two is quite apparent. It gives the *nitroso-indol* or *cholera-red reaction* very strongly.

Several *other non-pathogenic* organisms resembling the cholera germ are found in water, and they will be considered in connection with that organism.

The non-pathogenic bacteria which bear a resemblance to any of the pathogenic bacteria, so that they may be mistaken for them, will be described together with such organisms, so that their differentiation will be understood more clearly.

CHAPTER II.

MOULDS; FILAMENTOUS FUNGI; HYPHOMYCES.

Besides being the exciting cause of some *diseases*, especially those of the skin, moulds are frequently met in the laboratory in the way of *culture contaminations*.

Filamentous fungi is an infinitely better name than moulds; and is also, in a measure, descriptive of their appearance and method of reproduction. Their growths are often very beautiful, especially when pigments are formed. A filamentous fungus consists of thread-like cells or filaments which do not contain chlorophyl and which have an apical growth. They interlace very freely and grow luxuriantly, often forming dense, heavy, felt-like membranes. The growth is usually very dry, but occasionally the surface of the membrane is studded with minute dew-drop-like pearls; or the moisture may be diffused, giving the growth the appearance of a crust or scutulum. The threads forming the growth are termed *hyphæ*, and the growing or vegetative portion of the fungus is the *mycelium*. Arising from the mycelium is the fruit-bearer or *sporangium*. This carries the *spores* or *conidia*. From the spores are developed new hyphæ and new fungi.

These fungi are **classified** according to the structural difference in the sporangium. Several thousand different kinds of moulds have been described, only a small number of which possess clinical importance. They are divided also into *saprophytes* and *parasites*. The majority of mould fungi are saprophytes, and are not met with in man. The parasitic fungi may be the cause of disease in the human organism. This parasitism may be purely accidental, although a few fungi are really obligative parasites.

Moulds as a class are strict *aërobes*, and require an acid medium for their development. That is one reason why they

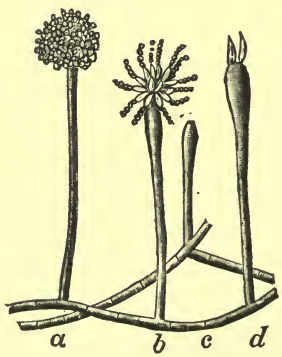
do not thrive in the body. The absence of oxygen and the alkalinity of the tissues speedily prove fatal.

Those that survive may **become pathogenic** by developing excessively, and thus acting as a foreign body. Levy and Klemperer say that there is no actual multiplication, but simply a germination. The number of spores injected is also of importance, as many disease foci are required to produce actual disease. The animal dies as a result of the extension of the foci of disease, and not from intoxication. Diseases of the surface of the body due to the filamentous fungi are common, but rarely prove fatal.

Moulds are distributed widely in nature, and cultures of all **varieties** are obtained readily. The principal moulds are the following:

Aspergillus or bulbous moulds (Fig. 59): The sporangium terminates in a club, which is surrounded and completely

FIG. 59.



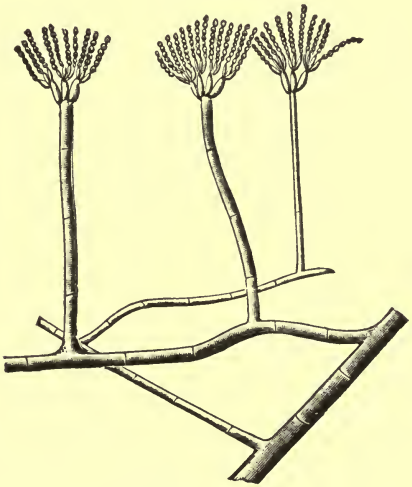
Aspergillus. At *b* a few of the spore-bearing sterigmata are shown; the usual picture is given at *a*. (Mez.)

covered by short, thick, flask-shaped structures radially arranged. These are known as *sterigmata*, and on the ends of them are found the conidia or spores. *Aspergillus fumigatus*, *Aspergillus glaucus*, and *Aspergillus niger* are examples of this class. The aspergillus moulds, especially *Aspergillus*

fumigatus, are the cause of most of the so-called *mycoses*, such as keratomycosis, otomycosis, myringomycosis, and pneumonomycosis. The growth is either black, gray, green, or yellow in color.

Penicillium or brush moulds (Fig. 60): In this variety the terminal extremity of the sporangium divides dichotomously into small endings, *basidia*, which form a brush. On the free end of each basidium is a long row of spores, as though

Fig. 60.



Penicillium. (Lehmann.)

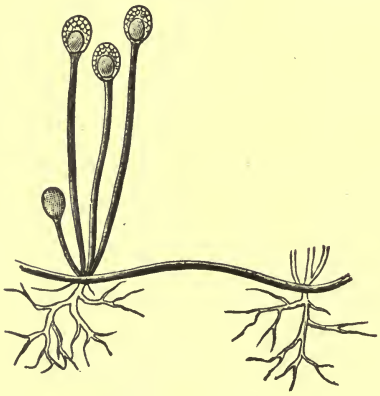
the end of the basidium had segmented into small globules. *Penicillium glaucum* is the example of this class. It is found widely distributed in nature, and is the most common mould. Its growth on bread is at first white, but as soon as sporulation occurs it assumes a greenish color.

Mucorini or globular moulds: These moulds (Fig. 61), although very common, are not met with so often as the preceding group. Their growth is white. The end of the sporangium enlarges to form a globular bulb, which is partitioned off into several compartments, each of which contains

a large oval spore. The bulb is enclosed in a cap, the *calumella,* which remains open when the spores ripen and become scattered.

Oidia or segmented moulds: The structure of these moulds is very simple. They represent a transition-stage between the moulds and the yeasts. At times they are typical moulds, and then again they resemble the yeasts in structure. The sporangium is very indistinct and often appears to be absent. The spores are formed directly from the sporangium or from the mycelium by a process of segmentation similar to that

FIG. 61.



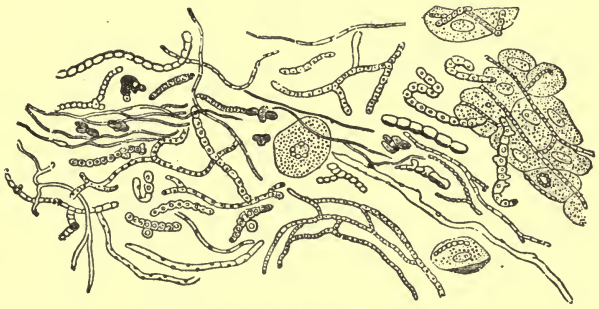
Mucor stolonifer. (Mez.)

seen in the penicillii. *Oidium albicans* or *thrush fungus* is the type of this class. *Oidium lactis* is the cause of sour milk and rancid butter.

The **actinomyces** has heretofore been considered a fungus, and because of its peculiar appearance was called the ray fungus. It is still often referred to as the streptothrix mould or fungus, but there can be no question that this organism belongs to the higher bacteria, and not to the moulds. It is really a transition-stage between the filamentous moulds and the bacteria or fission fungi. Hektoen thoroughly investi-

gated this organism some years ago, and found that in culture it very closely resembled the tubercle bacillus. Since then many bacteriologists look upon these two varieties and also the glanders bacillus as streptothrixes or mycobacteria. Other members of this group are *Streptothrix maduræ*, *Streptothrix farcinæ*, *Streptothrix Fœrsteri*, and *Streptothrix pseudotuberculosa*.

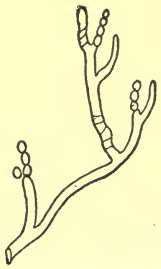
FIG. 62.



Achorion Schoenleinii. (After Kaposi.)

Moulds which closely resemble the mucorini are the **Achorion Schoenleinii** (Fig. 62), the cause of favus; **Tricophyton**

FIG. 63.



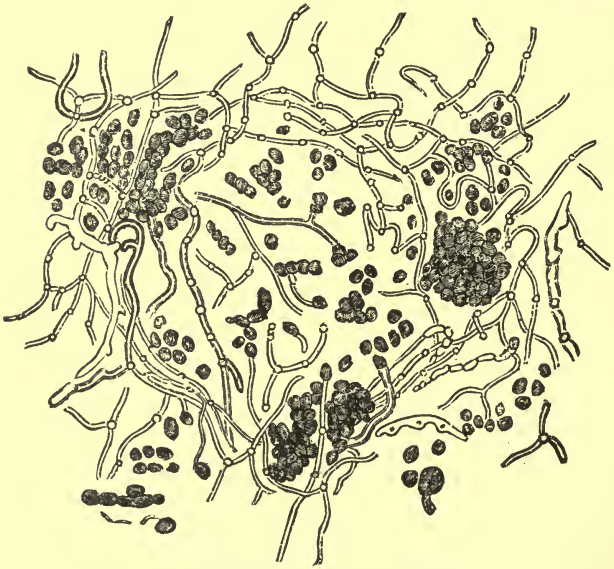
Trichophyton tonsurans. Diagrammatic. (Lehmann.)

tonsurans (Fig. 63), the cause of herpes tonsurans; and **Microsporon furfur** (Fig. 64), the cause of pityriasis versicolor.

The filamentous fungi are **examined** best in an unstained

condition, as they do not take stains well. A small portion of the mould colony is rubbed up gently with 50 per cent. alcohol containing a few drops of ammonia, and then mounted in glycerin. The cover-glass is rimmed with Tarrant's balsam

FIG. 64.



Microsporon furfur. (After Kaposi.)

or sealing-wax. A permanent preparation may also be made in *Unna's solution:*

Gelatin,	1 part;
Alcohol,	25 parts;
Solution of ammonia,	25 "
Glycerin,	25 "
Water,	35 "

Unna recommends that the cover-slip preparation be placed in 5 per cent. potassium hydroxide for one minute, rinsed in

water for five minutes, and then placed in a 5 per cent. acetic acid solution for a few minutes. They are stained with a strong anilin gentian-violet stain. The staining is greatly facilitated if the stain or the preparation covered with stain is heated gently.

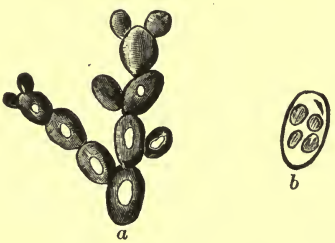
Moulds are **cultivated** just like the bacteria, except that the medium must have an acid reaction. Bread-pap is a most useful culture-medium, and is used very widely for this purpose. The aspergillus will outgrow any of the other moulds, and in order to get a pure culture of any one mould it will be necessary to inoculate an animal by injecting the mould in water into the abdominal cavity. The different varieties will separate and form colonies as the bacteria do on a plate or Petri dish culture. There is no necessity, however, for making pure cultures of the filamentous fungi, as they can be studied in a mixed culture just as well.

CHAPTER III.

YEASTS; BUDDING FUNGI; SACCHAROMYCES.

THE yeasts are known also as the **blastomyces** group of fungi. They, like the bacteria, contain no chlorophyll. The yeast-cells are slightly round, and multiply by budding like a tuber on a potato. They are the cause of alcoholic fermentation in sugar. The bud looks like a small sprout, and is detached finally from the parent cell to take on the functions

FIG. 65.



a. Saccharomyces. *b*. Cell with four spores. (Lehmann.)

of a matured cell (Figs. 65 and 66). Under certain conditions some of the yeasts may form hyphæ and mycelia. This usually occurs when the medium has an alkaline reaction or when it is deficient in sugar. The yeasts grow best at the room temperature and in the presence of oxygen. In fact, most of the budding fungi are strongly aërobic. The culture-medium must contain sufficient organic matter and must have an acid reaction. Putrefaction inhibits their growth. They are *cultivated* like the mould fungi. They can be *examined* in water or bouillon, or mounted in glycerin.

The most common and best known yeast is **Saccharomyces**

cerevisiæ or *beer yeast.* This variety has been found in the coating of the tongue, in vomited matter, in diarrhœal stools, in the vagina, and in diabetic urine. There are said to be several varieties of the cerevisiæ, each of which gives the malt product a characteristic taste. Brewers cultivate the

FIG. 66.

Saccharomyces albicans. (Grawitz.)

yeasts with this end in view, and the different kinds of beer are produced in this way.

The yeast which forms the mouldy growth on wine-preserves, and sour kraut is the **Mycoderma vini** or *Saccharomyces mycoderma.* Several of the yeasts produce pigments.

Prominent among the PATHOGENIC YEASTS are **Saccharomyces hominis,** which was found in a case of pyæmia; and **Saccharomyces subcutaneus tumefaciens,** found in a large myxomatous tumor of the thigh.

Yeasts have been observed in the blood, sputum, and urine

of a typhus patient; in pus from a case of otitis media; in the pseudomembranous angina occurring in a typhoid patient.

According to Demme, **Saccharomyces ruber**, occurring on red raspberries, was the cause of a severe epidemic of intestinal catarrh.

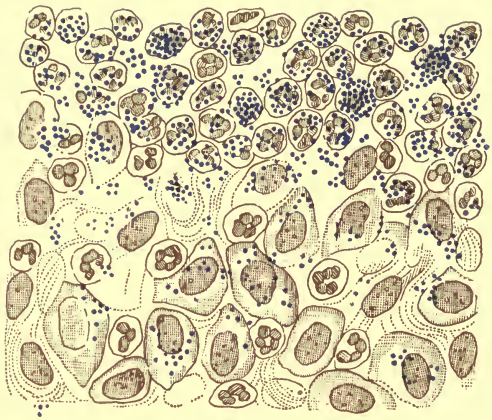
Busse, in 1895, found a pathogenic blastomyces in a large swelling of the tibia, which on microscopic examination showed a **sarcomatous** structure. The fungus was obtained from the tumor and isolated in culture. The blastomyces nodules resemble sarcomatous growths very closely.

The yeast-cell may be mistaken for a tumor-cell inclusion, which is seen so frequently in **malignant growths.** This finding is the reason for the belief that the blastomyces is the cause of malignant growths. The conclusions arrived at are based on a series of experiments conducted by several prominent pathologists, but a careful and unbiased analysis of their results forces us to the conclusion that the occurrence of yeasts in malignant growths is purely incidental and accidental, and should not be looked upon as an etiologic factor or even as a contributing cause.

Gilchrist, in 1884, and other investigators later, found the blastomyces or yeast fungus in certain peculiar **skin** affections. The pathology was that of scrofuloderma, a chronic diffuse inflammation. The yeast fungus was obtained in each case and cultivated. The disease is known as cutaneous blastomycosis or **blastomycetic dermatitis.** We mention these facts in order to impress the reader with the importance, clinically, of the yeast fungi, and to encourage research work in this direction.

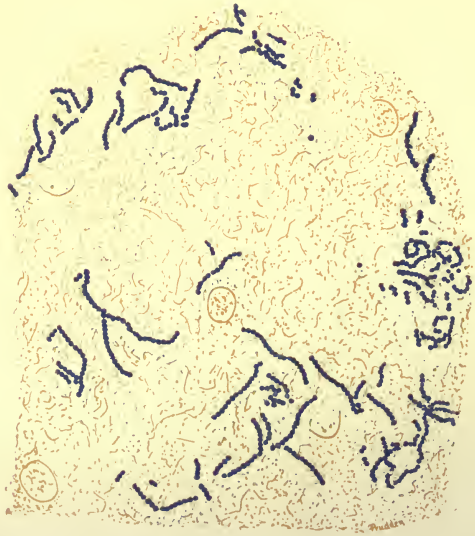
PLATE II.

FIG. 1.



Section through wall of abscess, showing staphylococcus pyogenes aureus. (Baumgarten.)

FIG. 2.



Streptococcus pyogenes. Streptococcus erysipelatis. (Prudden.)

PART III.

PATHOGENIC BACTERIA.

CHAPTER I.

SUPPURATION—PUS COCCI.

Suppuration, or the formation of pus, is not necessarily the result of bacterial activity. It may represent tissue-reaction to irritants other than bacteria, or to both combined. When suppuration is due to micro-organisms, it is called a specific process; when due to other agents, it is called a non-specific process. Turpentine, croton oil, carbolic acid, ammonia, and very strong solutions of mercuric chloride cause pus-formation. Identical results can also be produced by injecting cultures of the pus-forming bacteria that have been sterilized for two hours or more; by injecting sterilized products of bacterial activity, such as the albumoses, enzymes, etc. Some of the vegetable alkaloids, and any chemical substance which exerts a chemotactic action on the leucocytes or which induces necrosis of tissue, will cause a typical suppuration. No matter whether the pus is the result of the action of bacteria or chemicals, the composition of the pus is the same, except that non-specific pus does not contain bacteria and is not infectious.

Before the nature of bacteria and their part in the production of disease were thoroughly understood, pus-formation was looked upon as a necessary step in the healing of wounds and in the resolution of the infectious diseases. The physician did all in his power to facilitate the occurrence of suppuration, and to have as much pus as possible, so as to hasten

healing or recovery. Pus was taken to be significant of the discharge of the poison which was responsible for the infection, and the sooner this poison was gotten rid of the better. The wound could not heal until suppuration had occurred. In lobar pneumonia pus in the sputum was anxiously looked for, and its appearance hailed with delight. The advent of antisepsis completely revolutionized the treatment of wounds, and the physician now does all in his power to prevent suppuration. The occurrence of suppuration is considered a mark of inefficiency on the part of the attendant, and is known to interfere with and even prevent the healing of wounds. All the surgeon's efforts are directed toward the prevention of sepsis. Sterilization and disinfection are carried out so carefully and thoroughly that pus-formation or infection with the pus bacteria is a rather infrequent occurrence.

Many bacteria are responsible for the formation of pus, but those which are classed particularly as the **pus-producing organisms**, and which do not necessarily produce any general infectious disease, but only localized suppurations, are:

The pus cocci—the staphylococci and streptococci;
Bacillus pyocyaneus; blue-pus bacillus;
Micrococcus gonorrhœæ, or gonococcus;
Diplococcus pneumoniæ, or pneumococcus;
Bacillus of Friedlaender, or pneumobacillus;
Diplococcus intracellularis meningitidis; meningococcus.

Other bacteria which have been found in suppurative lesions are *Bacillus typhosus*, *Bacillus coli communis*, and the various organisms belonging to the so-called "colon group."

Staphylococcus Pyogenes.

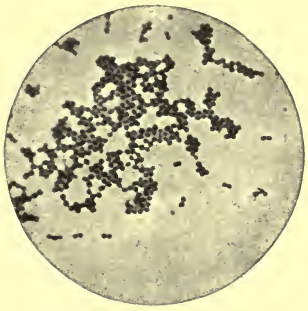
The **pyogenic staphylococci** are divided into several kinds depending upon their color-production. We have *Staphylococcus pyogenes aureus*, *Staphylococcus pyogenes albus*, *Staphylococcus pyogenes citreus*, *Staphylococcus cereus albus*, and *Staphylococcus cereus flavus*.

Habitat: The staphylococci are widely distributed in nature, but are never found in very large numbers in any

one locality. With but few exceptions they are always *parasitic.* They are always found on the surface of the body, in the mouth, nose, eyes, and ears, beneath the finger-nails, in the saliva, and occasionally in the feces; in the dust of the street, on the floors and walls of houses and hospitals, and wherever they may have been deposited from a previous infection. Occasionally they are found in the air and in water.

Biology and morphology: The staphylococci are from 0.7 μ to 1.2 μ in diameter, and have an arrangement resembling a bunch of grapes (staphylos), from which they derive their name. The typical grouping is seen only in preparations

FIG. 67.



Staphylococcus pyogenes. (Park.)

made directly from pus (Fig. 67). In culture specimens the staphylococcus occurs usually as a simple micrococcus, sometimes in masses, and sometimes as a typical staphylococcus. Staphylococci are not motile, and have no flagella. They divide by fission.

The entire group grows very readily on all the various culture-media, and equally well in the presence or absence of oxygen, but pigment is formed only in the presence of oxygen. The temperature optimum is that of the body, 37° C.; but they exhibit some growth in a temperature as low as 6° C. and as high as 44° C. They are stained readily with all the anilin dyes, and also by Gram's method.

On *gelatin plates* small round granular colonies, with a sharply defined border and a whitish-gray color, are seen to develop within forty-eight hours. The gelatin is gradually liquefied around these colonies. The colony is high in the centre and gradually slopes down toward the periphery. It is very thin at the edge. It resembles a small pile of sand. After a few days the golden-yellow color appears, beginning at the centre and gradually spreading toward the periphery. The color is not so pronounced in plate colonies as in tube cultures. The structure of these colonies is studied with the low power of the microscope.

On *agar-agar plates* the appearance of the colonies is similar to that of the colonies on the gelatin plates, but there is no liquefaction of the medium and the pigment is more intense.

In the *gelatin tube* culture the growth occurs along the entire length of the stab, with rapid liquefaction of the medium in the form of an inverted cone. The culture gradually settles to the bottom of this cone, where pigment-formation is evident. The supernatant liquid is always cloudy but not colored. Pigment-formation is not well marked in gelatin tube cultures.

On *agar stroke* cultures pigment-formation appears early and is very intense, beginning at the centre of the culture. The growth is limited to the needle track, and is very heavy, moist, and shining, with well-marked and quite regular edges. At first the growth is white, but in a few days the orange pigment begins to appear, especially if the tube is kept in a light place, with a plentiful supply of oxygen.

On *potato* the growth resembles that on agar, but it is not limited to the line of inoculation. It frequently forms a thick moist membrane that covers the entire surface of the potato. The culture gives off a peculiar sour odor.

Bouillon rapidly becomes clouded, but without the production of pigment.

Milk is coagulated, with the production of lactic and several other acids.

The production of the various *pigments* is not constant; the staphylococci varying in this respect. The cultures may

remain white, or when transplanted a white growth may become yellow.

Vitality: The staphylococcus is an exceedingly tenacious germ, retaining its vitality for a long time under the most adverse circumstances. It is rapidly killed by exposure to live or streaming steam and by a 3 per cent. solution of carbolic acid. Sutures contaminated by the staphylococcus are sterilized in one minute by a 3 per cent. solution of formaldehyde.

Pathogenesis: All the staphylococci cause local suppurative inflammations, and exhibit but little tendency to spread. Occasionally they are the cause of fatal septicæmia or pyæmia, especially when they find their way directly into the blood or lymph-current, or when the process is very virulent and accompanied by rapid absorption of the poisonous products of the germs.

These cocci usually gain entrance into the body through an abrasion of the skin or mucous membranes. Infection of the ducts of the glands in the skin results in the formation of a *carbuncle* or a *furuncle*. It is said that infection cannot occur unless the skin is broken, and yet infection may occur through an unbroken mucous surface, as in staphylococcus sore throat. Staphylococcus infection is not a very serious affair, because of the tendency of the process to remain localized.

The staphylococci are found in all abscesses (except in cold abscesses, which are sterile) and phlegmons, impetigo, ecthyma, acute suppurative inflammations of the nose, throat, and mouth, empyema, tonsillar abscess, phlyctenular conjunctivitis, suppurative inflammations of the middle ear, pelvic abscess, and generally in all those conditions that are described as localized suppurations, and in the mixed infections.

Staphylococcus pyogenes aureus: This is the most common and also the most virulent of the staphylococci. It is considered the type of the group. It is also called the *golden coccus*, because of the beautiful golden or orange pigment which it produces in culture. It is always found in the pus of acute abscesses.

Staphylococcus pyogenes albus: So far as its appearance and

growth are concerned, this germ is exactly like the staphylococcus aureus, except that it does not produce pigment. Its growth is always whitish. It is found everywhere, but possesses little virulence. It is usually the cause of long-standing suppurations, such as the suppuration occurring in a fistulous tract or a chronic otitis media. Welch described a somewhat similar organism, which he called the *Staphylococcus epidermidis albus*. It is constantly found both on the skin and in its deeper layers. He believes it to be an attenuated form of the *Staphylococcus pyogenes albus*.

Staphylococcus pyogenes citreus: This organism is also identical with the staphylococcus aureus, but in culture produces a lemon-yellow pigment. It is very uncommon, and is always associated with the other varieties of staphylococci.

Pathogenesis: These three varieties of staphylococci are always associated. In very acute suppurations the yellow coccus predominates; and in chronic suppurations the albus predominates. The citreus holds the middle ground. It is not so common as either of the other two. It is less pathogenic than the aureus, but more so than the albus.

Staphylococcus cereus albus and flavus: These two varieties are very uncommon. They have been found on the skin and in the external auditory canal. They do not liquefy gelatin and are very feebly pathogenic. They resemble the other varieties of staphylococci both in appearance and culture, but do not liquefy gelatin. The first named produces a waxy white growth and the other a waxy yellow growth.

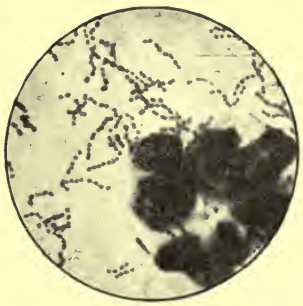
Streptococcus Pyogenes.

Biology and morphology: This organism is identical with the *Streptococcus erysipelatis of Fehleisen*, which was at one time considered a distinct species. It is from 0.4 μ to 1 μ in diameter, and always forms chains, from which characteristic it derived its name of the "chain coccus" (Figs. 68 and 69). These chains may be long or short, and a *streptococcus longus* and *brevis* may be distinguished. It stains well with the anilin dyes and also by Gram's method. It is not motile and has no flagella. Reproduction takes place by fission. Occasionally

some of the individual members of a chain are larger than the others. This is responsible for the belief that possibly these larger cocci are arthrospores. The name *streptococcus conglomeratus* has been given to snarls of chains.

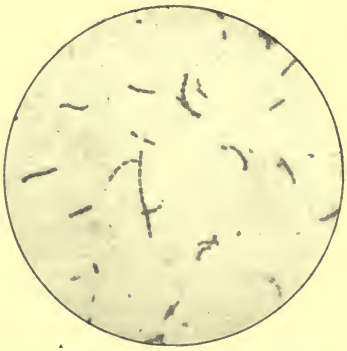
Unlike the staphylococcus, the streptococcus is not grown readily in culture. That is accounted for by the fact that it is an exceedingly virulent germ, possessing but little vegetative power. In order to preserve the culture, frequent transplantations are made, and this is always done at the expense of the virulence of the coccus. It becomes considerably attenuated, but the lost virulence can be regained by rapidly

FIG. 68.



Streptococci in peritoneal fluid, partly enclosed in leucocytes. × 1000. (Park.)

FIG. 69.



Streptococci in throat exudate smeared on cover-glass. × 1000. (Park.)

passing the coccus through rabbits until a very high degree of toxicity is obtained (Fig. 70). Marmorek says that the original virulence can be retained if a culture-medium consisting of 3 parts of human blood-serum and 1 part of bouillon is used, or ass' milk, ascitic fluid, or the fluid from a pleural effusion. The virulence of individual organisms is apparently subject to marked variations. The requirements as regards temperature are the same as those of the staphylococcus. It is a facultative anaërobe.

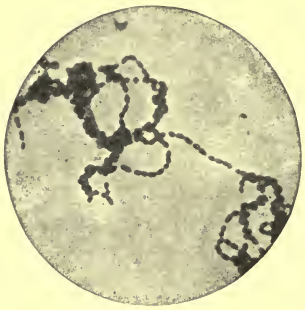
On *gelatin plates* the streptococcus forms very small, finely granular, translucent colonies of a very light color. They

are perfectly flat, round, with numerous fine projections at the periphery, due to chains of cocci which reach out from the colony into the medium. The gelatin is not liquefied. The appearance of the colony under the microscope is quite characteristic.

In *gelatin tube* cultures the growth is formed along the line of inoculation. It is very slight, and consists of many very small distinct spherical colonies that resemble the colony on the plate. They never become confluent. There is no liquefaction of the media. The surface growth is very slight.

In *agar-agar strokes* a very similar growth develops as in the gelatin, but not so rapidly, and the colonies are almost translucent. Sometimes the growth is so slight that the col-

FIG. 70.



Streptococcus. (Park.)

onies resemble minute drops of water. The growth is more rapid on glycerin-agar than on ordinary agar.

On *potato* the growth is almost invisible.

Bouillon becomes slightly cloudy and contains a flocculent precipitate. It is in the bouillon cultures that the variations in the formation and lengths of the streptococcus chains are seen. They are probably only cultural characteristics.

The growth on *blood-serum* is almost identical with that on agar. *Milk* is coagulated.

Vitality: An exposure of ten minutes to a temperature of 52° C. kills the coccus. It is more resistant to chemicals.

A 1 : 2500 bichloride solution is fatal in two hours; 1 : 300 carbolic acid, in two hours; 1 : 50 peroxide of hydrogen, in two hours.

Pathogenesis: The streptococcus is the cause of all severe and rapidly fatal inflammations, especially those of the lymphatic system, the so-called "spreading" inflammations. The germ has been found in hospital wards and in operating-rooms, in the mouth, nose, pharynx, intestinal canal, vagina, on the skin, and in the lesions caused by it. Infection occurs in the same manner as with the staphylococcus.

The streptococcus pyogenes is the specific *cause* of *erysipelas* (St. Anthony's fire), an acute inflammation involving especially the subcutaneous tissues, and it is always found in the erysipelatous patch, particularly at its periphery. It occupies the lymph-spaces and lymph-vessels of the skin, and subcutaneous tissues, in great numbers. The streptococcus found in erysipelas was formerly known as the *streptococcus erysipelatis* of Fehleisen; the term has been abandoned, as it is no longer recognized as a variety of streptococcus.

It is always found on the *heart valves* in ulcerative endocarditis; and sometimes in the *blood* in pneumonia, otitis media, meningitis, phlegmons, and the secondary infection in pulmonary tuberculosis.

It is always present in the uterus in *puerperal fever*, of which it has been said by some to be the specific cause. Puerperal fever is so extremely liable to follow infection with the streptococcus that physicians who have a "pus case" under their care, or any streptococcus infection, always refuse to take charge of obstetric cases during that time.

It is found very commonly in the diphtheritic membranes in the throat, and in non-diphtheritic angina, especially that of scarlet fever; in the blood of scarlet fever patients, and in the suppurative sequelæ of scarlet fever.

The streptococcus can always be obtained in *pure cultures* from the bleb or blister over an erysipelatous patch; or by introducing a fine needle into the subcutaneous tissues and withdrawing a small quantity of serum.

Serum: Marmorek has succeeded in obtaining an **antistreptococcus serum** which possesses a decided specific action

on all streptococcus infections. The serum is prepared by immunizing an animal to live cultures of a very virulent streptococcus. The streptococcus toxin is a diastase which is destroyed when it is exposed to a temperature of 70° C. Although all the streptococci are believed to be of one common species, the antistreptococcus serum apparently possesses bactericidal properties only for the streptococcus from which it is prepared. Many observers are of the opinion, however, that the serum of any one streptococcus antagonizes all streptococci more or less. The serum should always be kept in a cool dark place, as it deteriorates very rapidly.

Marmorek's observations have been confirmed by many reliable investigators. The serum has been *used* in scarlet fever, erysipelas, puerperal fever, tonsillitis, post-operative septicæmia, phthisis, and bronchopneumonia, with very gratifying results. It should be used only in suitable cases, and the serum must be fresh. A serum which is more than six weeks old should not be used, as it is practically inert after that time.

The daily *dose* varies with the severity of the condition. Ordinarily from 20 to 50 c.c. of the standardized serum can be used daily without exhibiting ill effects.

Some very excellent results have been obtained with Marmorek's serum in *streptococcus pneumonias.* It is practically devoid of effect in the pure pneumococcus pneumonia or in the pneumonia complicating la grippe or typhoid. In the stage of mixed infection in *phthisis,* the ulcerative stage, this serum can be used with benefit.

It may be of interest to refer briefly at this time to **Coley's serum.** It was noticed by a number of clinicians that an accidental infection of malignant tumors with *Streptococcus erysipelatis* was in some instances followed by a complete disappearance of the tumor. Coley verified these findings experimentally. He found further that the toxin of the streptococcus was preferable to the living culture. Also, that when mixed with *Bacillus prodigiosus* the efficiency of the serum is increased considerably.

Flasks containing slightly acid bouillon are inoculated with a virulent culture of the streptococcus obtained from the ery-

sipelas lesion, and are placed in the incubator for three weeks. The same flasks are then inoculated with *Bacillus prodigiosus* and replaced in the incubator for ten or twelve days. At the end of this time the flasks are well skaken and their contents poured into bottles of about one-half ounce capacity. These bottles are exposed to live steam for one hour. The toxin is injected directly into the tumor mass or into its periphery.

The best results from the use of this combined toxin have accrued in the treatment of accessible *sarcomata.* The injection is followed by necrosis and a gradual disappearance of the tumor. Quite a number of cases have been successfully treated in this manner. The serum has, however, proved most successful in the hands of its originator. The experience of the vast majority of the profession with its use has not warranted its continuance, and it has now been practically abandoned except by a few. Its use is restricted largely to inoperable cases, with the hope that some good may result.

Bacillus Pyocyaneus.

Strictly speaking, this is not a pus-producing germ, but it is frequently found in pus, to which it imparts a blue or green color. For this reason it is always described with the pus germs. It has also been found on the skin, especially in the axillæ, in the external auditory canal, and in the intestinal mucus. It is possible that very large numbers of the organism may induce suppuration.

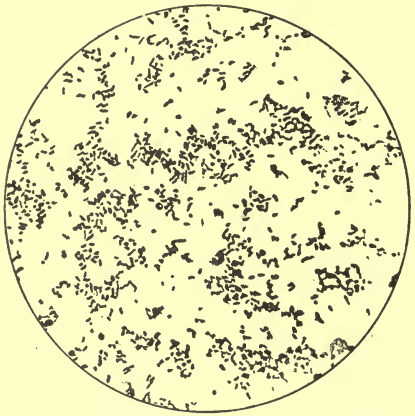
Biology and morphology: The *Bacillus pyocyaneus* is a very small, slender, and exceedingly motile bacterium, with rounded ends, and possessing a single terminal flagellum. It is 0.3 μ in length and 1.2 μ in width, and usually occurs singly, although occasionally it forms short chains of four or five. It does not sporulate, reproducing itself by fission. It is a facultative anaërobe, although developing best in the presence of oxygen. The anilin dyes stain it readily. It is decolorized by Gram's stain. Its temperature optimum is indefinite, as it grows equally well at either the room or body temperature (Fig. 71).

On *gelatin plates* it forms small flat round colonies of a

slightly greenish color. They are very granular, with an irregular border, and exhibit some radiation like a streptococcus colony. The gelatin is rapidly liquefied, the colony sinking into the medium as liquefaction progresses. The colony is darker at its centre than at the periphery.

In a *gelatin stab* culture the growth develops rapidly at the surface and rather slowly along the line of the needle inoculation. The medium is liquefied, the culture settling gradually to the bottom. The liquefaction is not characteristic.

FIG. 71.



Bacillus pyocyaneus, from an agar-agar culture. × 1000. (Itzerott and Niemann.)

The liquefied medium may be colored green or the solid portion blue, or the blue and green color may both be present in the same culture.

On *agar-agar* the growth develops very rapidly along the stroke. The growth remains white, while the medium is colored green because of the formation of fluorescin, a soluble pigment. If the medium contains an excess of peptone, the green color is displaced by a beautiful deep blue. *Bacillus pyocyaneus* forms two pigments, *fluorescin*, a green pigment, and *pyocyanin*, a blue pigment. Pyocyanin is crystallizable.

The growth on *potato* is very luxuriant and of a brownish

color. *Milk* is coagulated. *Bouillon* becomes clouded and is colored green.

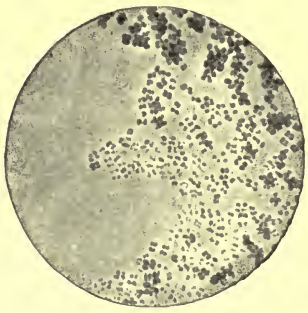
Pathogenesis: In man the organism appears to be purely saprophytic, whereas in animals it is intensely pathogenic, especially when injected into the subcutaneous tissues. It has been found in otitis media, pulmonary tuberculosis, pericarditis with effusion, acute angina, meningitis, bronchopneumonia, dysentery, diarrhœa, etc.

Micrococcus Tetragenus.

This germ belongs to the same class as *Bacillus pyocyaneus*. It is usually found in tubercular septicæmia in association with other bacteria, in the pus of empyema subsequent to pneumonia, and occasionally in the saliva of healthy persons.

Biology and morphology: In the tissues this organism occurs in squares of four cocci (Fig. 72). It is not motile; does

FIG. 72.



Tetracoccus. (Park.)

not sporulate; has no flagella; and stains readily with Gram's and the anilin dyes. It grows well on all the various nutrient media at either room or body temperature. It is a facultative aërobe. It measures about 1 μ in diameter, and is frequently surrounded by a gelatinous capsule.

In *blood cultures* it produces minute white colonies with a somewhat opalescent appearance. They are very finely granu-

lar. In a *gelatin stab* many small colonies form along the needle-track, and on the surface a little projection or button is formed, the typical nail-growth. On *potato* the growth is very luxuriant.

Pathogenesis: The tetracoccus is pathogenic for animals but not for man. It is very fatal for white mice in the laboratory. Lartigau believes that *Micrococcus tetragenus* may be the cause of a pseudomembranous angina. He has observed three cases of this kind. The tetracoccus is frequently seen in specimens of tubercular sputum. Its shape is very characteristic and cannot fail to attract attention.

CHAPTER II.

SUPPURATION (*Continued*).

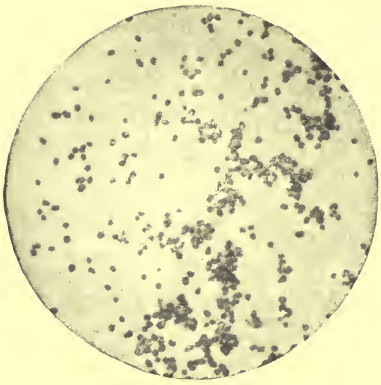
Micrococcus Gonorrhœæ.

Neisser, in 1879, observed an organism in the purulent discharge of gonorrhœa and purulent ophthalmia which he named the **gonococcus.** It was not cultivated and studied in pure culture, however, until 1885. It is seen in pairs, a diplococcus; although occasionally it forms tetrads, probably just after division of the diplococcus. The approximated surfaces of the cocci are slightly concave or flat, and almost touch, giving the organism a rather characteristic appearance, from which it has been dubbed the "biscuit coccus," or, as the Germans say, "semmel-kokken."

Biology and morphology: The gonococcus measures from 0.8 μ to 1.6 μ in length, and from 0.6 μ to 0.8 μ in breadth. It is not flagellated; nor is it motile; and it reproduces itself by binary division. It has never been seen to form spores. It is stained readily by all the anilin dyes (Fig. 73), but is promptly decolorized by Gram's stain. This is a very important point in the differentiation of the gonococcus from the pneumococcus and meningococcus. Methylene-blue is the best stain for specimens made directly from the purulent discharge. Eosin may be used as a contrast-stain. It is a facultative anaërobe.

It is very difficult to grow the gonococcus artificially. The temperature must be that of the body, 37° C., and even then the growth develops very slowly and sparsely. Except for experimental purposes, it is not necessary to make cultures of the gonococcus, as it is easily recognized (Fig. 74) in stained specimens mounted directly from the discharges of a gonorrhœal inflammation.

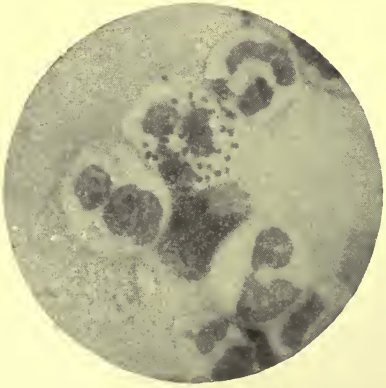
FIG. 73.



Smear from pure culture of gonococcus on agar. (Heiman.)

Plates are made in the usual way, but instead of agar or gelatin, a mixture consisting of equal parts of liquid human

FIG. 74.



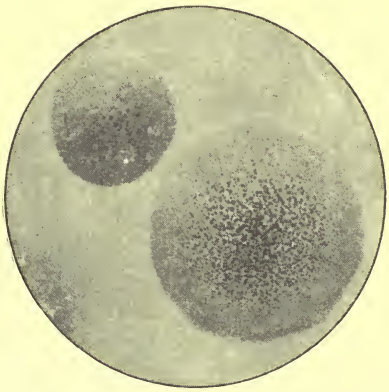
Gonococcus in pus-cells. × 1100. (Park.)

blood-serum and 2 per cent. peptone-agar is used. Within twenty-four hours a few very small dry colonies are seen on

the surface of the medium. These colonies are very finely granular and have a punctate centre (Fig. 75). The deeper colonies are very nodular and are of a grayish-white color. In a few days the colony looks like a blackberry.

On *blood-serum agar* (1 part of liquid blood-serum and 3 parts of agar solidified in the slanting position) a luxuriant growth is formed along the line of inoculation, consisting of individual grayish colonies, which finally coalesce to form a moist, sticky, glistening deposit. 1 part of human blood-serum and 2 parts of peptone-bouillon are also a good culture-

FIG. 75.



Colonies of gonococci on pleuritic fluid agar. (Heiman.)

medium. A pellicle forms on its surface, the medium remaining transparent.

Other media which have been used in the cultivation of this germ are: a mixture of urine and blood-serum; 2 parts of peptone-agar and 1 part of acid human urine; 1 part of ascitic fluid; and 1 part of nutrient glycerin-agar. Wertheim uses a mixture of placental blood-serum and 2 parts of peptone-agar for making pure cultures of the gonococcus. Hydrocele fluid and the serous effusion of pleurisy have been used either alone or in combination with other media. Acid urine or urine mixed with gelatin is also suitable.

The gonococcus cannot be grown on potato, plain gelatin, or agar-agar. It must be remembered that the growth is never very heavy. The results obtained with cultures are so variable that it is impossible to describe any characteristic appearance other than that seen in the plates.

Pathogenesis: The gonococcus is always found in the purulent discharge from gonorrhœal inflammations, and the position of the germ within the pus-cells is absolutely characteristic and a point in diagnosis. It must be borne in mind that gonorrhœal inflammations are not limited to the urethral canal, but may occur in any part of the body. These inflammations are always very serious, and should not be discussed lightly nor in a facetious spirit. In old cases of gonorrhœa it is occasionally impossible to find the gonococcus in the discharges because of its position within the tissue-cells covering the mucous membrane of the urethra or other parts of the body. This enclosure serves as a protecting barrier to the germ. If the gonococcus cannot be found, it is advisable to irritate the mucous membrane with instruments, such as the passage of sounds in the urethra, so as to induce free secretion. If the gonococcus is contained in the cells, such irritation will usually dislodge some cells and the germ will appear in the discharge.

The gonococcus is constantly present in all stages of the disease, and in large numbers during the acute stage. It is also present in the sequelæ of gonorrhœa. It is never present under normal conditions, although a number of organisms which resemble the gonococcus, but which differ as to pathogenicity and also in culture, are frequently found in the vaginal and urethral discharges.

The gonococcus will not develop on healthy mucous membranes. The conditions suitable for its development must exist. Congestion of these membranes furnishes the necessary conditions. Once it has found lodgement on or in a mucous membrane it is exceedingly difficult to dislodge it. The cessation of the purulent discharge is by no means an indication of the disappearance of the gonococcus. The gonococcus is very resistant to heat and chemicals. It also has a tendency to remain latent in the tissues for a long

time, even years, and yet retain its usual pathogenic power. The purulent inflammation may have subsided entirely, and the patient is apparently free from the disease, and yet it is possible to find the gonococcus in the mucous secretions if the membrane is irritated as previously described. A reappearance of the gonorrhœal discharge is not necessarily the result of reinfection, but may be evidence of renewed activity of the dormant germ.

The gonococcus is a very virulent germ. Its toxin is extremely toxic, and treatment must be thorough and continued for a long time to insure a complete cure. The gonococcus is never the cause of abscess-formation. It is said that the germ cannot penetrate membranes covered by columnar epithelium. This belief is apparently confirmed by the fact that urethral gonorrhœa is never met with in the female, the female urethra being lined with columnar epithelium. In gonorrhœa the entire vulva, including the urethral papillæ, is constantly bathed in pus, and yet the disease rarely extends to the urethral canal.

The characteristic biscuit shape, the position of the germ within the cells, and its failure to stain with Gram, make a positive diagnosis of it being the gonococcus justifiable.

Micrococcus Citreus Conglomeratus.

This germ is found in the urethra and vagina in health as well as in disease. It resembles the gonococcus in appearance, but differs in culture. It can be cultivated easily on all the ordinary culture-media, forming a solid yellow growth on solid media. Gelatin is rapidly liquefied. It is stained by Gram's method.

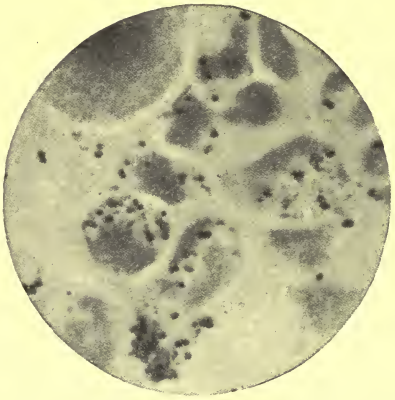
Other organisms which resemble the gonococcus, and which are also found in the vaginal and urethral secretions, are *Micrococcus subflavus*, *Diplococcus albicans amplus*, and *Diplococcus albicans tardissimus*. They are all stained by Gram's method, are not found within the cells, and rapidly liquefy gelatin. They form luxuriant growths on all ordinary media. We mention these various organisms for the purpose of emphasizing the importance of making an absolute diagnosis as

to the exciting cause in all cases of purulent inflammations of the urethra and vagina. The condition may be due to one of these germs and not to the gonococcus.

Diplococcus Intracellularis Meningitidis.

Meningitis may be due to a variety of organisms. There are streptococcus meningitis, tubercular meningitis, pneumococcus meningitis, and a form which is caused by a specific germ, the *Diplococcus intracellularis meningitidis.* The three varieties first mentioned are usually secondary to disease in some other part of the body; whereas the last is a primary

FIG. 76.



Diplococcus intracellularis meningitidis. × 1100. (Park.)

seropurulent inflammation of the meninges of the brain and cord, which was first described by Weichselbaum in 1887. This meningococcus is the specific cause of epidemic cerebrospinal meningitis, and is of special interest because of its resemblance to the pneumococcus and the gonococcus.

Biology and morphology: In form it is a diplococcus of the same shape as the gonococcus, and it is also enclosed within the leucocytes and tissue-cells (Fig. 76). Occasionally it is seen to occur singly or, like the gonococcus, in tetrads or

fours. It is readily stained with the anilin dyes, especially Loeffler's alkaline methylene-blue, and is decolorized by Gram's stain. It does not form spores, has no flagella, and is not motile. It differs from the gonococcus in that it is very easily cultivated.

The meningococcus grows well on *agar-agar* and on *glycerin-agar*, but not on potato, nor in gelatin or bouillon. It grows very luxuriantly on Loeffler's *blood-serum mixture*, but not at all in a mixture of bouillon and blood-serum. It absolutely requires a temperature equal to that of the body. It is a facultative anaërobe.

In *tube cultures* it forms minute round colonies having a very sharply defined regular border. The colonies are shining and almost translucent, and of a grayish-white color. They very closely resemble streptococcus colonies on an agar slant. The medium is not liquefied.

On *agar-agar tubes or plates* deep and superficial colonies are formed. The deep colonies are hardly visible. The superficial colonies are round, flat, and very finely granular, with dentate edges. They are yellowish-brown in color, and darker in the centre than at the periphery.

The meningococcus possesses very little resistance to either heat or chemicals, and dies very rapidly in culture unless frequently transplanted.

Pathogenesis: The meningococcus is pathogenic for man and animals. Weichselbaum concluded that infection occurred through the ear and upper air-passages, especially the nose. He succeeded in obtaining the diplococcus in pure culture from the nasal secretions of one case out of his series of six. It has also been found in the nares of healthy individuals and in a few cases of conjunctivitis. It has been found present in about 50 per cent. of all cases of cerebrospinal meningitis.

The **bacteriologic diagnosis** is made by examining the fluid obtained from the spinal canal by means of *lumbar puncture*. Park says that such a diagnosis is of clinical value, because about 40 per cent. of all cases of meningitis due to this coccus recover, while nearly all the cases due to the pneumococcus and streptococcus end fatally.

Cultures may also be made from the pus obtained from the

spinal canal at the autopsy. A large amount of the spinal fluid should be used, as ordinarily it contains very few living germs.

Lumbar puncture: Have the patient lie on the right side, with the knees drawn up and the left shoulder depressed, the same position which he would assume when squatting on his haunches. The site of puncture, the instruments, and the operator's hands should be carefully cleansed and disinfected. The needle should be of sufficient length—at least four centimeters, and have a long bevel. Locate the interspinous space between the third and fourth lumbar vertebræ and insert the needle slowly, about one centimeter to the right of the median line and directed slightly upward and inward toward the median line. Pressure is continued until the needle enters the subarachnoidean space, which is made evident by the outflow of a few drops of cerebrospinal fluid. About 5–15 c.c. of this fluid are collected in sterile tubes. Care must be taken not to introduce the needle too far, and not to draw blood, which interferes with the examination.

The needle must be introduced without any wiggling, and when it meets with an obstruction it should be immediately withdrawn and inserted again. The puncture is sealed with collodion. Patients experience no ill effects from this procedure.

Diplococcus Lanceolatus.

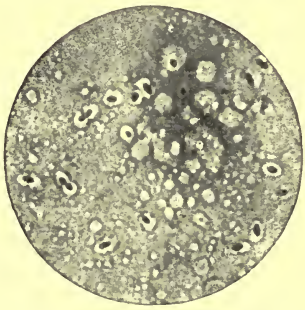
This organism is also known as **Micrococcus lanceolatus.** *Diplococcus pneumoniæ*, and the *pneumococcus* of Fraenkel, It is found in about 75 per cent. of all cases of lobar or croupous pneumonia, and is accepted as the specific cause of that affection, although it has been manifestly impossible to meet all the requirements of Koch's law as to specificity. Its morphology is extremely variable, and hence its many names (Fig. 78).

Biology and morphology: The pneumococcus (as it is usually designated) is an oval coccus which usually occurs in pairs. Sometimes it forms short chains of four or five, when it may be mistaken for the streptococcus. Each coccus has a leaf-

or lance-shaped extremity, but this shape is seen only in the disease product, and not in culture. Here the organism has more or less of an oval or spherical shape. Each diplococcus in its native state is also surrounded by a capsule (Fig. 77). When the germ is cultivated, the capsule is not visible. It is not motile, has no flagella, and does not sporulate. It stains with the anilin dyes and by Gram's method, differing in that respect from both the gonococcus and the meningococcus. It is a facultative anaërobe.

Although the pneumococcus will grow at a temperature as low as 71° F., its temperature optimum is more nearly the

FIG. 77.



Diplococcus of pneumonia, with surrounding capsule. (Park.)

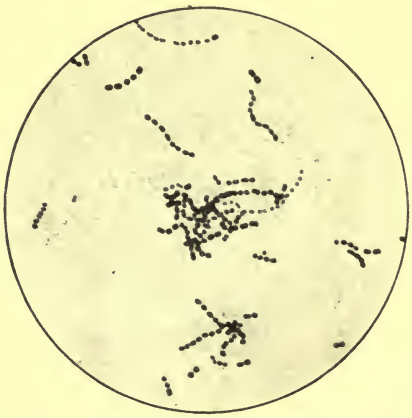
temperature of the body. A ten minute exposure to a temperature of 52° C. kills the germ. The conditions in culture must be absolutely favorable to the development of the germ, as it possesses but little vitality outside of the body.

The pneumococcus was observed first by Sternberg in the saliva of healthy persons. For culture purposes it is obtained best directly from pneumonic sputum. A rabbit is inoculated with the sputum, and after a few hours the germ is obtained from the blood of the animal. Kitasato advises washing the sputum of the pneumonic patient in sterile water until it has been freed from all contaminating organisms, then separating the mass and transplanting its central portion to the culture-medium.

It grows fairly well on all the ordinary culture-media except *potato;* but its growth is somewhat retarded by the formic acid which the organism produces in the course of its development. It is desirable for this reason to have a slightly alkaline medium. Like the meningococcus, it must be transplanted frequently in order to preserve its vitality. It is extremely sensitive to antiseptics and disinfectants.

A mixture consisting of one-third ascitic or pleuritic fluid and two-thirds bouillon is the best culture-medium. When

FIG. 78.



Pneumococcus from bouillon culture, resembling streptococcus. (Park.)

inoculated into this medium and then placed on ice, the pneumococcus will retain its vitality as well as its virulence for months. *Milk* is coagulated rapidly. The high temperature required for the growth of this organism makes it necessary to use a greater percentage of *gelatin* culture-medium ; 15 or 20 per cent. will not melt at a temperature of 24° C. When *plated* in this medium very minute round and finely granular white specks appear. On *agar-agar plates* the colonies are so transparent as to be scarcely perceptible. They are granulated and have a dark central portion.

In the *gelatin tube* culture little whitish colonies form along

the entire stab. They remain distinctly separate. Its limited growth is apparent even in the most suitable medium. On *agar-agar* and *blood-serum* the growth is often overlooked, the colonies are so very small and transparent. *Bouillon* is slightly clouded. Agar-agar may be covered with a thin film of blood-serum and used for a culture-medium. When desirable to increase the virulence of the germ, it must be passed through animals.

The *development* of the diplococcus is *checked* by a 1 : 400 solution of boric acid; 1 : 20,000 mercuric chloride solution or a 1 per cent. solution of carbolic acid destroys its vitality in two hours. In the dry state it retains its virulence for a long time. This is of importance when considering the methods of infection.

Pathogenesis: The pneumococcus is always found in the rusty sputum of lobar pneumonia, and is usually associated with other germs, especially the streptococcus and staphylococcus. It has also been found in meningitis, sore throat, endocarditis, otitis media, acute abscess, and in the sequelæ of croupous pneumonia. The bloodvessels and the lymphatics, especially the latter, carry the germ to other parts of the body from the original site of infection.

Infection usually occurs through the respiratory tract, although in secondary pneumonia the pneumococcus may find its way to the lung through the bloodvessels. But this method of infection is extremely infrequent. The air conveys the germ from place to place, and thus gives rise to epidemics of pneumonia. In order to prevent this dissemination it is absolutely necessary to prevent desiccation of the germ, and this can only be done by receiving the sputum in a proper receptacle containing an antiseptic solution. This solution not only prevents drying, but it also kills the germ. A 2 per cent. carbolic acid solution answers the purpose admirably. Neglect of these precautions is undoubtedly responsible for the so-called house epidemics of pneumonia, several instances of which have been recorded.

In view of the fact that the pneumococcus may be found in the mouths of *healthy individuals*, it is evident that conditions predisposing to infection must exist before the disease

will develop. The presence of the germ under normal conditions would also account for those isolated cases of pneumonia occurring in individuals who have not been exposed to infection, and who have not come in contact with a case of pneumonia. The possibility of contracting the disease has been present all the time and under favorable conditions the germ has become active. A few instances of transmission from the mother to the fœtus through the placenta have been recorded.

Immunity: It is believed by most investigators that immunity after an attack of pneumonia is present in a very slight degree and is of only short duration, or that it never exists. The brothers Klemperer isolated a substance from the serum of immunized rabbits which protected animals inoculated with the pneumococcus. They called this substance *pneumoprotein*. Washburne also prepared an *antipneumococcus serum* by inoculating a horse with virulent pneumococci. It appeared to be superior to the serum of the Klemperers inasmuch as it exerted a protective action in the human being as well as in animals. This question of immunization against the pneumococcus is still largely a matter of conjecture; but further investigation will undoubtedly place the antipneumococcus serum on a firmer foundation, at least so far as the prevention of the disease is concerned.

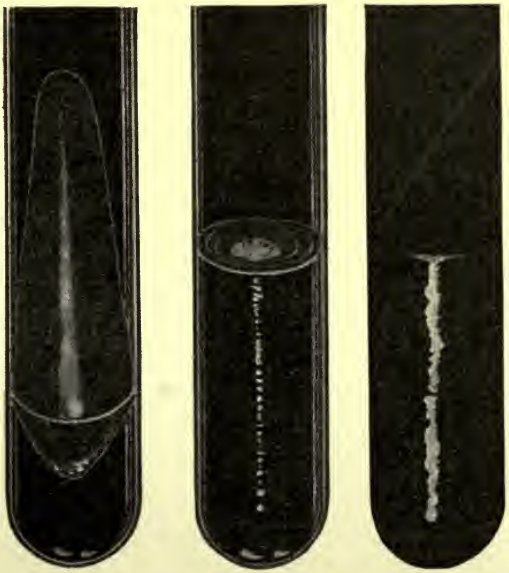
The use of the serum for **therapeutic purposes** has been attended with good results in the hands of a few clinicians; although in the great majority of instances the injection of even a large amount of serum does not appear to have altered the course of the disease in any way. The dyspnœa was lessened, but no other change was noted. It is perfectly harmless even in large doses, so that its use is not attended with danger. Another author, in an experience with 106 cases of lobar pneumonia, found that it lowered the temperature, relieved the pain, ameliorated all the symptoms, and hastened the crisis. The number of cases in which the serum has been used for therapeutic purposes is so small, however, that it is impossible to express any opinion as to its value either as a curative or preventive agent.

Bacillus of Friedlaender.

When Friedlaender discovered this organism in the sputum of pneumonia patients he believed it to be the specific cause of the disease. Further investigation disclosed the fact that

FIG. 79.

FIG. 80. FIG. 81. FIG. 82.



Bacillus of Friedlaender (Colonies).

it is an associated organism in only a very small percentage of the cases of lobar pneumonia.

Morphology and biology: The *pneumobacillus* is a very short bacillus with rounded ends, occurring in pairs or chains. The germ at times is so short as to resemble a coccus, and when seen in pairs it may be mistaken for the pneumococcus. In the sputum it is surrounded by a *capsule*. It stains with all the anilin dyes, but not by Gram's method. It does not sporulate, is non-motile, and has no flagella. It is a facultative anaërobe, growing equally well at the temperature of the body or the room temperature.

On *gelatin plates* it forms minute porcelain-like colonies, which have a very regular outline and are finely granular. On *agar-agar plates* the colonies are much larger, moist, and of a grayish color. In the *gelatin stab* a typical nail growth is seen (Figs. 79–82). The medium is not liquefied. A thick, heavy, moist growth is formed on *agar slants*. On *potato* a very luxuriant yellowish growth develops which soon covers the entire surface of the potato. It does not coagulate *milk*. It causes *fermentation* in media containing grape-sugar or milk-sugar. It produces *aromatics*, especially indol. Gas bubbles are occasionally formed in the gelatin cultures.

Pathogenesis: The bacillus has been found in the mouth and throat of healthy individuals, in the ear, and in the sputum of lobar pneumonia associated with the pneumococcus; also in gangrene of the lung, catarrhal pneumonia, malignant endocarditis, and the conditions already mentioned.

CHAPTER III.

BACILLUS TUBERCULOSIS.

THE **Bacillus tuberculosis** is the specific cause of all tubercular processes. It is one of the most commonly occurring germs, and therefore also one of the most dangerous. As early as 1868 Villemin showed that tuberculosis was an infectious disease, and that it might be produced experimentally by injecting tuberculous matter into healthy animals. Cohnheim later confirmed these findings, and in 1882 Robert Koch discovered the tubercle bacillus and succeeded in cultivating it.

The tubercle bacillus **is found** in all tubercular lesions and in the sputum of patients suffering from pulmonary or laryngeal tuberculosis; in the milk of tubercular cows; in rooms inhabited by tubercular patients (unless proper precautions are taken) who are not careful as to the disposition of their sputum; in food, especially the meat of tubercular cattle; and in the milk of a tubercular mother. It may also be found in the excretions of animals and persons suffering from intestinal tuberculosis; further, in all places and conveyances where tubercular persons expectorate promiscuously, thus favoring desiccation of the sputum and dissemination of the bacillus.

Biology and morphology: The tubercle bacillus is a very slender, rod-shaped organism, 1.5 μ to 4 μ in length, and 0.2 μ to 0.4 μ wide. It has rounded ends, and frequently is slightly curved. It is non-motile and has no flagella. It may be seen in a variety of arrangements. Usually it occurs singly; but it may be paired or form chains of three or four, especially in culture. At times the bacillus may be clubbed at one end, or it may exhibit central bulging suggestive of sporulation. This appearance of sporulation is more

marked in the case of those organisms which stain irregularly and present unstained areas, giving the bacterium a beaded appearance. This variety is usually seen only in old cultures or in the sputum of chronic cases of pulmonary tuberculosis. It probably represents an involution-form or a degeneration of the bacterium. The special spore-staining methods are not applicable. In these cases the peculiar branching or filamentous forms of the tubercle bacillus are also seen. These forms have suggested a relationship between the tubercle bacillus and the actinomyces. For this reason the tubercle bacillus has been placed by some among the streptothrixes, and the name mycobacterium has been suggested as being more correct than the appellation bacillus.

A most remarkable characteristic of the tubercle bacillus, and one which serves to differentiate it from all similar organisms, is its behavior to certain *staining solutions*. It is stained with great difficulty, but once it has taken up the stain it is almost impossible to decolorize it. Koch first used an anilin dye to which he added potassium hydrate. Ehrlich modified this method by staining with an anilin dye to which a saturated aqueous solution of anilin oil was added, and then decolorizing with a strong mineral acid, which removed the stain from everything except the tubercle bacillus. This was followed by a contrast-stain. This method has been modified in various ways by others, but the principle of overstaining and decolorizing with a mineral acid is the same in all the methods. The Ziehl-Neelson method is probably the best. Gram's stain is also applicable, but is not very satisfactory.

It is very difficult to obtain the tubercle bacillus in *pure culture*. It is an obligate parasite, aërobic, and absolutely requires a temperature of 37° C. for its development. It grows very slowly even under conditions favorable to its development. Hen's eggs are an excellent culture-medium for the bacillus. Either the yolk or the white, or both, may be used. Small round white colonies appear in from ten to fourteen days. After the tubercle bacillus has become habituated to being cultivated, it will grow quite readily on veal- or chicken-bouillon.

It is impossible to make a *plate culture* of this germ, because

of its slow growth, and, further, because the associated organisms outgrow the tubercle germ and thus suppress its growth. Except for experimental purposes and for the manufacture of the various tuberculin products, it is not necessary to make cultures of the bacillus; but when a *pure culture* is desired, a special line of procedure must be followed:

A number of animals which are very susceptible to tubercular infection, such as guinea-pigs, are inoculated with the tubercular material, at intervals of one day. Within about four or five weeks the animal inoculated first will die from tuberculosis, which is confirmed at the autopsy. One of the other animals is then killed, and under the strictest antiseptic precautions its abdomen and peritoneal cavity are opened. With sterile instruments the spleen is brought into view, as this is the organ which usually is affected most by the tubercular process. Examine the surface of the spleen for a tubercular nodule and excise it with sterile scissors. This nodule is then compressed between sterile glass slides and transferred to tubes containing blood-serum. Seal these tubes with rubber caps and place them in the incubator. It is perhaps needless to caution the operator never to allow his hands to come in contact with any tubercular material, as infection is extremely liable to occur. Everything should be handled with sterile instruments, so as to avoid contamination of the pure culture of the bacillus.

Pure cultures may also be obtained directly from *tubercular sputum.* The patient's mouth is first thoroughly disinfected. He is then instructed to expectorate into a sterile Petri dish. This sputum is subjected to repeated washings with sterile water until all the bacteria which may have been lodged on the surface of the sputum are removed. The mass is next carefully separated with a sterile needle, its centre removed and placed on glycerin-agar or blood-serum. After two or three weeks small grayish-white scaly colonies appear on the medium. These gradually coalesce to form an irregular scaly membranous growth.

After the bacillus has become habituated to being grown outside the body, it is preferable to perpetuate the cultures on glycerin-agar. The growth on *glycerin-agar* resembles

that on blood-serum, but occurs much more rapidly. In the course of time the whitish color is replaced by a light ochre-yellow. The growth soon spreads to form a heavy dry film which covers the entire surface of the medium and at times extends up the sides of the tube for a short distance.

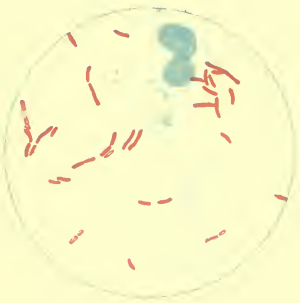
When transplanted into *veal-bouillon* or *glycerin-veal-bouillon*, a membrane resembling that seen on solid media is formed. A characteristic of the growth in fluid media is that development is limited to the surface, leaving the medium perfectly clear. The tubercle bacillus requires oxygen for its growth, and therefore will not grow beneath the surface of the culture.

Cultures may also be made on *potatoes* which are partly submerged in a 5 per cent. glycerin and 0.5 per cent. sodium chloride solution. A heavy film is formed on the potato and on the surface of the glycerin and salt solution. The fluid remains unclouded. Still another medium on which the tubercle bacillus can be grown is a mixture of commercial ammonium carbonate, 5 per cent.; primary potassium sulphate, 0.15 per cent.; magnesium sulphate, 2.5 per cent.; glycerin, 1.5 per cent.

Vitality of the germ: A ten-minute exposure to a temperature of 70° C. kills the germ; 95° C. is fatal in one minute. It is not affected by cold. Direct sunlight is fatal in a very short time, depending on the amount of material exposed. This effect on the germ of the sun's rays is now being made use of in the treatment of tuberculosis. Patients are exposed to the direct rays of the sun in so-called "sun parlors" for as long a time during the day as possible; or exposure is made to the concentrated rays for a short time. The supposition is that the germ will be either destroyed or inhibited in its growth, and that the system will thus have an opportunity to overcome the infection. The germ resists diffused daylight for a week or longer. Desiccation at ordinary temperatures is also resisted for a long time. Dry heat kills more slowly than moist heat. Bacteria placed in water at a temperature of 95° C. are killed in one minute, whereas dry heat at a temperature of 100° C. is often ineffective after an exposure of several hours. The bacilli contained in tubercular sputum are killed by a 3 per cent. solution of carbolic acid in about

PLATE III.

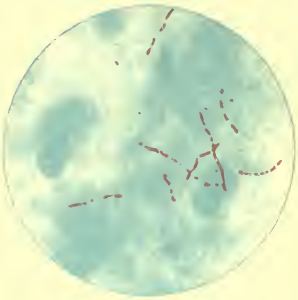
FIG. 1.



Tubercle bacilli, in red.
Strepto-bacilli, in blue. (Park.)

× 1100 diameters.

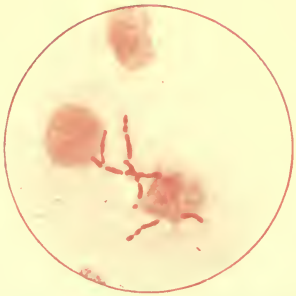
FIG. 2.



Tubercle bacilli, in red.
Tissue, in blue. (Park)

× 1100 diameters.

FIG 3



Very large tubercle bacilli. Cells in specimen are in blue, while bacilli are red. (Park.)

× 1100 diameters.

six hours; by a 5 per cent. solution in about one hour. The germ in the sputum is active for a long time, and infection may occur after two or three months.

Pathogenesis: The tubercle bacillus is the exciting cause of tuberculosis in man as well as in animals. It is a matter under discussion whether the organisms which cause tuberculosis in fish, birds, and cattle, are distinct species from *Bacillus tuberculosis* of man; or whether they are aberrant types of the same; or a modification of this germ due to changed surroundings—a question of adapting itself to its environment. It is a rather remarkable and at the same time significant fact that animals which in their native state are immune to tuberculosis, succumb very rapidly to the infection as soon as they are kept in captivity. The monkey is an excellent illustration of this. Nearly all captive monkeys die from tuberculosis, whereas the wild monkey is immune to the disease.

Guinea-pigs are naturally very susceptible to tuberculosis, and are used extensively for experimental and diagnostic purposes. An injection of a very small amount of tubercular material into a guinea-pig will cause death in about three or four weeks. Autopsy reveals extensive tubercular lesions in which the tubercle bacillus is always found. Tuberculosis in animals can be produced by feeding them with tubercular sputum or other material containing the bacillus; by causing them to inhale a very fine vapor spray in which the bacilli are suspended; and by inoculation into any part of their bodies.

Of the domestic animals, cattle and pigs are the most susceptible. Cold-blooded animals are naturally immune unless the bacillus has first been accustomed to grow at very low temperatures. Birds, with but few exceptions, are immune to tuberculosis.

Pathologic anatomy of the tubercle bacillus: The *tubercle* is the constant anatomic product of the tubercle bacillus. It is seen in all the organs and tissues. Lodgement of the bacillus in any tissue is followed by the production of many new cells, which comprise both regular, fixed, or connective-tissue cells, and modifications of the same—*i. e.*, the so-called epithelioid

or "epithelial" cells. This mass of proliferated cells is surrounded by a zone of leucocytes which collect for the purpose of attempting to stay and limit the infection. The entire mass of cells constitutes a tubercle, and represents a reaction of the tissues to the irritation caused by the tubercle bacillus and its toxins. The tubercle bacillus was given its name because it is the cause of, and is always found in these tubercles. The disease was named tubercle-osis or tuberculosis.

The continued multiplication of the tissue-cells, together with the continued secretion of the tubercle toxins and the growth of the bacillus, finally deprives the cells in the centre of the mass of their nutrition, so that they die from starvation; their death is called necrosis. Such death results in a cheesy mass which is surrounded by successive zones of proliferating cells and protecting leucocytes. Infiltration with lime salts may follow, or encapsulation of the tubercular area, when the disease is said to be cured.

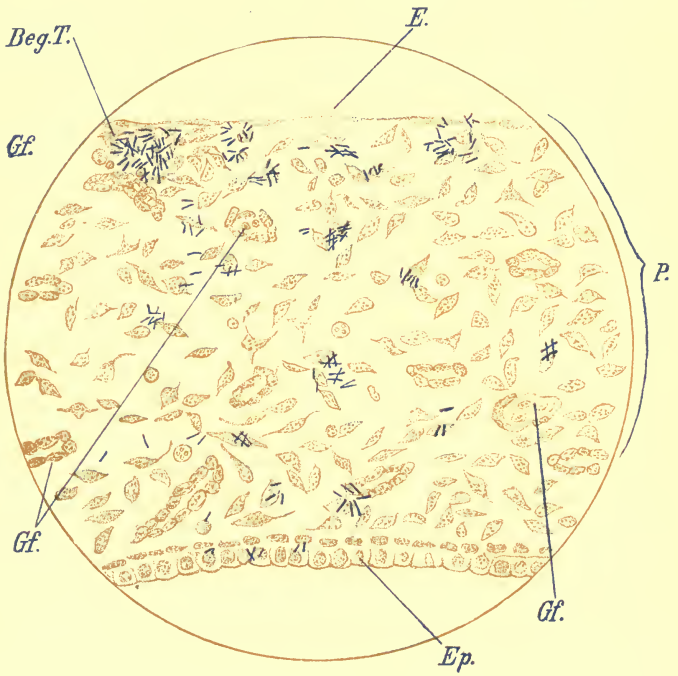
When the tubercle is infected with streptococci, there follow suppuration, the formation of cavities by expulsion of the broken-down material, and dissemination of the process to other parts of the body. Two influences are constantly operative, the resistance of the tissues and the destructive action of the germ and its products. Of these two processes, one or the other always predominates.

Because of their resemblance to a millet-seed the small tubercles are called *miliary tubercles.* The acute form of the disease is manifested by the formation of many such small growths, and is known as miliary tuberculosis. These small tubercles are grayish in color, and translucent and very firm. As they grow larger their centres degenerate, and they become soft and yellow. *Diffuse tubercle tissue* may also be formed. This is also composed of many cells, but does not tend to remain localized, nor does it break down.

In the *acute* tubercular processes the bacilli are very numerous. In the *chronic* lesions they are few in number, and oftentimes repeated examinations have to be made before it is possible to discover the bacillus. This is especially true of sputum examinations.

Infection: By far the most frequent channel of infection is

PLATE IV.



Tubercular eruption in the iris of a rabbit, fifth day after inoculation. Zeiss $^1/_{12}$. (Baumgarten.)

Beg. T. Formation of tubercles beginning (separation of white corpuscles).
E. Endothelium of the anterior surface of the iris.
Ep. Epithelium of the posterior surface of the iris.
P. Iris parenchyma.
Gf. Bloodvessels.

the respiratory tract. The bacillus is inhaled with the impalpable dust in which it is contained. It was formerly believed that all dust contained the bacillus, but this has been disproved by more recent investigations, which show that it is found only in the dust of places frequented by consumptives. It is also possible, although unusual, to inhale the germ directly from a patient when he sneezes or coughs. The mucus which is expelled with the effort may contain the bacillus. For this reason the physician should never stand in front of a patient when he is conducting an examination of the throat or chest. Patients should be urged to cover the face with a cloth or handkerchief when they cough or sneeze. Nuttall says that from one-half to three billion virulent tubercle bacilli are expectorated by a tubercular patient in the course of twenty-four hours. The necessity of extreme precautions to prevent infection, especially of the medical attendant and the patient's family, is very evident.

Persons susceptible to tuberculosis should be exceedingly careful not to increase the liability of infection by living with a tubercular patient or consorting with him longer than is absolutely necessary. The patient should do all in his power to prevent his becoming a nidus of infection for others. Bacilli contained within a small particle of mucus remain alive for a long time, and the danger of infection is much greater from this source than from the bacilli contained in dust which has been exposed to light, and especially sunlight, for some time.

Personal susceptibility is a very essential factor in the contraction of the disease. It is possible for a perfectly healthy individual to inhale the tubercle bacillus without acquiring the disease. His active phagocytes will dispose of the germ. At autopsies the bronchial and mediastinal lymph-glands frequently present evidences of tubercular invasion, although the patients may not have exhibited any symptoms of tuberculosis during their lifetime. This is an evidence of phagocytosis; or of infection with a bacillus which was considerably attenuated. It has been said that 80 per cent. of all persons post-mortemed show evidences of tubercular infection, and yet only (!) one-seventh of the population die from tubercu-

losis. Of course, this percentage of findings would be considerably less if it were possible to hold autopsies on all the dead. The existence of these lesions, without actual evidence, clinically, of disease, is sufficient to warrant the opinion that *infection with attenuated organisms* does occur, and that even when the bacilli are virulent the healthy body is able successfully to ward off the infection. Individual predisposition is of great importance.

Persons *not naturally susceptible* to tuberculosis may be deprived of their resistance to the disease by any of the causes which reduce resistance to infection in general. Diabetes and tuberculosis are rarely found associated. This is perhaps due to the large amount of sugar in the tissues of the diabetic.

When infection occurs through the *respiratory tract*, the infection usually remains localized in the lungs, but the organs adjacent to the lungs may sooner or later also become involved. General infection occurs when the tubercular lesion ruptures into a bloodvessel.

The healthy *nasal mucosa* appears to offer considerable resistance to tubercular infection. The *tonsils* are a frequent portal of infection. Tuberculosis of the cervical lymph-glands may have its origin in infection through the tonsils. Pulmonary tuberculosis may be secondary to tubercular cervical adenitis. Kissing a tubercular person should be refrained from, nor should a tuberculous individual ever be allowed to kiss a child. This interdiction applies to the parents and members of the family as well as to strangers.

Facial tuberculosis, or *lupus*, as it is more commonly called, is due to infection with the tubercle bacillus through the skin. Infection never occurs through the unbroken skin, but always at the site of a wound or an injury. Acne pimples may serve as an infection atrium. The surgeon is extremely liable to skin infection. Inoculation at autopsies has also been reported. These *anatomic tubercles* usually contain very few bacilli. The subcutaneous injection of dead tubercle bacilli results in the formation of an abscess.

That primary infection may occur through the *gastro-intestinal tract* is true. This may follow the ingestion of

tubercular food, such as the meat of tubercular cattle, or through the drinking of milk containing the bacillus. Intestinal tuberculosis or tuberculosis of the mesenteric and retroperitoneal lymph-glands is seen more frequently in infants than in adults. The infection may occur through the mother's milk, or through cows' milk if the child is being raised on the bottle. The large number of cases of tabes mesenterica in children confirm this statement. Primary intestinal tuberculosis in adults is not so common. The infection is usually a secondary one, and is the result of the swallowing of tubercular sputum. The danger of infection following the ingestion of tubercular meat is not nearly so great since the attention of the public has been called to the necessity of thoroughly cooking all food. Adults are not in the habit of consuming large quantities of milk, nor are they obliged to depend upon milk for their sustenance as is the child. This fact has led many clinicians to make the erroneous statement that primary intestinal tuberculosis is never met with in the adult; that it is always secondary to pulmonary tuberculosis. Yet undoubted cases of primary intestinal tuberculosis are on record.

Milk must be recognized as an important factor in the spread of tuberculosis, and the oft-repeated admonition to sterilize milk before using is not without reason. If a cow is tubercular, no matter where the lesion is situated, whether in the udder or elsewhere, its milk is very liable to contain the tubercle bacillus. A tubercular mother should never be allowed to nurse her child, and when the child is fed on cows' milk the milk should always be rendered sterile before using. From the intestinal canal the infection may spread to other parts of the body.

Heredity: The hereditary transmission of tuberculosis is still a matter of dispute. A few cases have been reported in which infection took place through the placenta; also two cases of placental tuberculosis. These findings have been confirmed experimentally in animals. It is extremely doubtful that infection ever occurs from the father, even when he is suffering from tuberculosis of the testicle or seminal vesicles, although he may infect the wife. It is self-evident,

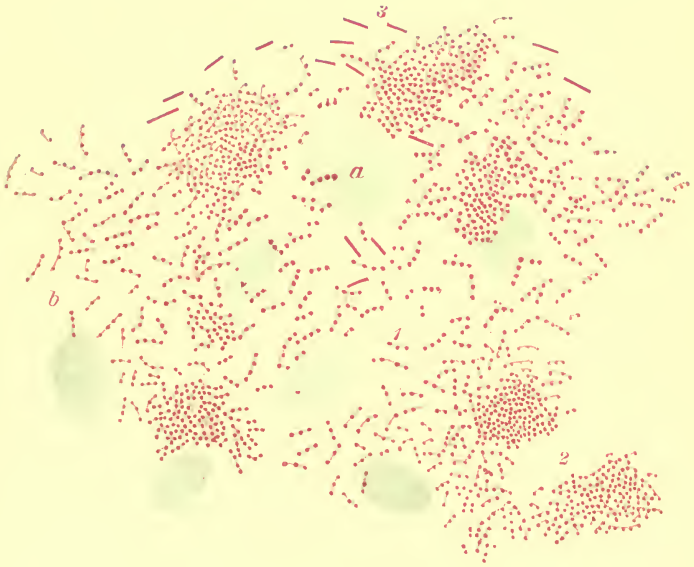
however, that children born of tubercular parents, or of a tubercular father or mother, are predisposed to tuberculosis because of the lack of sufficient vitality to resist that or any other infection.

Baumgarten believes that the disease is inherited directly, but that the great vitality of the tissues of the developing child inhibits the growth of the tubercle bacillus, so that the germ remains latent in the bone-marrow, lymph-glands, and other parts of the body until such time when, either through traumatism or disease, the natural barriers to infection are removed. He and others have found living but inactive tubercle bacilli in the organs of babies born of tubercular mothers. Gærtner is of the opinion that the bacillus is not transmitted to the fœtus by the mother, but that during parturition the bacillus may, in consequence of tears in the placenta, be conveyed to the child. This is the most plausible explanation of those infrequent cases of primary tuberculosis of the skin, bones, joints, liver and other organs, which occur in young children who present no other evidence of tuberculosis.

Mixed infection: The occurrence of mixed infection in tuberculosis is quite common, and materially lessens the chances of recovery from the original infection. This is especially true in pulmonary tuberculosis when a septicæmia is added to the tuberculosis. The organisms which are most frequently associated with the tubercle bacillus are the streptococcus, staphylococcus, *Micrococcus tetragenus*, pneumococcus, influenza bacillus, and *Bacillus pyocyaneus.*

Demonstration of the bacillus: *Sputum:* The demonstration of the bacillus in the tubercular material or tissue is positive evidence of the existence of the disease. Failure to find the germ in stained specimens is not positive evidence of the non-existence of the disease. It should be borne in mind that frequently, especially in the incipient stages of tuberculosis repeated examinations must be made before it is possible to find the organism. It is always well to make five or six stained specimens, and if the bacillus is not found in these, several more should be prepared, and finally the microscopic finding should be substantiated by the injection of guinea-pigs.

PLATE V.



Tubercle bacilli from a tubercular cavity Carbol-fuchsin, nitric acid, methyl-blue. Zeiss $^{1}/_{18}$ O. 4. (Senn.)

A negation should always be withheld until every possible means of finding the germ has been exhausted. A $\frac{1}{12}$ inch oil-immersion lens should be used, together with a mechanical stage, to insure examination of all parts of the specimen.

The sputum is collected in a clean, wide-mouthed, glass-stoppered bottle. If expectoration is very scanty, the sputum should be collected for the entire twenty-four hours, otherwise the sputum expectorated immediately on rising in the morning is taken. The sputum should be examined as soon as possible. One of the small yellow nodules usually contained in tubercular sputum is placed on a clean slide or cover-glass with the platinum needle or with forceps. While selecting these nodules, the sputum is placed on a black background to facilitate selection. The addition of a small amount of carbolic acid to the sputum will coagulate the albuminous masses, and one of these can be selected for examination. In the absence of nodules, several loopfuls of the sputum are placed on the slide. The sputum is spread uniformly and as thinly as possible, dried in the air, fixed in the flame, and stained in accordance with any of the methods detailed in the chapter on staining. In order to obtain the bacillus free from associated bacteria, the sputum should be washed in sterile water as already described.

Feces: A flake of rectal mucus or pus is examined in the same manner as the sputum. It is exceedingly difficult to find the tubercle bacillus in feces, and frequent examinations are necessary.

Urine: The urine is first centrifuged or allowed to settle, and the sediment is then examined for tubercle bacilli. When present they usually appear in bunches or masses. The microscopic examination of urine or any other fluid rarely gives positive results even after repeated and careful centrifugation. It is much more satisfactory and time-saving to inject the suspected material into a guinea-pig and arrive at a diagnosis in that way.

The bacillus can also be stained in *sections of tissue.* The sections are prepared in the manner described in text-books on histology, and are stained by the cold method. Decolorize, counterstain, wash, dehydrate, and mount in Canada balsam.

Milk is centrifuged and the sediment examined for the bacilli. The film is placed first in chloroform for from four to six minutes in order to extract the fat. The chloroform is then evaporated over the flame, and the preparation is stained and mounted in the usual way.

Animal inoculation may often have to be resorted to in the examination of tubercular material before a positive diagnosis can be made. Confirmatory evidence is also obtained by inoculation of animals.

CHAPTER IV.

BACILLUS TUBERCULOSIS (*Continued*).

Prophylaxis against tuberculosis: In view of the bacterial origin of tuberculosis, it may not be amiss to say a few words about prophylaxis. If proper disinfection were practised, it would be possible to stamp out effectually the disease by simply killing all the tubercle bacilli. Prophylaxis should be directed especially against the sputum as the most common source of infection. If all sputum could be rendered innocuous, tuberculosis would be stamped out completely.

The patient should be impressed with the importance of disposing of his **sputum** so that it will not be an element of danger to others. In hospitals and sanitoria it is customary to supply the patient with a spit-cup containing either water or an antiseptic such as carbolic acid. If the sputum is kept wet, it cannot desiccate and be blown about the room every time the door is opened or by the draught caused by women's skirts. These spit-cups are made either of glass or porcelain. They are not very satisfactory, however, as the disinfectant action of the carbolic acid is not very great, and also because of the coagulation of the albumins which it causes. The contents of these spit-cups are usually emptied into a water-closet or they are disinfected by boiling.

A much better spit-cup is the one in use by the Boston Board of Health. It consists of a light metal frame in which a pasteboard box is placed. When the cup is full, or at the end of each day, it is removed, and burnt. This insures perfect disinfection, and the cups are so inexpensive as to be within the reach of everybody (see also Fig. 19).

On the street the tuberculous individual is a source of greater danger than in the house. He should never be allowed to expectorate on the street, nor in any public place or con-

veyance. He should be instructed to spit into a piece of linen or muslin, or an old handkerchief or a Japanese paper napkin, which is burnt at the first opportunity. The patient may, if preferred, carry in his pocket a small wide-mouthed flask, stoppered with a glass or other impervious stopper. The flask may contain water or an antiseptic fluid. Dettweiler's spit-cup is especially designed for that purpose. The patient should be cautioned against swallowing the sputum.

The **room** occupied by tubercular patients should be aired thoroughly every day, and scrubbed with soap and water, followed by an antiseptic solution, at least twice a week. The room should contain as little furniture and hangings as possible. Plenty of sunlight should be admitted. In hospitals, asylums, and prisons it is possible to isolate the tubercular patient. Each patient should have his own set of dishes, and anything coming in contact with the patient or his excreta should either be thoroughly disinfected or destroyed by burning. After his demise the room occupied by him should be disinfected with formalin vapor.

The **excreta** of cases of intestinal or genito-urinary tuberculosis should receive the same care as the sputum in pulmonary tuberculosis. The patient should not be allowed to use the same toilet as the other members of the family. The feces and urine should be voided into vessels containing water, and then be disinfected with lime, or formalin, or sulphate of copper. Sexual intercourse should be abstained from entirely by persons suffering from genito-urinary tuberculosis.

Notification of the health authorities of every case of tuberculosis would do much to prevent spread of the disease. The cases could be watched, and inspectors could visit them from time to time and instruct them in the principles of disinfection so far as pertains to the individual case.

The careful **inspection of cattle** carried out by the government has made infection from the ingestion of tuberculous meat or milk less common than was the case before these preventive measures were adopted. In fact, the only *positive* safeguard against infection through meat or milk is—never to partake of any meat or milk unless it has been thoroughly cooked.

Immunization and Cure.

As soon as it was learned that filtered cultures of the tubercle bacillus contained a substance which was capable of producing the disease in animals, various attempts were made to utilize this substance for the **production of immunity** to the disease. Up to the present time, however, all attempts at immunization have been fruitless, and the question of immunization against tuberculosis is still in *statu quo*. The blood-serum of animals not susceptible to tuberculosis, and attenuated and sterilized cultures of the tubercle bacillus, have been used with the hope of producing a condition of immunity, but without success.

Koch's researches in this direction have been most valuable, and may in the course of time be productive of the desired result. He found that the remarkable pathogenic power of the tubercle bacillus was due to a toxin produced by the germ. Animals were injected at intervals with mixtures containing live tubercle bacilli, with the result that the condition produced after the first injection disappeared. The same was true when dead bacilli were injected. If only the first injection had been given, the animal would certainly have succumbed, but, as it was, some of them remained alive for as long a period as nineteen weeks.

A 50 per cent. glycerin extract of tubercle bacilli cultures produced the same result. Koch named this substance **tuberculin.** It is a proteid substance which is insoluble in absolute alcohol, and resists a temperature of 120° C. for hours. Chemically it resembles the albumins.

Healthy animals do not *react* to subcutaneous injections of tuberculin, even as much as 2 c.c.; but tubercular animals succumb very rapidly to as small a dose as 0.6 c.c. The injection of tubercular animals with very small doses of tuberculin was followed by improvement in the general condition, although complete recovery rarely occurred. Injection into diseased animals is followed by a febrile reaction which is sufficiently characteristic to be of *diagnostic* value.

Tuberculin is *prepared* in the following manner: A very wide 1000 c.c. flask is half filled with veal-bouillon contain-

ing from 4 to 6 per cent. of glycerin. The surface of the liquid is inoculated with a pure culture of the tubercle bacillus and placed in an incubator for from six to eight weeks, when development ceases. The thick dry pellicle which has formed on the surface of the liquid sinks. The bouillon is evaporated over a water-bath to one-tenth of its volume, and filtered through porcelain, gravel, or sterilized filter-paper. The filtrate is tuberculin. It contains from 40 to 50 per cent. of glycerin and keeps quite well.

Koch believed that it was possible to produce *immunity* against the *toxin* but not against the *bacillus*. Tuberculin is not bactericidal. Tuberculin induces coagulation-necrosis in the vicinity of the tubercular lesion, and interferes with the further development of the bacilli, many of them dying. The vitality of the tissue-cells surrounding the tubercular spot is also seriously diminished; there are hyperæmia and degeneration of the tissue, and finally absorption of the poisons into the blood, with consequent liability of spread of the process to other parts of the body.

As a **diagnostic agent** tuberculin is of great value. The subcutaneous injection of tuberculin in doses of from 1 to 5 milligrams into a non-tubercular individual is not followed by appreciable reaction. A like dose injected into a tubercular patient is always followed by a decided reaction, such as increased temperature, headache, lassitude, and at times nausea and vomiting, and chilliness or distinct chills and rigors. In doubtful cases tuberculin is probably the only means at our command for making a positive diagnosis. It is advisable to begin with a very minute dose, in order to ascertain the susceptibility of the patient. Subsequent doses can be regulated accordingly.

Before injection the *temperature-standard* of the patient should first be determined. Therefore the temperature is taken every two hours from 4 o'clock in the morning to 10 o'clock at night, for two or three days prior to the use of the tuberculin. In this way we obtain both the highest and lowest temperature-record of that person, and this temperature-record is a valuable aid in determining the degree of reaction, if any.

The tuberculin is *injected* subcutaneously, either into the flank or between the shoulder-blades, with an ordinary hypodermic or an antitoxin syringe, both the instrument and the site of injection having first been rendered sterile. The reaction usually results in from six to sixteen hours; on an average in about twelve hours. It is advisable to give the injection at midnight, so that the reaction will occur at a time when both the patient and the attendant are wide awake and alert to note any change in the patient's condition. The reaction is then due some time between 12 noon and 4 o'clock in the afternoon.

A feeling of chilliness, headache, lassitude, rise in temperature, and increase in the pulse-rate and respiration, sometimes nausea and vomiting, constitute *the reaction*. An increase of two degrees in the temperature is a positive indication of the presence of tuberculosis. These symptoms usually continue for about thirty hours, and subside gradually. It is best to begin with a small dose, and if no reaction follows, the injection may be repeated with a larger dose at intervals of three or four days until 5 milligrams, the maximum dose, have been administered. If no reaction occurs then, it may be accepted as positive proof that tuberculosis is not present.

If a reaction follows the injection, the same result cannot be obtained again *within at least thirty days*. This has been used as a means of perpetrating fraud in the case of cattle which are known to be tubercular. They are injected with tuberculin, and if another examination is made by the health authorities within the next thirty days their finding will be negative.

The *objection* has been raised that the reaction will occur in individuals in whom the tubercular focus or foci may be encapsulated, and that the injection would under these circumstances be the cause of active manifestations of the disease with possibly fatal results. This objection is based on the fact that the reaction has appeared in persons who exhibited absolutely no symptom of the disease. The autopsy on such individuals would doubtless disclose tubercular bronchial or mediastinal lymph-glands.

Another objection is that the tuberculin may contain viru-

lent tubercle bacilli, and that the disease may thus be produced in persons who, up to that time, have been free from it. The first-named objection is given considerable credence by those whose experience in the use of tuberculin has been very limited. Clinicians who have used tuberculin as a diagnostic agent very freely are outspoken in their opinion that it never produces disease where there is none, nor does it arouse a latent tuberculosis into fatal activity. As to the second objection, that can easily be overruled, because the manufacturers of tuberculin are extremely careful in its preparation, testing it on guinea-pigs before it is marketed. Anders has used the tuberculin-test in a large number of cases, and his experience has been exceedingly satisfactory. The same is true of Wood, who had charge of the Cook County (Illinois) Consumptive Hospital for many years; and of many others.

The **agglutination-test** has also been used as a diagnostic agent, but the results have been such that little can be said as to its value in diagnosis. The agglutination of the bacilli occurs neither constantly nor regularly, and cannot be relied upon.

"TR" and "TO": After a further study of tuberculin, Koch came to the conclusion that its non-bactericidal action was due to the very tough cell-membrane of the bacillus, which prevented the body fluids from exerting any influence upon it. Neither could the toxin elaborated by the bacillus be carried into the tissues in sufficient quantity to cause the formation of an antitoxin. Koch then conceived the idea of breaking up the bacillus, either mechanically or chemically. He ground up the dried bacilli very finely in a glass mortar, and then made a watery extract of the soluble parts of the germ. The operation of fragmenting dried bacilli in this way is attended by great risks to the operator because of the possibility of their inhalation. This watery extract was centrifuged. The sediment he named "TR" (tuberculin residue), and the supernatant clear fluid "TO" (tuberculin ober or upper). This latter was found to contain the tuberculin, but no bacilli, either intact or fragmented.

The *residue* contained both whole and fragmented bacilli. With this preparation Koch was able to produce *immunity* in

animals to virulent bacilli. But his experiments lack sufficient confirmation to warrant their repetition in man.

Both "TR" and "TO" are preserved in 20 per cent. glycerin.

Temporary benefit has followed the use of TR in *lupus.*

Some striking results have accrued from the use of TR as a *therapeutic measure;* and it is regarded by some as of equal importance with hygiene and diet, with which it should invariably be combined. Koch says that tuberculin is of great value as a therapeutic agent in early, uncomplicated cases. In more advanced cases it is necessary to wait until the temperature becomes normal. The treatment should be extended over considerable periods, with intervals of from three to four months, until the injections no longer give any reaction. The consensus of opinion seems to be that the various sera used in the treatment of tuberculosis are of value only in incipient cases and when used in conjunction with other measures. They are all powerless to remove dead tissue or newly formed tubercular tissue.

Fisch *immunized* a horse with TR and then used its serum, which he called **antiphthisin.** He has used this serum in the *treatment* of tuberculosis, and, if his reports are reliable, the results have been good in each case. Klebs used antiphthisin, but failed to get beneficial results. Antiphthisin is really a very dilute tuberculin.

Klebs advocated the use of another product of the tubercle bacillus, known as **tuberculocidin,** which in the hands of some clinicians has yielded remarkably good results.

Maragliano's **antitubercle serum** is obtained from horses immunized to tuberculosis with old or attenuated cultures grown in glycerin-bouillon. The clinical results obtained with these sera have been so uncertain that it is impossible to make any definite statement as to their efficiency or utility.

Bovine Tuberculosis.

Tuberculosis in **cattle** is of special interest, because the animal is one of the sources, and an important one, of our food-supply.

Although it has been known since Koch's first studies in tuberculosis that there was a slight biologic and morphologic difference between the bacillus tuberculosis of man and that of cattle, yet there was no doubt as to the identity of these organisms.

The bacillus of bovine tuberculosis is constant in its shape; it is shorter than the bacillus of human tuberculosis, and does not exhibit the variations in form so frequently met in the human variety. It stains more readily and evenly, although occasionally it is seen to contain deeply staining bodies suggestive of spores. It has been demonstrated experimentally that it is much more virulent than the bacillus of tuberculosis in man. It produces extensive lesions and induces rapid coagulation-necrosis. It grows more slowly in culture. The tissue lesions are identical.

Koch recently startled the medical world by claiming that the bacillus of human tuberculosis and the bacillus of bovine tuberculosis are distinct organisms, and that reciprocal infection never occurs. He based his claim on the finding that injections of pure cultures of human tubercle bacilli into cattle were not followed by a typical tuberculosis. Although there are not many cases on record where tuberculosis in man can be traced directly to cattle, there is no question that this occurs. Cases of unquestionable primary intestinal tuberculosis, in an individual who is not tubercular, cannot be explained in any other way than by assuming that the germ was ingested with the food—*i. e.*, beef. Tabes mesenterica in artificially fed babies undoubtedly has its origin in the milk-supply. Until more accurate studies can be made in this direction it is advisable to regard bovine and human tuberculosis as reciprocally infectious diseases.

Fowl Tuberculosis.

The bacillus of fowl tuberculosis is morphologically similar to the bacillus of human tuberculosis. It is long and slender, and frequently shows branching forms. It grows quite readily on all culture-media. It stains like the bacillus tuberculosis, but takes the stain more readily. Its principal distinctive

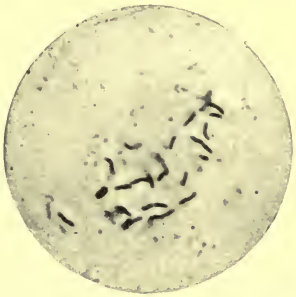
feature is that it grows at temperatures which are fatal to the human tubercle bacillus. This difference may be explained by the fact that as the body temperature of fowls is higher than that of man, the germ may have habituated itself to the higher temperature.

Pseudotuberculosis.

This term usually has reference to a **pathologic condition**, and not to its exciting cause. It is seen in animals, and is characterized by the formation of small nodules resembling tubercles. They are caused by inanimate bodies, animal parasites, bacteria, and highly organized vegetable parasites.

Bacillus pseudotuberculosis is a short, thick rod, which does not form spores; stains readily with the anilin dyes, but not

FIG. 83.



Smegma bacilli, similar in appearance to syphilis bacilli. × 1000. (Park.)

with Gram's solution. It does not liquefy gelatin. In a *gelatin stab* it grows along the puncture and also on the surface of the medium. On *agar* a heavy gray growth develops. On *potato* the growth is luxuriant and of a yellowish color. In *bouillon* the growth gradually settles, leaving the supernatant fluid clear and transparent. The bacillus is pathogenic for animals, especially mice, rats, and guinea-pigs.

Various *streptothrices* and *Aspergillus glaucus* and *A. fumigatus* have also been found in these pseudotubercles.

Bacillus Smegmatis.

This bacillus is often mistaken for *Bacillus tuberculosis.* It is found beneath the prepuce of man, and between the labia and under the fourchette of woman. In appearance it resembles the tubercle bacillus (Fig. 83), stains like it, and resists the mineral acids, but is decolorized by absolute alcohol. It is non-pathogenic, but is of great importance in diseases of the genito-urinary organs, when it must be differentiated from the tubercle bacillus. Animal inoculation will offer conclusive evidence as to its identity.

CHAPTER V.

ORGANISMS RESEMBLING THE BACILLUS TUBERCULOSIS.

Bacillus of Leprosy.

THE specific cause of leprosy is the bacillus of leprosy, or the **lepra bacillus**, discovered by Hansen in 1879. It is always found within the leprous tubercles, contained within the cells, and is also found in the blood during the febrile attacks. It is very slender, a little shorter than the tubercle bacillus, straight, and has rounded ends. It does not form spores, but in the stained preparation it shows unstained spaces, just like the tubercle bacillus. It stains readily with aqueous solutions of the anilin dyes, but retains its color when treated with the mineral acids. Gram's method is also applicable. It is not motile, and always occurs singly or in groups (Figs. 84–87).

Various attempts have been made to cultivate the bacillus on **artificial media**, but the results have been extremely unsatisfactory.

Thus far it has been impossible to **transmit leprosy to animals by inoculation.**

Infection in man usually occurs through an abrasion of the *skin* or the *nasal mucous membrane.* Infection through the respiratory and intestinal tracts has never been known to occur. Sticker believes that infection always occurs through the nose. He bases this opinion on the facts—that the nasal lesion is the only constant one in both forms of leprosy (see below); that the symptoms of the disease always have their origin in the nose; that the relapses begin with nasal symptoms; that it is the only characteristic lesion; and that the bacilli can be found in the leprous nodules in the

nose long before they can be demonstrated in any other part of the body.

Varieties of leprosy and the nodule: The characteristic leprous nodule, which very closely resembles a tubercle in structure, is usually found only in the skin and subcutaneous tissues;

FIG. 84.

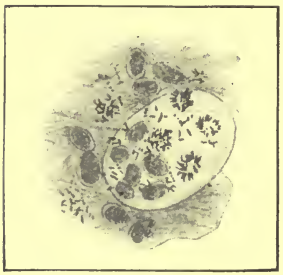


FIG. 85.

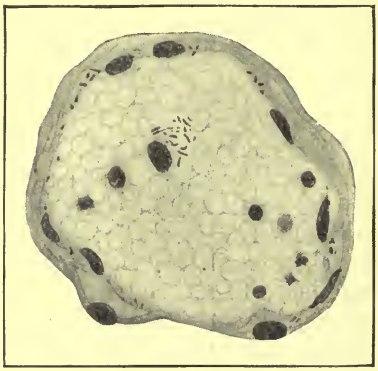


FIG. 86.

FIG. 87.

Lepra bacillus.

but may in exceptional instances occur in the internal organs, especially the spleen. One *variety* of leprosy is characterized by extensive *ulceration* of the tubercles. In the so-called *anæsthetic leprosy* the bacilli are found in the nerve substance, in the spinal cord, and in the brain. They have also been

found enclosed in the leucocytes in the intestines, lungs, and in the sputum.

The *leprous nodule* or tubercle differs from the genuine tubercle in that it is vascular and contains much fibrous tissue. The bacillus is enclosed within large cells, which have been called lepra-cells. They resemble a tubercular giant cell in size, but are not always multinucleated. The ulceration appears to be due rather to the poor vitality of the tissue than to any action of the bacillus. In the *anæsthetic* form of leprosy the nodules are located on the peripheral nerves, and the formation of fibrous tissue is responsible for the subsequent anæsthesia. The various skin lesions occurring in this form are always the result of injury. Burns are very common because of the anæsthesia.

Arning **inoculated** a condemned criminal, who was perfectly healthy, with leprous material, and in five years the disease was fully developed. Other attempts to *infect healthy individuals* have failed. It is believed that leprosy is contagious in the same sense that tuberculosis is contagious; and, therefore, that contact is probably not so much a source of infection as was formerly supposed.

Baumgarten is of the opinion that it is also **inherited**, and cases have been reported in which that assumption seems to be the only explanation for the occurrence of the disease. Opposed to this view is the fact that leprosy is never known to occur in infants.

Individual susceptibility is an important factor. Persons who have been in contact with lepers for years have not contracted the disease. Sexual intercourse appears to be a very common method of infection in leprosy.

Diagnosis: Preparations made from the serum obtained from a leprous nodule are stained in the same manner as for tubercle bacilli. The lepra bacillus is always found in great numbers, which serves to differentiate it from the tubercle bacillus. The nasal mucus always contains lepra bacilli. The serum of a leprous patient will agglutinate the bacilli in dilutions of 1 : 60. Leprous patients usually die from exhaustion or some intercurrent affection, especially inhalation pneumonia.

Distribution: Although leprosy is commonly believed to be

limited to certain countries, it is in reality comparatively widespread in all parts of the globe. It is exceedingly common in Egypt, Syria, China, Siam, Norway, Sweden, the Sandwich Islands, Turkey, and parts of Italy and the United States, and India. The leper colony in the Sandwich Islands contains 11,000 patients. Isolated cases have been reported in many States of this country, especially in Louisiana, where there is now established a small leper colony. Lepers should be promptly isolated.

Bacillus of Syphilis.

In 1884 Lustgarten discovered a **bacillus** in the lesions of syphilis which he believed to be the specific cause of the disease. It has not yet been accepted as the cause of syphilis, and for that reason it is said that the exciting cause of syphilis is unknown. Lustgarten's bacillus resembles the tubercle and smegma bacilli. Many other organisms have been described as causative, but Lustgarten's is the only one which is deserving of consideration. He did not succeed in isolating the germ and cultivating it, nor did inoculation experiments with the syphilitic virus produce the disease. He based his claim almost entirely on the constancy of the bacillus in the lesions and discharges of syphilis. It stains in a very peculiar manner, but the method is also applicable to the tubercle and lepra bacilli.

Lustgarten's bacillus is from 3 μ to 5 μ long, and from 0.2 μ to 0.3 μ broad, slightly curved, often pointed at one end, and presenting unstained spaces in the stained specimen, which he believed to be spores. The bacilli usually occur singly or in groups within the large cells (Fig. 88). He found the organism in all the lesions of syphilis, both internal and external.

Preparations are made from the tissues and the discharges. The film is fixed by passing it through the flame only once, and is then kept at the room temperature for twenty-four hours. It is *stained* with warmed anilin-water gentian-violet, decolorized in absolute alcohol, and exposed to the action of a 1.5 per cent. aqueous solution of potassium permanganate for ten seconds. A second decolorization is effected

with an aqueous solution of sulphurous acid. Wash in water, return to the permanganate solution for a few seconds, and then place in the sulphurous acid again until the film is thoroughly decolorized. It is dehydrated in alcohol, cleared in oil of cloves, and mounted in Canada balsam. Nitric acid readily decolorizes the syphilis bacillus, but not the tubercle or lepra bacilli.

Van Niesen cultivated a bacillus about the size of the tubercle bacillus from the blood of a number of cases of syphilis. The blood is obtained by puncture from the finger, and kept in a sterile dish at a temperature of 13° to 15° C.

FIG. 88.

Syphilis bacilli from a papule, after a preparation from Lustgarten. × 2500.

for ten days. It is then ready to be transplanted. In *bouillon* this bacillus produces grayish-white threads; some of them forming a membrane on the surface and others floating in the medium. In a *gelatin stroke* culture it forms a fine grayish, streaky-looking mass, which consists of threads, some of which penetrate into the medium. The gelatin is liquefied very slowly. The growth on *agar* consists of a central grayish mass with projecting rays. Potato, milk, urine, serum, and water are also available as culture-media.

The colonies in the *plate culture* are quite characteristic, turning from gray to yellow, and finally to brown. The

organism is motile and forms spores. Later it changes its form to a coccus, or branched forms, or it may resemble a mould. It stains readily with the anilin dyes and with Gram's, and is decolorized by the mineral acids.

Inoculation experiments in animals produced abortion in pregnant rabbits, extragenital primary nodular lesions on the ears, secondary ulcers and tumor formations, and irregular lesions.

A **white diplococcus** has also been found and successfully cultivated on agar and potato from the blood of syphilitics. Perhaps this is one of the variations of Van Niesen's bacillus.

Infection: Infection always occurs by *contact* with the products of syphilitic lesions or the blood of syphilitics. An abrasion is necessary before infection occurs. *Sexual intercourse* is the most frequent method of infection, although the *kissing* and *nursing* of a syphilitic infant, the *handling of infected instruments* and other objects, the depraved habit of *tongue-sucking*, and inoculation of the fingers of physicians and midwives (*extragenital chancres*) by coming in contact with the syphilitic virus, must not be overlooked as equally dangerous, and by no means infrequent, methods by which infection is conveyed.

The **primary lesion** is found most frequently on the genitals, and less often on the lips, tongue, tonsils, nipples, fingers, etc. In from three to six weeks the syphilitic virus is disseminated throughout the entire body, and then the so-called *secondaries* appear. Some time afterward these are followed by the lesions or manifestations of the *tertiary stage.*

In **hereditary syphilis** the portal of entrance is the blood, and here the primary lesion is wanting, the secondaries inaugurating the attack. Whatever the exciting cause of syphilis may be, it is endowed with an indefinite term of life, and the disease is transmissible throughout this entire period.

Immunity: Syphilis is the one disease to which all persons, all races, and all nationalities are susceptible. There is no natural immunity to syphilis, but one attack usually confers immunity against another, and a second attack is a rarity. Second attacks are usually relapses, which are not at all

uncommon in syphilis, as the syphilis germ (?) is exceedingly tenacious.

Colles is authority for the statement that a mother giving birth to a child which inherited syphilis from the father, is herself rendered immune. The bacilli apparently do not pass from the fœtus to the mother, but the toxins do, and the immunity is conferred in that way. Such a mother may also nurse her infant without contracting the disease, although a wet-nurse would certainly become syphilitic from the same source. It is held by some authorities that the immunity of the mother is not due to the toxin which has been transmitted to her from the child, but that she may herself have been inoculated with syphilis by the husband. All attempts to produce immunity in non-syphilitics by injection of the serum of syphilitics have failed.

Heredity : The question of the heredity of syphilis presents some interesting points which may now be discussed profitably. It is a well-known fact that the children of syphilitics are either born with evidences of syphilis ; or they do not manifest any syphilitic taint at birth, but show evidences of the disease later on. It is claimed that the disease is transmitted from the father through the spermatozoa. Experiments tending to prove this assertion have been unsuccessful, but clinically there is sufficient evidence at hand to warrant the statement that syphilitic fathers propagate syphilitic children, the mothers escaping infection. On the other hand, it is held that in the absence of syphilis in the mother the child will remain free from syphilis. The mother may not have had any evidences of the primary or secondary lesions, but well-marked tertiary symptoms appear later on, proving that the mother at the time of birth of the child really was a syphilitic, and that her healthy condition was apparent only.

The opinions of eminent syphilographers are divided about equally on these propositions ; but if the second is true, the first is unquestionably untenable. Furthermore, the children of a syphilitic mother are always syphilitic even when syphilis in the father can be ruled out absolutely. The infection is transmitted through the ovum.

Infection of the child may occur during its intra-uterine life. If the mother is infected while she is *enceinte,* the child is also infected, except when infection occurs during the last two months of pregnancy, during which time the infection of the child is not so apt to occur. The mother is infected through sexual intercourse, and the disease is carried to the fœtus through the placenta. It is less probable that the fœtus is infected first, and that the mother is infected through the fœtus. When the child is suffering from an intra-uterine infection the primary lesion is invariably absent at birth. If the primary lesion makes its appearance after the birth of the child, the infection has undoubtedly occurred extra-uterine.

CHAPTER VI.

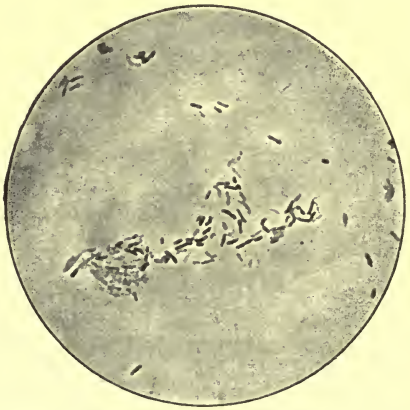
GLANDERS AND ACTINOMYCOSIS.

Bacillus of Glanders (Bacillus Mallei).

THE specific cause of **glanders** is the *Bacillus mallei*, discovered by Loeffler and Schuetz in 1882, in the lesions and discharges of glanders.

The **glanders bacillus** is a small, thick rod, with rounded ends, measuring from 1.5 μ to 3 μ in length, and from 0.2 μ to 4 μ in thickness. It is not motile, has no demonstrable flagella, and is without definite arrangement or grouping

FIG. 89.



Glanders bacilli. Agar culture. × 1000. (Park.)

(Fig. 89). Some observers have found evidences of sporulation and others have not.

It stains best with Loeffler's methylene-blue. It is very easily decolorized. Gram's method is not applicable. The

stained specimen appears to be fragmented, and this has given rise to the belief that it produces spores. *Bacillus mallei* is a facultative anaërobe. It grows readily on all culture-media at a temperature anywhere between 25° and 40° C., the optimum being 37° C.

Vitality: The bacillus is killed in ten minutes by exposure to a temperature of 55° C.; in five minutes by a 5 per cent. carbolic acid solution; in two minutes by a 1 : 5000 solution of mercuric chloride. It will resist drying for months. The organism does not thrive as a saprophyte, and must be classed as an obligative parasite.

Pure cultures are made from the nasal mucus or the tissues of animals suffering from glanders. A very rapid method is to inject the suspected material into the peritoneal cavity of a male guinea-pig. If the glanders bacillus is present, the testicles will be involved early. They break down and frequently discharge through the skin. The animal is killed, and on removing the testicles the tunica vaginalis is found full of fluid pus, from which the germ is obtained in pure culture. It will grow on any media, but best on glycerin-agar, blood-serum, and potato.

On *glycerin-agar plates* it forms small, glistening, finely granular, yellowish colonies within forty-eight hours. In *stroke cultures* on glycerin-agar and blood-serum the growth develops along the entire needle-track as a very thick moist white membrane. *Gelatin* is slightly liquefied. *Bouillon* is clouded.

On *potato* the growth is quite characteristic. It first forms a luxuriant moist yellow coating, which gradually turns to a deep reddish-brown. The potato around the culture takes on a greenish-yellow tinge. *Milk* is coagulated with the production of acid. The glanders bacillus loses its virulence in culture, but regains it when it is passed through animals.

Pathogenesis: Glanders is primarily only a disease of animals, especially the horse; but man is extremely liable to contract the disease from infected animals. Cases of glanders have also been reported frequently in the persons of laboratory assistants. The greatest care should be taken in the handling of this or any other pathogenic organism. The glanders

bacillus produces a small nodule (Fig. 90), which somewhat resembles the tubercular nodule, and which finally softens and breaks down. The bacillus is found in the centre of the nodule.

In the *horse* the disease first manifests itself by the formation of small ulcers on the nasal mucous membrane and an excessive discharge of nasal mucus. The submaxillary and other lymph-glands soon become enlarged and suppurate.

FIG. 90.

Bacilli of glanders: *a*, section from glandrous nodule, × 700; *b*, bacilli of glanders, stained with methyl-blue. (Fluegge.)

The disease may extend from the nose to other parts of the body, especially the lung.

The chronic form of the disease which affects the body generally is called *farcy*. It is characterized by the formation in different parts of the body of small circumscribed swellings, known as *farcy buds*. These finally suppurate and form ulcers, which discharge quite freely.

Infection: In man the skin is the most frequent portal of entry for the infection. An abrasion is always necessary. Infection may also occur through the mucous surfaces. Cases are on record where hostlers drank from the same pail as a horse affected with glanders, and acquired the disease. It is

also possible, though rather infrequent, that infection may occur through the respiratory and gastro-intestinal tracts. The organism is transported in the body through the lymph-vessels.

In *man* the disease is characterized by the formation of multiple abscesses in the skin, muscles, joints, and the internal organs, with a decided tendency to a fatal ending. The mucous membranes, especially that of the nose, may be the seat of many small ulcers. The infection resembles a streptococcus infection, and death results from septicæmia. The disease may be transmitted from man to man through the secretions and the excretions.

Diagnosis: The methods detailed above for obtaining the organism for culture are the same as those used in making a diagnosis. Animal *inoculation* is by far the most reliable.

The organism may be *stained in tissue*. Loeffler says to place the section in a solution of alkaline methylene-blue for five minutes, and then transfer to the following mixture for five seconds:

Concentrated sulphuric acid,	2 drops;
5 per cent. solution of oxalic acid,	1 drop;
Distilled water,	10 c.c.

Dehydrate in absolute alcohol, clear in xylol or clove oil, and mount in Canada balsam.

Kuehne's method is more complicated. He places the section in alkaline methylene-blue for thirty minutes; washes well in water and decolorizes in a weak solution of hydrochloric acid; immerses in an aqueous solution of lithium carbonate (8 drops of the saturated solution in 10 c.c. of water); washes in distilled water; dips in absolute alcohol, slightly colored with methylene-blue, for a few seconds; dehydrates in anilin oil containing a trace of methylene-blue; washes in pure anilin oil, clears in xylol, and mounts in balsam.

Immunity and Mallein: One attack of glanders confers immunity of from three to six weeks' duration. It has thus far been impossible to produce an artificial immunity. *Mallein* is made with cultures of the glanders bacillus just like Koch's original tuberculin. A six-weeks-old culture of the glanders

bacillus, grown in 5 per cent. nutrient glycerin-veal-bouillon, is evaporated to one-tenth its volume. The result is mallein.

It is very largely employed as a *diagnostic agent.* The method of administration and the reaction in positive cases are the same as with tuberculin. In an animal affected with glanders there usually appears at the site of inoculation or injection a very large painful swelling accompanied by considerable inflammation of the lymph-vessels and glands and œdema. This reaction persists for from three to ten days. In animals free from glanders the small œdematous tumors disappear in twenty-four hours.

Prophylaxis: A rigid quarantine should be instituted in stables in which glanders is found. The affected animals should be killed immediately and the carcass destroyed by fire. The attendants should be warned of the danger of contracting the disease, and precautions taken to prevent infection from spreading to the other stock.

Actinomycosis (Streptothrix Actinomyces).

Actinomycosis is a disease of cattle, but is occasionally seen in man.

The cause of this peculiar affection is the **actinomyces** or "ray fungus." Actinomycosis is no longer regarded as a mould disease, but is now placed in the same class as tuberculosis.

The exciting cause is not a mould, but a *streptothrix*, one of the higher class of bacteria. In the course of its growth it is seen to assume a variety of forms, sometimes a coccus, or a bacillus, or at other times a distinct fungous arrangement. Hektoen's valuable work in connection with the study of this organism has enabled us to make a proper classification of the actinomyces.

Although the infectious character of the disease was known as early as 1845, it was not until many years later, in 1877, that the specific cause was discovered. The disease was first described in man in 1885 by Israel. Four years later, Boström succeeded in cultivating the **actinomyces**, and he has given a very detailed account of his work. The organism,

because of its size, is easily detected in the lesions as small sulphur-yellow granules ranging from 0.5 to 2 mm. in diameter. When one of these granules is transferred to a slide and examined microscopically (Fig. 91), it is seen to be made up of a central granular mass from which radiate a large number of club-shaped threads. It really consists of three zones: the central granular zone; next to that a zone composed of freely interlacing threads. The free ends of these threads are directed outward to form the third or outer zone, which consists of the club-shaped extremities of these threads.

FIG. 91.

Three actinomyces from a case of pulmonary actinomycosis. Below, three finger-like buds and dichomatous branching of actinomyces threads. × 450. (Baumgarten.)

It is easily *stained* with the usual anilin dyes, and also by Gram's method. When the organism is to be stained in tissue, picrocarmine gives very good results, the tissue being stained carmine and the actinomyces yellow. Gram's stain, followed by Weigert's method also brings out the organism to good advantage.

Pure cultures are made from the actinomyces granules. These are removed from the tissues, slightly crushed between sterile glass slides, and transferred to the culture-medium. The organism is strongly aërobic, developing rapidly at the room temperature on all the usual media. A strictly anaërobic variety has also been described.

In *plate culture* the colonies first appear as very small filamentous grayish masses. On *blood-serum*, *gelatin*, *agar-agar*, and *glycerin-agar*, the growth develops in the form of small yellowish or grayish colonies, which soon coalesce to cover the entire surface of the medium with a thick dry wrinkled fluffy membrane, which adheres to the media very firmly. It is utterly impossible to remove the growth without tearing it into shreds. Projecting from the surface growth down into the medium are many fine threads. Blood-serum and gelatin are liquefied. The surface of *bouillon* is covered by a membrane just like that on the solid media, but the fluid is perfectly clear. Frequent shaking of the flask breaks the membrane into many small granular masses.

The growth on *potato* is yellowish-red in color, and is covered by a very fine white fur. The actinomyces grows quite readily in both raw and boiled *eggs*. It is introduced into the egg through a small opening made with a hot needle. The opening can be sealed with collodion, sealing-wax, or paraffin. *Milk* is peptonized. In all *stab cultures* the growth forms on the surface of the medium, and the track of the needle soon becomes filled with a grayish turbid fluid. The cultures are quite resistant to drying. They are killed in five minutes by a temperature of 75° C.

The many **variations** of this organism, or what might be called transition-stages, can readily be studied in the cultures. There are long wavy filaments of uniform size; short, thick rods, either perfectly straight or slightly curved like the tubercle bacillus; filaments with segmented ends like a spore-containing mycelium in the thrush mould; many micrococcus-like bodies, branching forms, rods and filaments with club-shaped extremities, all of which must be considered as distinct stages in the development of this remarkable organism.

Pathogenesis: The disease is common in cattle, and is usually located in the jaw, where it forms a distinct swelling. It is commonly known as *lumpy jaw*. The actinomyces is ingested with the food (cereals), and then finds its way into the jaw-bone through a carious tooth or an injury or abrasion of the gum. It develops rapidly, forming a granulomatous tumor, somewhat similar to a tubercle, which contains many

small round cells, giant cells, and leucocytes. This tumor eventually undergoes necrosis, breaks down, and discharges on the surface through a sinus. The tumor closely resembles an osteosarcoma in its structure, and for a long time was considered as such. The actinomycetes are found imbedded in this tumor mass.

In man infection may occur through the *mouth*, through the *respiratory* or *gastro-intestinal tract*, and through the *skin*.

Clinical observation of the disease in man has shown that actinomycosis not infrequently occurs in individuals who have been in the habit of chewing wisps of hay or straw, or different kinds of cereals. Certain it is that the disease is seen only in persons who have to do with cattle a great deal and in farmers. In many instances it has been possible to prove the existence of a straw-chewing habit. People who are in the habit of chewing a tooth-pick or some other sharp-pointed or rough object are also liable to the infection because of the presence of some injury of the gum produced by the foreign body in the mouth. The lower jaw is affected more often than the upper.

Infection through the *skin* is not very common. It always occurs through an abrasion or a wound. Running a splinter of wood into the finger or foot is the usual method of infection.

Infection through the *respiratory tract* occurs as a rule by inhalation, although the disease may extend from the jaw along the muscles of the neck and into the lungs and pleura. From there it may continue down through the diaphragm and into the abdominal cavity. When the disease appears in distant parts of the body it is due to the rupture of an actinomycial mass into the bloodvessels, a metastasis such as is seen in malignant tumors. Lodgement of the actinomyces in the lung is followed by a fatal bronchopneumonia.

The *gastro-intestinal* tract serves as a portal of infection when food containing the actinomyces is eaten. The inspection of cattle is very stringent, and cases of lumpy jaw are always promptly condemned; but unfortunately some persons are avaricious enough to dispose of such cattle for food, and infection from that source is possible. The disease may

appear in the intestines or in any of the abdominal viscera, especially the liver.

Direct transmission from cattle to man has not been demonstrated.

Diagnosis: The microscopic diagnosis is readily made by examining the little sulphur-colored granules contained in the discharge from the lesion in the jaw; or by excising a portion of the tumor and either making sections of the tissue or crushing a small piece between two glass slides.

Streptothrix Maduræ; Mycetoma or Madura Foot.

This disease is peculiar to certain parts of India and very closely resembles actinomycosis. So far as the pathology of the two conditions is concerned, it is practically identical.

Many investigators are of the opinion that the **streptothrix** found in the lesions of mycetoma is identical with *Streptothrix actinomyces.*

Mycetoma affects usually only one foot, less frequently both, and very rarely the hand, shoulder, or hip. It invariably follows an injury, such as is caused by stepping on a nail, piece of wood, or a thorn. Small nodules or tubercles are formed, which later attain a considerable size, break down, and discharge freely. The discharge is either purulent or seropurulent, and contains small pinkish granules, **Streptothrix maduræ.**

Vincent succeeded in cultivating this organism on *acid vegetable infusions*, which are apparently most suited for its growth. It is a strong anaërobe. A surface growth develops on *liquid media.* It is white at first, but soon changes to a faint red. On *solid media* small round colorless colonies are formed which do not coalesce. Very old colonies are perfectly white. The growth clings to the medium like the actinomyces. In *bouillon* the growth is very peculiar. It gradually settles to the bottom of the tube and has the appearance of a small white powder-puff ball.

The growth on *potato* is very meagre unless the potato has an acid reaction. A heavy white dry woolly membrane is formed which never changes color.

The *stained specimen* very closely resembles the actino-

myces, presenting the same branched forms, clubs, spore-like bodies, and filaments. It also stains like the actinomyces. Most authorities are inclined to regard the *Streptothrix maduræ* and the *Streptothrix actinomyces* as separate species, but there are so many points of similarity between the conditions produced by them that one is justified in assuming that *Streptothrix maduræ* is simply a variation of the actinomyces, such variation being due to conditions which influence the growth of the organism.

Farcin du Bœuf; Streptothrix Farcinæ.

This is another disease caused by an organism which resembles the actinomyces.

The exciting cause is **Streptothrix farcinæ** or the *bacille du farcin des bœufs Nocard.*

The **disease** is one of cattle, and is never seen in man. Pathologically it is an intense inflammation of the lymphatic

FIG. 92.

Streptothrix farcinæ.

structures, especially the lymph-glands in the axillæ and those at the root of the lung. The glands are considerably enlarged, and finally break down, discharging a creamy pus.

The **specific organism** is found in the centre of the lesion. It consists of very long, slender filaments, which branch freely and contain spore-like bodies (Fig. 92). It is strongly aërobic, and grows best at the temperature of the body. It is stained by Gram's method, and also with the ordinary anilin dyes.

On *agar-agar* the growth first develops as small very dry discrete irregular masses that tend to become confluent and develop a yellow color. On *blood-serum* the growth is not so luxuriant as on agar, but has the same formation and appearance. Similar appearing masses are developed in *bouillon*, some of them floating on the surface of the medium and others sinking to the bottom. *Milk* is not coagulated, nor is its reaction changed. On *potato* large dry scales of a pale-yellow color are formed.

Injection into animals of a pure culture of the streptothrix produces the disease, thus positively establishing its etiology. The horse, dog, ass, and rabbit are immune. Intraperitoneal injection into susceptible animals is followed by extensive development of little tubercles in the omentum and the peritoneal covering of the abdominal viscera. Intravenous injection is followed by the development of tubercles in all parts of the body, resembling an acute miliary tuberculosis.

Rhinoscleroma.

This **disease** is characterized by the development of small circumscribed tumors on the nasal mucous membrane. From this point the disease gradually spreads to the surrounding tissues, sometimes as far as the pharynx. The tumors resemble the lesions of glanders, but do not ulcerate.

Von Frisch discovered an **organism** in the lesions which resembles Friedlaender's pneumobacillus in every particular. It is surrounded by a capsule, stains readily by Gram's method, and is always found within the tissue-cells. It grows readily on all the various culture-media and induces fermentation of sugar. Milk is coagulated. It has been impossible to produce the disease in animals by inoculation.

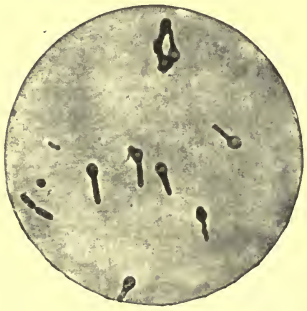
CHAPTER VII.

BACILLUS OF TETANUS.

Bacillus tetani, or the bacillus of Nicolaier, is the exciting cause of tetanus. It was first obtained in pure culture by Kitasato, in 1889, but was discovered by Nicolaier as early as 1884. It is the accepted cause of tetanus. It is found in the purulent discharge of tetanus, at the site of inoculation; in the soil, especially garden earth; and in the excretions of horses and cattle. Its appearance is very characteristic.

Biology and morphology: It is a very slender germ, about the size of a small red blood-corpuscle, from 3 to 5 μ in length,

FIG. 93.



Tetanus bacilli with spores in distended ends. × 1100. (Park.)

with an enlargement at one end containing a spore, the typical drum-stick shape (Fig. 93). When the bacterium is not sporulating, its ends are rounded and it is regular in outline. It has no flagella, but is motile nevertheless. It usually occurs singly, rarely forming chains. It is an obligative anaërobe, and will not grow in the presence of the slightest amount of

oxygen. That is one reason why it is so difficult to cultivate this germ, although it will grow on all kinds of culture-media. It can be habituated to oxygen, but that is done at the expense of its virulence.

It grows best at the temperature of the body. It is readily stained with the anilin dyes, and also by Gram's method. The spores are stained in the usual manner. When the tetanus bacillus is grown at very high temperatures it presents distinct involution-forms. It is rapidly destroyed by temperatures above 55° C.

The bacillus of tetanus is grown best in an atmosphere of hydrogen.

On *gelatin plates* small white colonies develop in about five days, which are quite characteristic. They have the appearance of a thistle, very fine lines radiating from a dense central mass. The gelatin is gradually liquefied. On *agar plates* the colonies develop more slowly; the medium is not liquefied.

In *gelatin stab* cultures development occurs along the line of inoculation, without any surface growth, in the form of fine radiating threads which extend from the central line of growth out into the gelatin, an appearance which suggests a fir tree (Figs. 94 and 95). This characteristic appearance is lost when liquefaction occurs at about the end of the second week. A grayish-white viscid liquid fills the centre of the medium, and the culture accumulates at the bottom of the liquefied mass. *Agar* cultures are not liquefied.

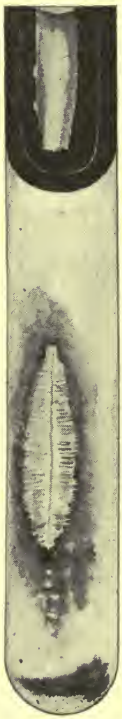
Plain bouillon or *glucose-bouillon* is first rendered turbid, but becomes quite clear when the culture settles. The supernatant fluid contains the tetanus toxin. The growth in bouillon is accompanied by the evolution of much gas. A very disagreeable, peculiar odor is given off by all tetanus cultures.

On *potato* a moist invisible growth develops. *Milk* is not changed in appearance.

Vitality of spores: The spores are quite resistant, and will remain alive for a long time. When exposed to live steam at a temperature of 100° C., they are killed in from five to eight minutes. A much longer exposure is required for tempera-

tures lower than this. A 5 per cent. carbolic acid solution destroys the spore in about fifteen hours; a 1 per cent. solution of mercuric chloride in about three hours. The addition of

FIG. 94.



Bacillus tetani: six-days-old puncture culture in glucose-gelatin. (Fraenkel and Pfeiffer.)

FIG. 95.

Bacillus tetani: culture four days old in glucose-gelatin. (Fraenkel and Pfeiffer.)

a 0.5 per cent. aqueous solution of hydrochloric acid to these antiseptics hastens the destruction of the spores considerably.

Kitasato's method for *isolating* the bacillus takes advantage of this resistance of the spores to high temperatures. Agar

tubes are inoculated with the material containing the tetanus bacillus and placed in the incubator for two days. This will cause the development of not only the tetanus bacillus, but also of all other organisms contained in the culture. This mixed culture is heated on a water-bath at a temperature of 80° C. for one hour. This destroys all the bacteria, including the tetanus bacillus, but does not affect the spores of the latter. Pure cultures are made from this spore-containing culture according to the methods previously described for the cultivation of anaërobic bacteria.

Pathogenesis: Man and nearly all the domestic animals, except dogs and birds, are susceptible to tetanus. The infection, in order to become manifest, must take place through a wound. The organism is a very common saprophyte in the soil, and cattle, when feeding on grass, are quite liable to swallow the germ, which passes through the gastro-intestinal tract without exhibiting any evidence of infection. The fact that the tetanus bacillus is so frequently found in manure proves that it may be ingested by animals without producing the disease. It grows luxuriantly in manured ground. Tetanus can also be produced artificially by injecting the germ into the circulation or into the peritoneal or other cavities.

Infection: The most frequent portal of entry of the germ is through the skin. Usually the site of infection is easily recognized, but occasionally cases of tetanus are seen in which it is impossible to find any evidence of an injury which may have served as an infection atrium. Penetrating wounds offer the most suitable lodgement for the bacillus. Stepping on a rusty nail or the prongs of a pitchfork or garden-rake are well-known methods of infection in tetanus, or lock-jaw, as the disease is designated by the laity. It was at one time believed that tetanus was the inevitable result of such an injury. The wound may, however, be produced by any other object than those mentioned. Neither is it essential that the nail or prong be rusty, although the danger of infection in that case is much greater, as the rusty nail has lain on the ground for some time, and is more liable to be the "host" of the tetanus bacillus than a clean nail.

The wire wrapped around baled hay is also responsible

for cases of tetanus. Fish-bones, wood, and any object that has been partially buried in the ground for some time, may convey the infection. Many cases have been recorded from localities where people are accustomed to bathe, such as sandy beaches, *e. g.*, the shores of Long Island, where, as is known, tetanus has followed a fish-bone injury of the foot.

The bites of insects, such as the sand flea or the chigger, may be instrumental in producing tetanus. This is particularly common in warm countries, where the inhabitants are in the habit of going barefoot. Many cases were reported from Mexico, when the soldiery went without shoes or stockings, following the bites of the chigger. The prick of a pin is sufficient to cause infection. All that is necessary is a penetrating wound in which the bacillus can develop in an oxygen-free environment.

Cases of tetanus are very common around the Fourth of July, and usually follow an injury of the hand produced by the explosion of a fire-cracker or pistol. Naturally the hand of the celebrant is not very clean after a day's shooting, and all the accumulated dirt is street-dirt, which may contain the tetanus bacillus. The force of the explosion drives this grime into the wound and tetanus results. Superficial wounds or extensive open wounds are rarely followed by tetanus, because the conditions in such a wound are unfavorable for the development of the germ. Penetrating wounds always close immediately after the instrument inflicting the injury is withdrawn, thus leaving the bacillus in the bottom of the wound and in a most favorable environment. The bottom of the wound is not exposed to the air and the germ flourishes. Sometimes these wounds close so tightly that it is impossible to locate them even within a few hours after the injury. In such cases the treatment is necessarily more difficult than when the wound can be located.

If the infection is with the tetanus germ only, very little or no suppuration occurs; but in a *mixed infection* suppuration is always more or less profuse. Mixed infections are always serious, because they favor the development of the tetanus bacillus. The aërobic organisms consume the oxygen in the wound, and the tetanus bacillus is furnished with an

environment in which it can grow and produce its toxin. Such mixed infections probably account for those cases where the tetanus does not manifest itself until some time after the injury has occurred. The bacilli die in the presence of oxygen, but the spores survive, although they remain inactive until such time when conditions are favorable for their development. If the infection has occurred in an open wound, the disease may not manifest itself until after the wound has closed.

The tetanus bacillus is an extremely toxic germ, and the **manifestations of the disease** are entirely due to absorption of the *toxin.*

The *bacillus* does not spread throughout the body, but remains confined to the point of injury. It is never found in the tissues of the body, nor in the blood unless injected experimentally. $\frac{1}{100}$ of a milligram of a filtered culture is sufficient to kill a mouse.

A man weighing 175 pounds would be killed by 0.23 of a milligram of tetanus toxin.

This *toxin* is very easily destroyed; and its chemical composition is unknown.

It is produced in large quantities by the bacillus, and its action is very rapid.

It first affects the muscles nearest to the point of infection, and then, in order, all the parts of the body. *Strychnine-poisoning* resembles tetanus in its manifestations; and, like strychnine, the tetanus toxin has a predilective action on the spinal cord. The action of the tetanus toxin is localized in the spinal cord. That some of the toxin is taken up by the blood and lymph-currents has been proved by injecting blood from a case of tetanus into an animal, which promptly developed the disease. The toxin is *excreted* by the kidneys.

Immunity: In view of the fact that the bacillus remains localized at the site of infection, and that the circulating toxin is responsible for the manifestations of the disease, immunity to tetanus must be against the toxin and not the germ. The serum of animals immunized to tetanus confers immunity, and it also exerts a curative action; but for curative purposes a larger amount of serum, or a more active

serum, must be used. Enough serum must be injected to neutralize the toxin in the body.

The *toxin* is *prepared* from active bouillon cultures, which are filtered through porcelain. It is preserved by the addition of 0.5 per cent. of carbolic acid.

The *antitoxin* is *prepared* by inoculating horses with gradually increasing doses of the toxin until the desired degree of immunity is attained. The method of preparation of antitoxins has been described in a preceding chapter. The same product is used for immunization and for therapeutic purposes.

This serum has yielded remarkably good results as an immunizing agent; but not as a therapeutic measure. The reason for this is evident when we remember that unfortunately cases of tetanus do not present themselves for treatment as soon as the injury has occurred. The patient is not seen until he presents the active manifestations of the disease, and then it is impossible to administer enough antitoxin to neutralize all the toxin which is already in circulation. Persons should present themselves for treatment as soon as the injury has occurred, so that prompt measures may be taken to prevent development of the disease. The antitoxin must be used early and in sufficient quantity to be of any service as a curative agent.

Roux and others have suggested that the antitoxin be *injected under the dura mater* through a trephine-opening. It is believed that if the antitoxin is brought into direct contact with the cerebral substance a cure is more apt to result. Most authorities recommend making the injection directly *into a vein*, so as to insure prompt action. When injected *subcutaneously*, the antitoxin must be absorbed first and thus the action is delayed.

Wassermann is of the opinion that it is possible to obtain an *immunizing substance* from the nerve-cells of the cord and brain of cases of tetanus. He removed the brain and cord from tetanized animals and rubbed them up with physiologic salt solution. This mixture was injected into animals, and Wassermann found that in doses of 1 c.c. it neutralized ten times the amount of tetanus toxin necessary to kill an animal,

and that it also conferred an immunity of twenty-four hours' duration.

For *immunizing purposes*, the *dose* of the tetanus antitoxin is 10 c.c. of a serum of the strength of 1 : 1,000,000,000. This dose may be repeated in a week. For *therapeutic purposes*, the initial dose should be 50 c.c.; and depending on the severity of the case, from 20 to 50 c.c. should be injected every day until the disease is under control.

Since this antitoxin has been used the number of fatal cases of tetanus has diminished considerably, although the results accruing from its use are not so good as those attained in diphtheria with the diphtheria antitoxin. Very probably a similarly favorable condition of affairs would obtain if every case of tetanus could be treated early, before the germ has developed and produced sufficient toxin to cause active manifestations of the disease.

We also wish to call attention to the fact that *treatment* should not be limited to the use of *antitoxin*. The bacillus must be prevented from manufacturing any more toxin. *Surgical intervention* is absolutely indicated. Penetrating wounds are either excised completely or laid widely open, so that the air may have free access, and thus prevent development of the germ. The cautery and strong disinfecting solutions should also be used. Every effort should be made to prevent formation of the toxin, to prevent its absorption, and to neutralize the toxin which has been absorbed.

Pseudotetanus Bacillus.

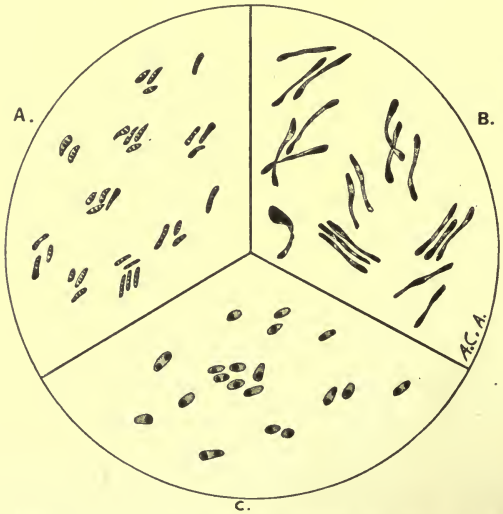
This is a more slender organism than the tetanus bacillus. It is club-shaped, with a large spore in one end. It also has flagella. It stains with the anilin dyes, but not with Gram. It is sometimes found in large numbers in the appendix, and was considered by its discoverer to be the cause of appendicitis. It does not resemble the tetanus bacillus in culture. It is a facultative anaërobe.

CHAPTER VIII.

BACILLUS DIPHTHERIÆ, OR KLEBS-LOEFFLER BACILLUS.

In 1883 Klebs discovered the constant presence of a very peculiar appearing organism in the superficial strata or layers of the pseudomembrane removed from the throats of patients

Fig. 96.



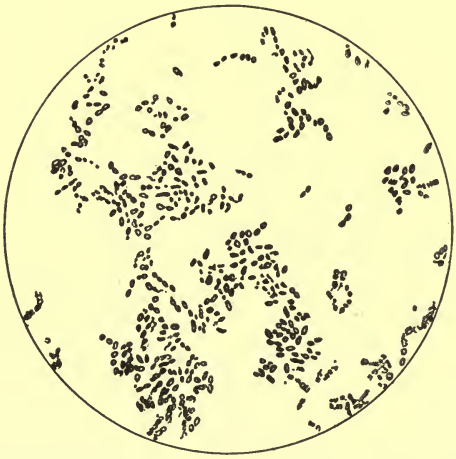
Bacillus diphtheriæ: A, its morphology on glycerin-agar-agar; B, its morphology on Loeffler's blood-serum; C, its morphology on acid blood-serum mixture. (Abbott.)

suffering from diphtheria. One year later Loeffler succeeded in isolating and cultivating this same organism, and it has

since been known as the Klebs-Loeffler bacillus. Loeffler's findings have been verified by others, and **Bacillus diphtheriæ** has been accepted as the specific cause of diphtheritic sore throat.

The bacillus is always present in the lesions of diphtheria, and occasionally is found in the healthy throat and in the throats of persons who have recently recovered from an attack

FIG. 97.



Bacillus diphtheriæ, from a culture upon blood-serum. × 1000.
(Fraenkel and Pfeiffer.)

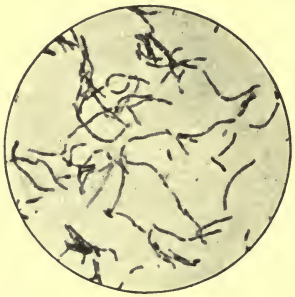
of diphtheria. Abbott cites the accidental occurrence of diphtheria in one of his assistants who unintentionally sucked a few drops of a virulent culture of the diphtheria bacillus through a defective pipette, thus fulfilling all the requirements of Koch's law as to the specificity of any germ.

Biology and morphology: The diphtheria bacillus (Figs. 96 and 97) is a rather thick rod, of about the length of the tubercle bacillus and with a marked tendency to variation in form. The rods may be straight or slightly curved; of a uniform size or irregular; pointed at one end and clubbed at the

other; of a dumb-bell shape or bulging in the middle. Branching forms are also met with occasionally. It usually occurs singly and rarely forms chains. It exhibits a slight tendency to parallelism. It has been suggested that there might be some relationship between the tubercle bacillus, the actinomyces, and the diphtheria bacillus, because all three exhibit the same variability as to form (Fig. 98).

Bacillus diphtheriæ has no flagella and is not motile. Spore-formation has not been observed. It is easily stained with the anilin dyes, but best with Loeffler's alkaline methylene-blue. Gram's method is also applicable. It does not, however,

FIG. 98.



Extremely long form of diphtheria bacillus. This culture has grown on artificial media for four years and produces strong toxin. × 1100. (Park.)

stain uniformly, but presents spaces suggestive of sporulation or fragmentation of the germ. This is especially marked in the larger organisms. Polar bodies are common in the smaller, more regular varieties. They are the *Babes-Ernst bodies*. They have a diameter greater than that of the bacillus and give rise to the dumb-bell shape. These irregularities in staining are more apparent in germs which are cultivated artificially than in those obtained directly from the lesions. As the culture ages the staining becomes less characteristic.

Neisser's stain brings out the polar bodies very strongly. The film is stained for two or three minutes in the following solution:

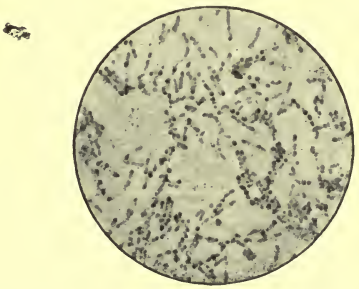
Alcohol (96 per cent.),	20 parts;
Methylene-blue (Gruebler),	1 part;
Distilled water,	950 parts;
Glacial acetic acid,	50 "

Wash and stain with the following for from three to five seconds:

Bismarck-brown,	1 part;
Boiling distilled water,	500 parts.

This is followed by repeated washings in water. The bacilli are stained dark brown with a dark-blue body at one or both ends (Fig. 99). The bacilli taken from recent cultures

FIG. 99.



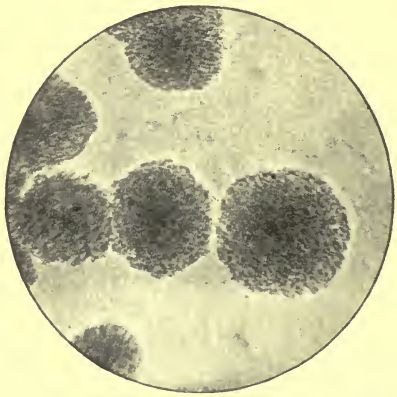
Non-virulent diphtheria bacilli, showing stain with Neisser's solutions, supposed to be characteristic of virulent bacilli. Bodies of bacilli in smear, faint brown; points, dark blue. (Park.)

invariably show this polar staining. The pseudobacilli are stained a uniform brown.

The germ attains its *maximum development* at the temperature of the body. It is a facultative anaërobe. One marked cultural characteristic is the exceedingly rapid growth on blood-serum. The diphtheria colonies appear a long time before those of any other organism. A growth sufficient for diagnostic purposes always occurs in from nine to twelve hours.

Loeffler's blood-serum mixture, consisting of 3 parts of blood-serum and 1 part of 1 per cent. glucose-bouillon, is the most satisfactory culture-medium for the diphtheria bacillus. This is the medium which is used for making bacteriologic examinations of material obtained from the throats of persons suspected of having diphtheria. A growth is present in five hours, although it is invisible. The colonies are plainly to be seen nine hours after inoculation of the blood-serum. They appear as pearly-gray or yellowish-gray, slightly raised dots with irregular borders (Fig. 100), steadily increase

FIG. 100.



Colonies of diphtheria bacilli. × 200. (Park.)

in size, and finally become confluent, forming a nodular yellowish-gray growth.

On *agar-agar* and *glycerin-agar* development occurs more slowly unless the culture was transplanted from blood-serum, when development takes place more rapidly. The colonies are large, granular, and much darker in the centre than in the periphery. The growth may be so luxuriant as to resemble a colony of a staphylococcus.

In *gelatin stab* cultures small whitish colonies form along the line of inoculation, with a slight surface growth. The gelatin is not liquefied.

Bouillon is first clouded, and then the growth gradually accumulates on the sides and bottom of the tube in the shape of a light flocculent precipitate, leaving the bouillon clear and transparent. Not infrequently the surface of the medium is covered by a very thin, fragile membrane. Picking up the tube without more than the ordinary agitation caused thereby causes the membrane to fall to pieces.

On *alkaline potato* a delicate surface coating is formed. The organism grows in *milk* without changing its appearance. *Litmus milk* is first turned a faint red, and then blue again. The bacillus grows well on both raw and boiled *eggs*.

Vitality: A striking characteristic of the diphtheria bacillus is its very feeble resistance to heat and chemicals. It is destroyed by a temperature of 58° C. in ten minutes; by 1 : 1000 solution of bichloride in twenty seconds, and as rapidly by 5 per cent. solution of potassium permanganate, 5 per cent. carbolic acid, 3 per cent. carbolic acid in 30 per cent. alcohol, and 4 per cent. cresol in 40 per cent. alcohol. Pure lemon juice is rapidly fatal. It cannot withstand drying for any length of time, but will remain alive for months if enclosed in a shred of the diphtheritic membrane. It is not affected by cold.

Pathogenesis: The diphtheria bacillus is pathogenic for man as well as animals, especially cats, chickens, and pigeons. Animals are frequent sources of infection, particularly when they are petted. The organism gains entrance to the throat, and when conditions are favorable for its development it produces the characteristic pseudomembrane of diphtheria. Other varieties of diphtheria, as well as chemicals, not infrequently are the cause of the formation of a pseudomembrane in the throat which cannot, by its appearance, be differentiated from the diphtheritic membrane. This membrane usually is formed on the fauces first, but may appear first in the pharynx, nares, larynx, or on the tonsils. In rare instances the membrane may be formed in the vagina or rectum, on the conjunctiva, or in a wound of the skin. This membrane has a dirty white or grayish color suggestive of decomposition, and when it is forcibly detached it leaves a raw, bleeding surface.

The *diphtheritic infection*, like tetanus, is purely a local one.

The membrane represents the local reaction, and the constitutional symptoms are due to absorption of the toxin. When the germ lodges on the mucous membrane, it excites intense congestion and inflammation in the upper layers. This is followed by exudation of serum into these layers and a consequent lessened nutrition to the tissues. This lack of nutrition, together with the toxin elaborated by the germ, is responsible for the coagulation-necrosis which follows, and represents the final step in the process. This necrosed tissue is the so-called pseudomembrane of diphtheria. There is no deposit on the mucosa. It is a death of its superficial layers. The bacilli are found in greatest number in the older portions of the membrane. From the original site of infection the membrane gradually spreads to other parts of the throat, nares, and larynx. The appearance of this membrane is not a criterion of the toxicity or virulence of the bacilli.

The *toxin* elaborated in this membrane is absorbed by the bloodvessels and lymphatics, and distributed throughout the body. Its entrance into the circulation is marked by the appearance of constitutional symptoms. The bacilli are rarely, if ever, found in the blood.

Diphtheria is seldom a pure infection. Associated with the diphtheria bacillus we find the staphylococcus, streptococcus, pneumococcus, and occasionally *Bacillus coli communis*. These associated organisms play a very important role in diphtheria. They are largely responsible for the complications of diphtheria, except the paralyses which are due to the toxin, and for those severe, aggravated cases which resist all treatment. The streptococcus is most to be feared, because, as has been demonstrated experimentally, when the diphtheria bacillus and the streptococcus are associated the latter increases the virulence of the former.

Infection: The disease is always conveyed from one person to another either directly or indirectly. Kissing a convalescent is a common source of infection. The bacillus has been found in the throat for as long a period as six months after subsidence of the disease. Persons who have come in contact with the disease may not themselves become infected, but may convey the disease to others. The

patient's toys, eating utensils, dishes, clothing, or bed-linen may convey the disease. The infecting agent is the small pieces of membrane coughed up by the patient. They may be so small as to be invisible, and when they lodge on the clothing of the nurse or physician, or on the furniture or hangings in the room, they escape notice. When the patient sneezes or coughs, a cloth saturated with bichloride should be held before the face. Infection from this source should be guarded against very carefully.

It is very unwise, as well as dangerous, for the physician, when examining the throat, to stand directly before the patient. The examination usually induces a coughing spell or gagging severe enough to detach small pieces of membrane, which lodge on the clothing, hair, or beard of the physician. He may escape the infection, but he serves as a walking incubator, and any one coming in contact with him may become infected.

It should be borne in mind that in many cases of diphtheria the throat manifestations are very mild, and the disease may not be suspected. For this reason it is advisable to regard all cases of angina with suspicion until their identity has been established. It has been suggested that the infection may be conveyed through milk, although that mode would seem rather improbable unless a piece of membrane was accidentally deposited in the milk.

Cats may be a frequent source of infection. Their wandering propensities predispose them to infection from any case in the vicinity, and when a child plays with the cat it may contract the disease. During diphtheria epidemics, or when cases are known to exist in the immediate neighborhood, it is well to watch these household pets carefully, and children should not be allowed to play with them.

The *toxin* of the diphtheria bacillus is intensely poisonous. A toxalbumin has also been isolated. The chemical composition of the toxin is unknown. It is obtained by filtering bouillon cultures through a porcelain filter. It is destroyed in five minutes by boiling. When kept in a cold dark place it retains its toxicity for years.

Immunization and therapy: Behring was the first to discover that the blood-serum of animals immunized to diph-

theria contains a substance which neutralizes the effects of the diphtheria bacillus and its toxin. Intensely virulent bacilli are grown in alkaline bouillon in the incubator for five to seven days until the medium is strongly alkaline. A 0.4 per cent. solution of trikresol is then added and the mixture filtered through porcelain. The filtrate contains the toxin, which is used for immunizing purposes.

One attack of diphtheria confers immunity, which is, however, of only temporary duration. If it is desired to immunize persons who have been exposed to the disease, only a small amount of the antitoxin is injected. Five hundred units will confer protective immunity for about six weeks. This injection should be made as promptly as possible, as it will otherwise fail of producing the desired result. For therapeutic purposes a larger amount—that is, a stronger serum—must be used.

The *antitoxin treatment* of diphtheria is an absolute specific, and when used properly never fails to give the results which are claimed for it. Notwithstanding the opinion of those who are still prejudiced against it, the antitoxin should be used in every case of diphtheria. Its use is never followed by untoward results. The reported results which ostensibly contraindicate the use of antitoxin are entirely due to faulty administration and to its being used too late. It may be well to refer briefly to its correct use.

Use of antitoxin: Most important of all is that it should be used early; and, second, in sufficient strength. Although only a short time is required for making a bacteriologic diagnosis, it is better, in order to be on the safe side, to use the serum at once if diphtheria is suspected. Even if the microscopic diagnosis negatives diphtheria, the injection of serum will not have done any harm. The most common mistake made in using the serum is in waiting until the medicinal treatment has failed before the serum injection is made. Too many practitioners look upon antitoxin as a last resort, and when used as such it proves ineffectual. In severe cases of diphtheria death usually results; but if the serum has been used, it is sure to be blamed for the fatal outcome. Perhaps the case would have been saved if the serum had been used in time.

The most thorough antisepsis should obtain when the serum is injected. Many of the unsatisfactory results following injection can be ascribed to a careless injection. Abscesses may develop at the site of the injection and death may follow from septicæmia or even pyæmia. These are the cases that are "made worse" by the injection. A hypodermic injection should always be looked upon as a surgical procedure, and the strictest asepsis carried out.

The injection can be made into the buttock or the loose tissues of the abdomen, but the best place is the subcutaneous tissue of the back between the shoulder-blades. The patient should be held absolutely quiet during the injection. The child can be wrapped in a sheet or cloth, and held by the assistant in such a manner that only the back of the patient is exposed. The instruments, hands of the operator, and the site of inoculation are rendered sterile. The smallest quantity of antitoxin, consistent with the dose it is desired to inject, should be used. The injection should be made slowly and the needle withdrawn gradually, the skin at the puncture being held firmly between the first finger and thumb of the left hand. By gently manipulating the tissues absorption of the antitoxin will be facilitated considerably. The wound is sealed with collodion and covered with a protective dressing. It is useless to give the antitoxin by mouth, because it is digested in the alimentary canal without exhibiting any action.

No matter what the age of the patient, the *initial dose* should be from 1500 to 2000 units. In very severe cases at least 3000 units should be used. If the reaction does not occur within twenty-four hours after the injection is made, another 1000 units should be injected. This may be repeated, if necessary, on the following day. It is a matter of record that since the advent of the antitoxin treatment of diphtheria the mortality from this disease has been reduced at least one-half; and in the hands of some clinicians the percentage of fatalities is even less. The necessity for intubation or tracheotomy has been correspondingly diminished; and in cases in which these procedures have been performed the mortality is less now than what it was when the antitoxin was not used.

The observation has been made that *paralysis* occurs more often after the use of antitoxin than when it is not used. McFarland has studied this subject very carefully, and arrives at the conclusion that this is to be expected, as these palsies usually follow very severe cases, and that they would have occurred anyway no matter whether or not the antitoxin was used; so that the antitoxin is not responsible for the paralyses. Furthermore, he says that these cases were so severe that they would not have recovered even if the antitoxin had not been used. In cases in which a large amount of toxin has been elaborated and absorbed, the antitoxin is not able to neutralize all the toxin, and the result is *nil;* but when used in sufficient quantity it never fails to produce the expected results.

If small quantities of a high grade of antitoxin are used, the skin rashes which occasionally follow the administration of antitoxin are not so apt to occur as when large quantities of an antitoxin having a low degree of immunizing power are used. These skin rashes are of no significance, however, so far as the course of the disease is concerned; but they may be exceedingly annoying, as, for instance, an extensive urticaria.

Bacteriologic diagnosis: A bacteriologic diagnosis should be made as soon as the case is seen. For the sake of convenience, special diphtheria diagnosis outfits are put up by nearly all boards of health. Each outfit comprises a small platinum box, containing blood-serum, which is sealed with a heavy rubber band to prevent evaporation of the medium; and a swab or inoculator consisting of a small iron rod or wooden stick, one end of which is wound with cotton. This inoculator is absolutely sterile, and is enclosed in a sterile glass tube. A wooden tongue-depressor completes the outfit, which is placed in a heavy Manilla envelope. The latter may be opened conveniently without destroying it, and on the outside is stamped the date after which it is no longer advisable to use the culture-medium.

A small portion of the membrane is removed from the throat with the inoculator and transferred to the culture-medium. If no membrane is visible, the inoculator should

be thoroughly but gently rubbed over the inflamed mucous membrane of the fauces and a smear made on the blood-serum. The tongue-depressor is burnt immediately after it has been used, likewise the swab. The platinum box is sealed with the rubber and placed in the incubator for from nine to twelve hours, when the surface of the blood-serum is seen to be studded with a number of small white translucent colonies, from which a slide is prepared.

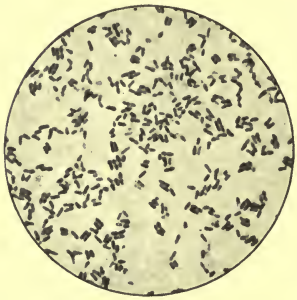
As most physicians do not possess an incubator, it will suffice to carry the box containing the culture in the waist-coat pocket or as near the body as possible; or it can be placed in the axilla and strapped there. This makes an efficient incubator. If the inoculation is made at night, the box can be placed in the pocket of the night-gown. In the morning the culture will have developed sufficiently to be examined. Ohlmacher says that sufficient growth has taken place at the end of five hours to permit of a diagnosis. The growth is not visible, but if a sterile platinum needle is carefully rubbed over the surface of the blood-serum and mixed with a drop of sterile water on a slide, the diphtheria bacilli will be found in the stained specimen.

A diagnosis can also frequently be made by examining a stained piece of membrane. It is not necessary to tear off a large piece of membrane. The bacteria are in the superficial layer, and a little scraping of membrane will suffice for examination. In an emergency a piece of membrane can be well wrapped in a cloth and the inoculation or examination made as soon as the means are at hand. In large cities diphtheria examinations are made in the municipal laboratory. The inoculated boxes and a special blank properly filled out are sent in by the physician, and a report can be had the next day.

Pseudodiphtheria Bacillus.

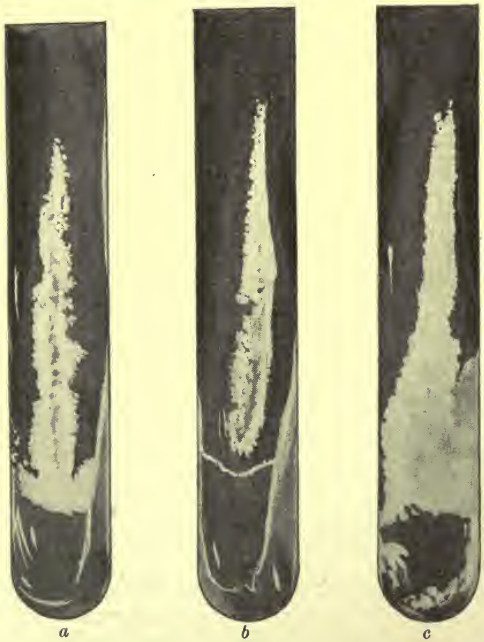
This organism resembles *Bacillus diphtheriæ* so closely in every respect that the question has been raised as to whether this organism is not the diphtheria bacillus in an attenuated form (Figs. 101 and 102). It has been found in the diph-

FIG. 101.



Small type of pseudodiphtheria bacilli. × 1000. (Park.)

FIG. 102.



a *b* *c*

Colonies: Diphtheria bacilli: *a*, pseudobacillus; *b*, true bacillus; *c*, pseudobacillus. (Dunham.)

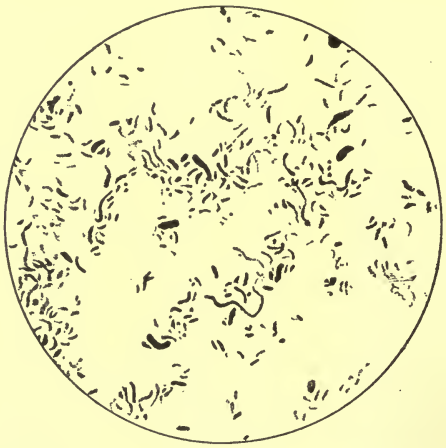
theritic membrane, in healthy throats, in the nose, on the skin, and on the conjunctivæ, especially in xerosis conjunctivæ. It has also been found in impetigo, acne, pustules of variola, in gangrene of the lung, and in pneumonia. The pseudodiphtheria bacillus does not elaborate a toxin. It is not virulent.

CHAPTER IX.

SPIRILLUM CHOLERÆ ASIATICÆ.

THIS organism, also known as the **cholera vibrio** or the **comma bacillus** of Koch, was discovered in 1883 in the dejecta and intestines of cholera patients. It is the accepted specific cause of Asiatic cholera. It is never found in the healthy

FIG. 103.



Spirillum of Asiatic cholera, from a bouillon culture three weeks old, showing numbers of long spirals. × 1000. (Fraenkel and Pfeiffer.)

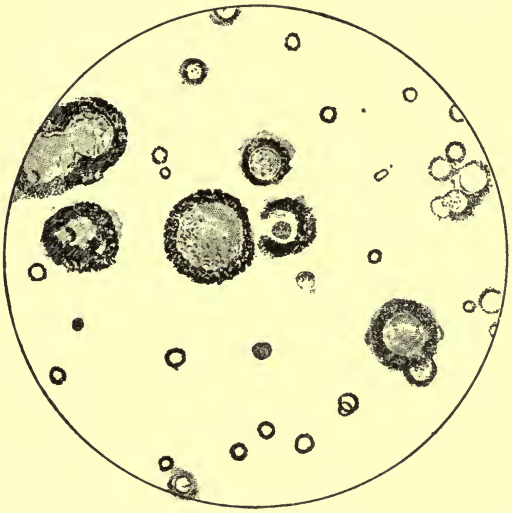
human body nor in any other part of the cholera patient except the intestines and their contents.

Morphology and biology: The cholera bacillus is a very small, slightly bent rod, with rounded ends, and resembles a comma, whence its name (Fig. 103). When two of these organisms

are joined end to end they resemble the letter S (Fig. 103). Long chains are occasionally formed in culture (Fig. 106).

The cholera bacillus can be *stained* with the anilin dyes, but with some difficulty. It stains best in a hot carbol-fuchsin solution. It does not stain by Gram's method. It is exceedingly motile and has terminal flagella. It is one of

FIG. 104.



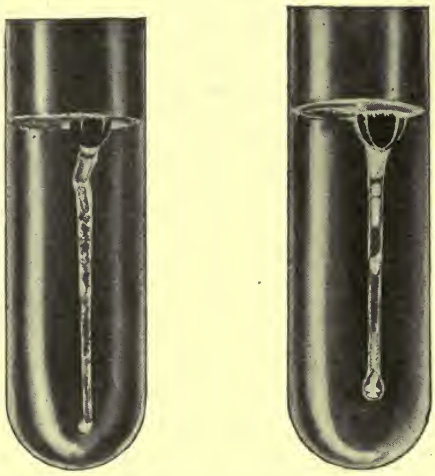
Spirillum of Asiatic cholera: colonies two days old upon a gelatin plate. × 35. (Heim.)

the monotrichia, which have only one or two flagella projecting from one end. It does not form spores, although Hueppe believed that arthrospore-formation existed.

It is strongly *aërobic*, growing quite readily on all culture-media either at the room or the body temperature, but the medium must be slightly alkaline, as the cholera spirillum is quite sensitive to even small amounts of acid.

Schottelius gives the following method for making *pure cultures* of the cholera germ: A small quantity of the intestinal mucus or fecal matter is transferred to a flask or tube

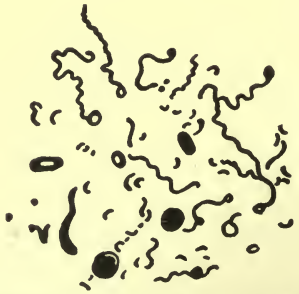
FIG. 105.



Spirillum choleræ Asiaticæ: gelatin puncture-cultures aged forty-eight and sixty hours. (Shakespeare.)

containing bouillon which is slightly alkaline, and placed in the incubator for twenty-four hours. The growth forms

FIG. 106.

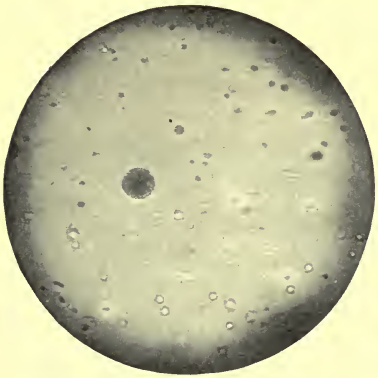


Spirillum choleræ Asiaticæ: involution-forms. × 700. (Van Ermengen.)

rapidly in the shape of a luxuriant surface membrane. From this membrane plate cultures are made.

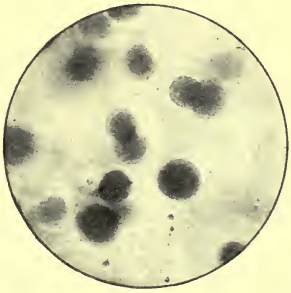
In *gelatin plates* colonies appear within twenty-four hours (Figs. 104 and 107). They are very small, granular, with irregular borders, of a whitish color, and develop in the medium.

FIG. 107.



Cholera colonies in gelatin: twenty-four hours' growth. (Dunham.)

FIG. 108.



Cholera colonies in gelatin: thirty-six to forty-eight hours' growth. × about 30. (Park.)

These colonies gradually turn from white to yellow and from yellow to brown, at the same time liquefying the gelatin quite rapidly until each colony occupies a small conical depression. The old colonies are very granular and glistening (Fig. 108).

FIG. 109.

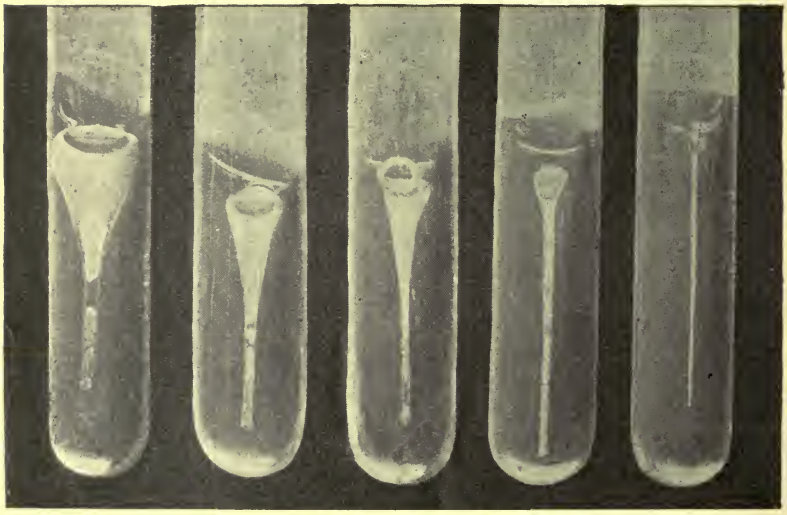
Cholera colony in gelatin. × 30. (Dunham.)

Their appearance at this time is that of a dirty granular mass strewn with small bits of glass. Each colony is surrounded by a transparent halo (Fig. 109). The colony gradually sinks

deeper into the medium as liquefaction progresses, and at last turns a very peculiar pink.

The *gelatin stab* culture is also quite characteristic. The growth first takes place along the entire needle-track, being more profuse on and near the surface, where the supply of oxygen is greater. The liquefaction of the gelatin keeps pace with the growth, beginning at the top as a small funnel-shaped

FIG. 110.



A characteristic series of cholera cultures in gelatin: one, two, three, four, and six days' growth. (Dunham.)

depression. This funnel or inverted cone gradually increases in size until all the gelatin is liquefied. The culture slowly settles to the bottom of the funnel, the end of which usually shows a slight bulbous enlargement (Figs. 105, 110, and 111). The liquefied gelatin evaporates slowly, so that the funnel is empty at the top. It looks as though it contained an enormous air bubble. At the end of four or five weeks liquefaction of the gelatin is complete. According to different

authorities, the organism remains alive in the gelatin tube for from eight weeks to two years.

On *agar-agar* stroke cultures a shining, moist, grayish-white growth develops along the line of inoculation (Fig. 112). The growth on *blood-serum* is exactly like that on agar, but the blood-serum is liquefied.

Bouillon, even when very dilute, is soon clouded, with the formation of a surface membrane. The culture in *milk* is destroyed by the formation of lactic acid. On *potato* a grayish-brown, translucent, very delicate membrane is formed, even when the potato is acid.

If it is desired to preserve the cholera spirillum for a long time, it should be transplanted into *sterilized water*, in which it develops quite rapidly and persistently. It also retains its vitality for a long time if wrapped in moist linen.

The cholera germ also produces *nitrites* and *indol*. The addition of a few drops of sulphuric or hydrochloric acid to cholera cultures containing peptone gives a beautiful red color, the so-called *cholera-red reaction*, which is characteristic of this organism. The cholera bacillus is one of a very few germs which give the *nitroso-indol* reaction. This is used as a means of differentiation.

Vitality: The cholera spirillum possesses little resisting power. A four minute exposure to a temperature of 52° C. is fatal. Drying also destroys it very quickly—within a few hours. The addition of a very small quantity of acid to the culture-medium immediately inhibits the growth of the organism. Pettenkofer claimed that when the cholera organism is brought in contact with normal gastric juice it is destroyed immediately. He demonstrated the truth of this by swallowing a pure culture of cholera. In the presence of a little moisture the germ retains its vitality for a long time. When associated in culture with saprophytic germs its development ceases. Freezing destroys the germ in a few days. A 10 per cent. solution of mercuric chloride or a 3 per cent. solution of carbolic acid is effective immediately. Milk of lime and sulphate of copper are excellent disinfectants for choleraic discharges. High temperatures always favor the development of the germ.

Pathogenesis: Cholera is pathogenic for man only. The disease is endemic in India, and all epidemics of cholera have had their origin in that country. The habits of the Hindoos and their religious customs are largely responsible for the constant presence of this disease. Infection follows ingestion of the germ in either water or food. The disease is probably never conveyed through the air, because the bacillus succumbs so rapidly to desiccation. Furthermore, the germ must find its way to the intestinal tract before the disease will develop. It is possible that small numbers of the germ may be swallowed with impunity; but it is more probable, in view of its feeble resistance to the mineral acids, that there must be some abnormal condition of the stomach which is accompanied by a diminution in the secretion of hydrochloric acid or its total suppression, before infection can take place.

The cholera germ multiplies rapidly in the intestinal tract, with elaboration of its toxin, which is absorbed and gives rise to the symptoms of the disease. All the pathologic lesions of cholera are due to this absorption of the toxin.

Infection: The stools of the cholera patient are the infecting medium, and soiled clothing or bed-linen is the usual means of conveying the disease to others. Emptying the dejecta into a sewer or depositing them on the ground without disinfecting them, causes contamination of the water-supply if the sewerage is imperfect or when the water is obtained from wells or from small streams or creeks.

Insects which feed on the dejecta may carry the germs and deposit them on food or in water many miles distant from the original source of the disease.

Pollution of the water-supply is by far the most common and also the most dangerous source of infection. The famous epidemic of cholera in London had its origin in the pollution by cholera dejecta of the water-supply derived from the Broad Street pump. Nearly every individual using this water became infected with the disease.

In India the natives bathe in the river Ganges as a religious practice, and the dead are also buried in the river no matter what the cause of death may have been. At those times of

the year when the Hindoos flock to the Ganges to worship they live in crowded camps, and under these circumstances cases of cholera are always very numerous.

Pettenkofer believes that the disease is never transmitted from one individual to another, but that the germ must first mature in the earth for a certain length of time before infection can occur. He also believes that infection occurs through the respiratory tract. His theory is known as the *ground-water theory.*

Garden vegetables sprinkled with water containing the cholera germ; and milk which is either diluted with or contained in cans that have been washed in infected water, may

FIG. 111.

Stab cultures of three cholera spirilla in gelatin, showing in upper portion of growth considerable liquefaction of nutrient gelatin. (Park.)

be the source of infection. A number of investigators have become infected with pure cultures of cholera while conducting experiments in the laboratory on animals.

Immunity: The serum of the blood of animals that have recovered from cholera contains a substance which has decided bactericidal properties. This substance is not an antitoxin. The immunity is entirely dependent upon lysogenicity, or the formation of lysogenic bodies in the blood which possess a

bactericidal action. This substance is not found in the blood until after the first week of convalescence, and disappears within four weeks. There is no natural immunity to cholera. Koch believes that one attack conveys a permanent immunity. The blood-serum of immunized animals confers immunity.

Immunity in man is produced by a form of *vaccination* which was first proposed by Haffkine. He begins by injecting a dead culture, and five days afterward he follows it with an injection of a virulent culture, which is repeated on the tenth day. The immunity obtained in this way is not permanent, but serves merely as a prophylactic measure or a protective during epidemics of cholera. The production of immunity to cholera by means of an antitoxin which causes

FIG. 112.

Contact smear of colony of cholera spirilla from agar. ×700. (Dunham.)

the formation of lysogenic bodies in the blood is as yet a matter of dispute, although Haffkine's results have been very good. It is at best only a protective, the efficacy of which cannot be depended upon at all times.

Diagnosis: A flake of intestinal mucus is spread on a slide and stained with a hot carbol-fuchsin solution. A positive diagnosis can be made in about 50 per cent. of all cases. On the whole, it is preferable to cultivate the organism, as the results obtained in this manner are positive in each case. Pure cultures are made as already described. The distinctive appearance of the plate culture, the gelatin stab culture, and the cholera-red reaction are sufficiently characteristic to be of diagnostic value. Animal experimentation may also

be resorted to in making a diagnosis. A small quantity of the intestinal discharge is injected into the abdominal cavity of a guinea-pig, and in the presence of the cholera germ a choleraic peritonitis develops.

Cholera Nostras and Cholera Morbus.

A large and varied number of bacteria have been found in the intestinal discharges of patients affected with cholera nostras and cholera morbus, *e. g.*, members of the so-called colon group, especially *Bacterium coli communis.* Streptococci have also been found and a large variety of vibrios, but not the cholera vibrio. The acute enteritis of children, commonly known as summer complaint, is believed to be due to *Bacterium lactis* (of Fluegge) or a peptonizing milk bacterium.

At the present writing, reports are current of the discovery of a specific germ in summer complaint, but no authentic statement has as yet been published in this matter, and it is, therefore, impossible to give even a brief description of this organism. The meagre information at hand indicates that the germ is in all probability *Bacillus dysenteriæ* or some member of the colon group.

CHAPTER X.

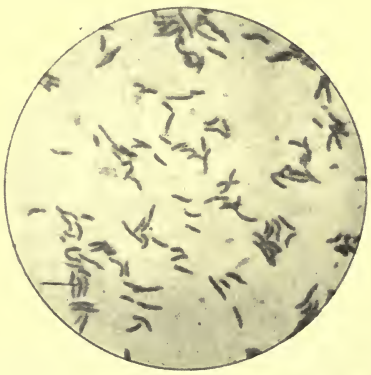
ORGANISMS RESEMBLING THE CHOLERA SPIRILLUM.

Spirillum of Finkler-Prior (Vibrio Proteus).

This germ was found in the intestinal discharges of patients sick with cholera nostras.

Biology and morphology: The Finkler-Prior spirillum resembles the cholera organism very closely in contour, but is much shorter and thicker, and it does not form such long chains and spirals. Its ends are pointed and it bulges slightly in the centre. Extending from one end of the

Fig. 113.



Spirillum of Finkler and Prior. × 1100. (Park.)

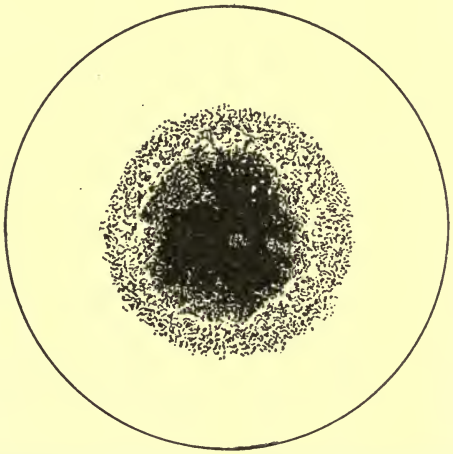
organism is a single flagellum (Fig. 113). The spirillum is exceedingly motile. It does not form spores. The ordinary anilin dyes are used in staining.

It is a facultative anaërobe, growing readily on all culture-

media. Its growth in gelatin is characterized by an extremely rapid and characteristic liquefaction of the medium.

On *gelatin plates* small white colonies form in the medium. These colonies are finely granular, with very sharply marked borders and of a yellowish-brown color, which is more intense in the centre than at the periphery of the colony. They are surrounded within a few days by a zone of liquefaction. These colonies can be differentiated from those of the cholera spirillum by a more sharply defined border and

FIG. 114.



Spirillum of Finkler and Prior: colony twenty-four hours old, as seen upon a gelatin plate. × 100. (Fraenkel and Pfeiffer.)

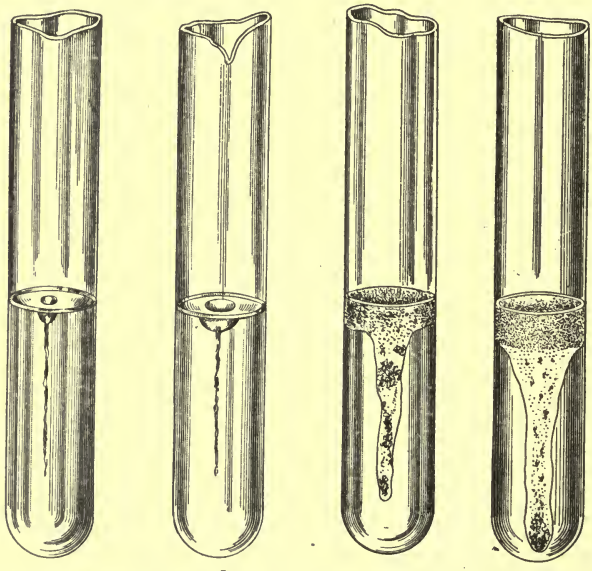
a darker color, and they are also less granular (Fig. 114). After a time they resemble each other so closely that differentiation is impossible.

In the *gelatin stab* liquefaction progresses very rapidly and forms a typical stocking-shape, which is filled with a cloudy liquid. The surface of the medium is usually covered with a thick whitish membranous growth. Liquefaction occurs much more rapidly than in cholera cultures. The stocking-shaped liquefaction is peculiar to this organism, and while

the liquefied gelatin is clouded in this culture, in the cholera culture it is perfectly clear, the culture having settled to the bottom of the liquefaction (Fig. 115).

The growth on *agar* is very luxuriant. It forms a heavy slimy whitish membrane which soon covers the entire surface of the medium. On *potato* an extensive moist grayish-yellow

FIG. 115.



Stab culture of the Finkler-Prior bacillus in gelatin, at 18° to 20° C.: *a*, after twenty-four hours; *b*, after forty-eight hours; *c*, after seventy-two hours; *d*, after ninety-six hours. (Abbott.)

coating is formed. The growth on potato develops at the room temperature, whereas the cholera spirillum will not develop on potato at a temperature lower than that of the body. Even then the growth is very slight and of a brownish color.

Blood-serum is liquefied rapidly. The growth in *milk* is

very slight. No growth occurs in either tap-water or sterilized water. There is no cholera-red reaction. When grown in a medium containing glucose, acid is produced. The organism resists desiccation much better than the cholera spirillum.

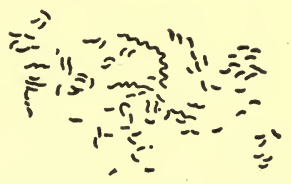
Pathogenesis: Although the organism is pathogenic for some animals, it does not appear to be the cause of disease in man. It is most frequently found in the intestinal discharges of persons suffering from diarrhœa or cholera nostras. It may occasionally appear in the feces of healthy patients.

Spirillum Deneke (Vibrio Tyrogenum).

Deneke obtained this organism from old cheese.

Biology and morphology: The *Spirillum Deneke* resembles the cholera spirillum even more than does the Finkler-Prior spirillum. It is a very short curved rod, a number of which may form tightly coiled chains and spirals (Fig. 116). It is

FIG. 116.



Spirillum tyrogenum. × 700. (Fluegge.)

flagellated and actively motile; stains like the cholera bacillus; grows equally well at the room and body temperature; and does not form spores. It is a facultative anaërobe.

The colonies on the *gelatin plate* differ from those of cholera in that they develop more rapidly, are of a yellowish-green color, and are irregular in contour, with sharply defined borders (Fig. 117). The medium is also liquefied more rapidly.

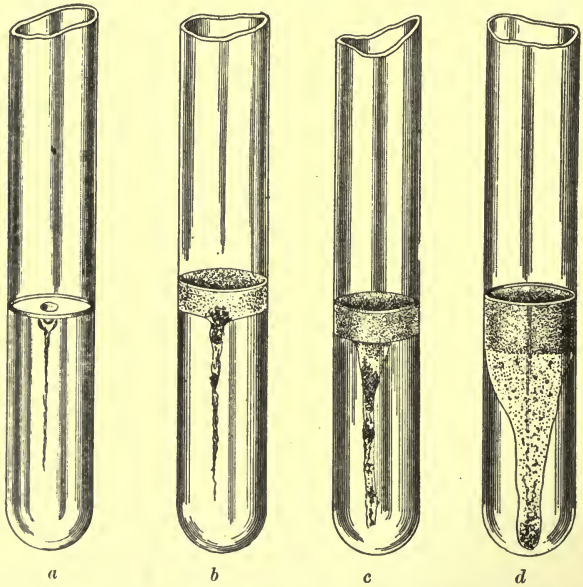
In the *gelatin stab* the stocking-shaped liquefaction is formed, which is filled with the cloudy liquid, the culture col-

FIG. 117.

Spirillum tyrogenum, colonies on gelatin plate: *a*, end of sixteen hours; *b*, end of twenty-four hours; *c*, end of thirty-six hours. × 80. (Fluegge.)

lecting in the bottom in the form of a coiled mass. The gelatin is liquefied completely in about two weeks (Fig. 118).

FIG. 118.



Stab culture of Deneke's cheese spirillum in gelatin, at 18° to 20° C.: *a*, after twenty-four hours; *b*, after forty-eight hours; *c*, after seventy-two hours; *d*, after ninety-six hours. (Abbott.)

A very sparse yellowish membrane is formed on *agar*.
Blood-serum is rapidly liquefied.
A very luxuriant moist yellow film, containing many long

spirals, is formed on *potato* when grown at the body temperature. No indol is produced.

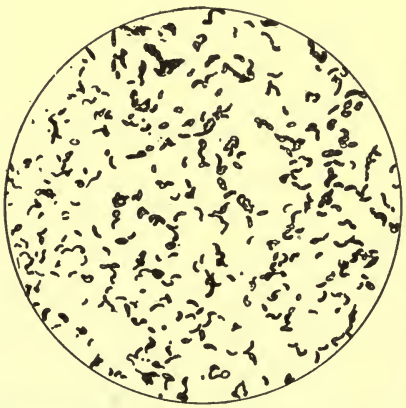
The *Spirillum Deneke* is of absolutely no importance clinically except for its resemblance to the cholera germ. It has never been found in the secretions or excretions of either man or animals.

Spirillum Metschnikovi (Spirillum of Gamaleia).

This organism was discovered in the excretions and intestinal canal of chickens affected with a diarrhœaic disease epidemic in southern Russia during the summer months.

Biology and morphology: The *Spirillum Metschnikovi* is shorter, thicker, and more curved than the cholera spirillum. It forms long chains and spirals, is motile, and possesses a

FIG. 119.



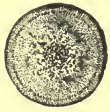
Spirillum Metschnikovi, from an agar-agar culture. × 1000. (Itzerott and Niemann.)

terminal flagellum. It does not form spores. It is only feebly resistant to heat and chemicals. It grows at both the body and the room temperatures: stains with all the anilin dyes, but not with Gram. It is a facultative anaërobe.

Gelatin plate colonies appear in about twelve hours as small

whitish dots (Fig. 120), which rapidly increase in size to form yellowish-brown masses. When liquefaction occurs, the

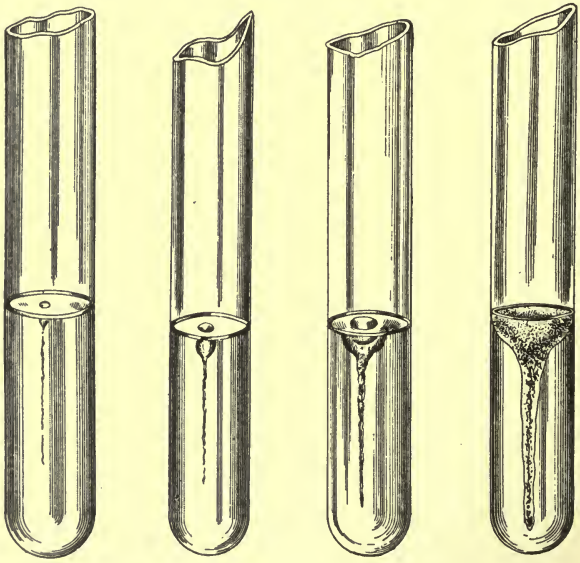
FIG. 120.



Colony of *Vibrio Metschnikovi* in gelatin, after thirty hours at 20° to 22° C. × about 75. (Abbott.)

colony occupies a shallow, saucer-shaped depression. The edges of the colony are irregular and fringed with radiating filaments.

FIG. 121.



Stab culture of *Vibrio Metschnikovi* in gelatin, at 18° to 20° C.: *a*, after twenty-four hours; *b*, after forty-eight hours; *c*, after seventy-two hours; *d*, after ninety-six hours. (Abbott.)

In *tube cultures* on agar, gelatin, and blood-serum the growth closely resembles that of cholera, but it develops more

rapidly (Figs. 119 and 121). On *potato*, at the incubator temperature, a heavy yellowish-brown or chocolate-colored growth develops.

Bouillon becomes clouded and opaque, a thin wrinkled membrane forming on the surface. The spirillum is not gas-producing, but forms acid very rapidly. *Milk* is coagulated. The *cholera-red reaction* is very apparent.

The organism is highly **pathogenic** for animals, especially chickens, but not for man.

Other Organisms which Resemble the Cholera Germ.

Spirillum Berolinensis was discovered by Neisser in river-water. It very closely resembles the spirilla already described. It is not pathogenic for man.

Spirillum Dunbar is also a water vibrio. It differs from the cholera organism in its staining reaction. It stains very poorly and exhibits polar bodies. Cultures grown at low temperatures are said to be phosphorescent.

Spirillum Danubicus, found in water; **Spirillum Wernicke** and **Spirillum Bonhoffi**; **Spirillum Weibeli**; **Spirillum Milleri**; **Spirillum Aquatilis**; **Spirillum terrigenus,** and **Vibrio Schuylkiliensis,** are all non-pathogenic for man, and are met only occasionally. Many of them have not been described since they were discovered. Most of them are found in water, a few in the soil.

The absolutely *characteristic growth* of the **cholera organism** will in each instance serve to differentiate it from these less common forms.

CHAPTER XI.

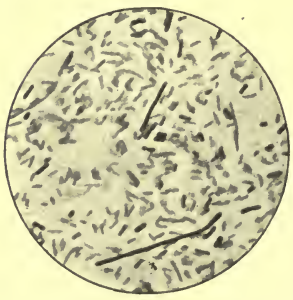
BACILLUS TYPHOSUS.

(Bacillus Typhi Abdominalis; Bacillus of Eberth; Eberth-Gaffky Bacillus.)

THE **bacillus of typhoid fever** was discovered by both Eberth and Koch, in 1880, in the spleen and mesenteric lymph-glands of typhoid cadavers, and was first isolated and studied in pure cultures by Gaffky four years later.

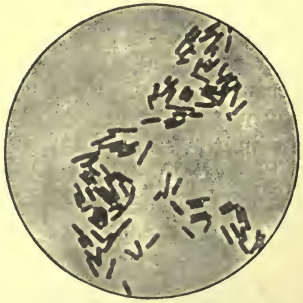
Biology and morphology: *Bacillus typhosus* is a small thick rod, with pointed ends and from ten to eighteen terminal and lateral flagella. It is exceedingly motile. It measures from

FIG. 122.



Typhoid bacilli from nutrient agar. × 1100. (Park.)

FIG. 123.

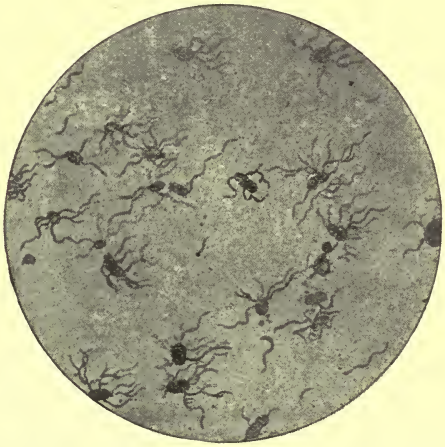


Typhoid bacilli from nutrient gelatin. × 1100. (Park.)

1 μ to 3 μ in length, and from 0.5 μ to 0.8 μ in width. There is no evidence of sporulation. The organism is extremely variable in cultures. In specimens made directly from the tissues or typhoid excreta the germ usually occurs singly; but in culture specimens it is frequently seen to form chains, both long and short (Figs. 122, 123).

The bacillus can be *stained* with all the anilin dyes, but must be exposed to the action of the stain for a long time. To facilitate staining, it is advisable to use slightly warmed staining solutions. It is decolorized easily, and for that reason should not be washed in anything but water. Heavily staining granules have been seen in the body of the organism, and also polar granules. These were at one time believed to be spores, but because of their feeble resistance to heat and chemicals this view is untenable. They are undoubtedly

FIG. 124.



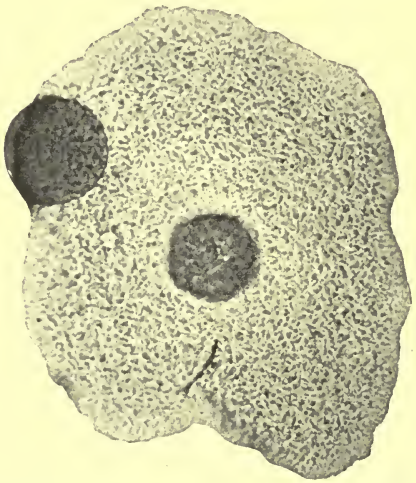
Bacillus typhi abdominalis, from an agar-agar culture six hours old, showing the flagella stained by Loeffler's method. × 1000. (Fraenkel and Pfeiffer.)

polar granules or vacuoles caused by the drying and fixing of the specimen preparatory to staining. Gram's stain is not applicable. The flagella (Fig. 124) are easily stained by the usual methods. The typhoid bacillus is used to demonstrate the existence of flagella on bacteria.

Bacillus typhosus is both *saprophytic* and *parasitic*. It is a facultative anaërobe, but has very strong aërobic tendencies. It will develop equally well at both the room and body temperature, although the growth is most luxuriant at the latter.

On *gelatin plates* both deep and superficial colonies develop. The deep colonies possess no particular distinguishing feature. They are quite small, round, and finely granular. They are light brown in color. The superficial colonies are much larger, transparent, with irregular, serrated edges, and bluish-white in color. The colonies are usually described as having the shape of a grape leaf (Fig. 125). The centre of the colony is a very light yellowish-brown; the periphery is

FIG. 125.



A superficial and a deep colony of typhoid bacilli in gelatin. × 50. (Park.)

colorless and presents a reticular arrangement. The gelatin is not liquefied.

In *Elsner's medium* the colonies of the colon bacillus appear within twenty-four hours, whereas those of the typhoid bacillus do not appear until after forty-eight hours. The typhoid colonies are very small, round, and finely granular. Unfortunately for purposes of differentiation the colonies of the colon bacillus occasionally come out in successive crops, so that they may be mistaken for typhoid colonies. Close watching will aid in the differentiation.

In *gelatin stab* cultures the principal growth occurs on the surface of the medium. Along the track of the needle development is extremely slight. The surface growth resembles the superficial colonies on the plate. The gelatin is not liquefied.

Stroke cultures on agar-agar and blood-serum are not characteristic. A thin transparent grayish coating is formed along the line of inoculation. *Bouillon* is clouded, with the occasional formation of a delicate surface membrane.

The growth on *potato* is quite characteristic. It is usually referred to as the "invisible growth." Even after two or three days there is no apparent growth on the surface of the potato, but when the platinum needle is slowly and gently passed over the surface it meets with resistance, due to a heavy moist but absolutely colorless film which has formed. By reflected light the growth is made visible. Occasionally the growth is of an ochre color, or even brown with a greenish tinge. This growth resembles that of the colon bacillus on potato.

The typhoid bacillus flourishes in *milk* without changing the medium in any way. At times it may produce a very slight amount of acid, although as a rule it is not acid-producing. Neither does it cause fermentation. It does not produce aromatics. These characteristics serve to identify the typhoid bacillus, and to differentiate it from the colon bacillus, which resembles it in every other respect.

Vitality: One of the peculiarities of this organism is its resisting power. It will remain alive in distilled water for three months. In ordinary water the bacilli disappear within a week or two because of the vigorous growth of the other bacteria contained in the water. In quiescent pools of water the typhoid bacillus will remain alive for a month. When contained in water for any length of time, its appearance is so changed that recognition is impossible. It has been found to retain its vitality in milk for five weeks. Transplanted into the upper layers of the soil, it remains alive for nearly six months. When nutrient bouillon is poured over the soil the germ will retain its vitality for twelve months. In feces it

persists for three or four months if not too many other saprophytic germs are contained in it.

Repeated freezing and thawing does not affect the vitality of the germ, but a ten-minute exposure to a temperature of 60° C. is invariably fatal. Neither can it resist desiccation. Carbolic acid, in a 1 or 2 per cent. solution, has no effect on the germ. This resistance to carbolic acid is utilized in obtaining pure cultures. Dried in a thin layer, the typhoid bacilli preserve their vitality on linen for from sixty to seventy days, on wood for thirty-two days, and on buckskin for eighty days. Hermetically sealed bouillon cultures remain alive for more than a year.

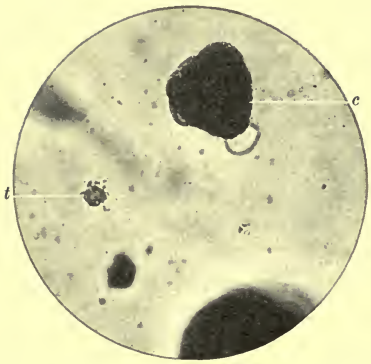
Isolation: *Pure cultures* can be procured from the feces of typhoid patients, but not until the second week, and even then with difficulty unless the feces contain very few contaminating germs. Repeated attempts may have to be made before the organism is finally isolated.

It is preferable to make the culture from the *spleen* or the *mesenteric lymph-glands* or *Peyer's patches* of typhoid fever cadavers, but the autopsy must be made as soon as possible.

The *method* of making the pure culture is as follows: Add a 0.05 per cent. solution of carbolic acid to each of several tubes of liquefied gelatin. A small piece of tissue or several loopfuls of feces are transplanted to tube No. 1. Tube No. 2 is inoculated from tube No. 1, and tube No. 3 from tube No 2. The contents of each tube are then plated or rolled. The saprophytes do not develop because of the carbolic acid which has been added to the medium, but the growth of the typhoid bacillus and *Bacillus coli communis* is not interfered with in the least. The colonies of these two varieties must be differentiated.

In order to aid in this *differentiation*, Elsner uses a special medium for plating. It is prepared as follows: 1 kilogram of potato, preferably the small, red, German variety, is macerated in 1 liter of water for twelve hours. The juice is thoroughly expressed and filtered cold, to separate as much starch as possible. The filtrate is boiled and filtered again. The resulting clear fluid is neutralized by adding $2\frac{1}{2}$ to 3 c.c. of a decinormal solution of sodium hydrate to each 10 c.c. of

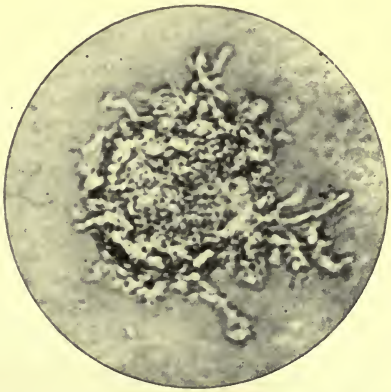
FIG. 126.



Hiss' plate media: small light colony (*t*) is composed of typhoid bacilli; large colony (*c*) of colon bacilli. (Hiss.)

juice. Either litmus-paper or phenolphthalein may be used to determine the reaction, which should be just perceptibly

FIG. 127.



Colony of typhoid bacilli highly magnified. (Hiss.)

acid. The solution is then heated and 10 per cent. of nutrient gelatin added, after which the medium is again neutralized

to the same point as before, filtered, and 1 per cent. of potassium iodide added. The typhoid and colon bacillus are the only organisms that will grow on this medium, the colonies of the colon bacillus appearing some time before those of the typhoid bacillus (Figs. 126 and 127).

Another method for isolating the typhoid bacillus from the feces is proposed by *Drigalski* and *Conradi:* Agar containing 2 per cent. of peptone and 1 per cent. of nutrose is used. To this are added 130 c.c. of litmus solution, containing 15 grams of milk-sugar, 2 c.c. of a 10 per cent. soda solution, and 10 c.c. per liter of "Krystall violett-Hoechst" solution. This medium is plated in Petri dishes. After it has hardened, the material is spread over the surface of the plate in a thin film. After this film has dried thoroughly the plates are placed in the incubator. In about fifteen hours the colonies are distinctly visible. Those of the colon bacillus are from 2 to 6 mm. in diameter and red in color. The typhoid colonies are about half that size, smooth, dew-drop-like, and blue. The colonies are examined microscopically and the results verified.

Pathogenesis: Typhoid fever is unquestionably due to *Bacillus typhosus,* although thus far it has been impossible to produce typhoid fever experimentally in animals. It is never found in the tissues of a healthy individual, and it is constant in the lesions of typhoid fever. During the convalescent period it can also be found in the excreta, especially the urine. When an animal is inoculated with a pure culture of the germ, it dies with symptoms of intoxication. In man, ingestion of the bacillus is followed by the typical disease, providing the conditions are favorable to the development of the organism. To all appearances, man possesses a certain degree of immunity to typhoid, and a special predisposition, either general or local, such as convalescence from some other disease, or an acute intestinal catarrh, is necessary before infection can take place.

Infection: Although infection may occur by way of the respiratory tract by inhaling dust containing the typhoid germ, yet the *digestive tract* is the usual portal of entry for the germ. The natural resistance of the germ to acids enables it

to pass through the stomach and to reach the intestinal tract, where it finds a lodgement and develops. The bacillus usually gains entrance into the body through the agency of food or drink, or infection may occur by coming in contact with any article which has become contaminated by the discharges of a person sick with typhoid, such as clothing, bed-linen, or eating utensils.

Epidemics of typhoid fever are usually due to contamination of drinking-water or milk by the alvine discharges of typhoid patients. The washing of milk-cans with water contaminated with the excreta of typhoids or with the typhoid bacillus is a common mode of infection. Several epidemics of unusual severity have been recorded which had their origin in the water used to wash milk-cans or to dilute milk. In such epidemics the cases of typhoid are usually scattered or occur in groups, distributed along the route of the milkman. Garden vegetables which have been sprinkled with infected water may also convey the disease; they should never be eaten uncooked.

Insufficient disinfection or careless disposition of the feces and urine of typhoids is the source of contamination of the water-supply. If the excreta of every case of typhoid fever were thoroughly disinfected before they are disposed of, the disease could be stamped out. Infection of the water-supply may occur in many ways. Occasionally it is extremely difficult to account for the occurrence of epidemics of typhoid fever, but a diligent search will always result in locating the source of the infection. It must be borne in mind that when the excreta are thrown on the ground or into a privy vault they invariably filter through the ground and in that way may reach the water-supply. Of course, modern sanitation and efficient drainage have done much to eradicate this evil, but in rural districts these do not obtain. A well or rain-water barrel may be on a lower level than the privy vault or sewer, or not far away from it, and the water-supply be polluted in that way. Every case of typhoid fever has its origin in a previous case, and it is important to find the source of contamination to prevent an epidemic of the disease.

Unhygienic surroundings are also an important factor in the causation of typhoid fever. Damp, dark, and dirty houses, and tenements especially, undermine the natural resisting power of the individual, and he more readily falls a prey to infections, particularly typhoid fever, which is a filth disease. Personal cleanliness means health and the power to ward off infections.

After the typhoid bacillus *has gained entrance to the intestinal canal* it lodges in the Peyer's patches and the solitary lymph-follicles in the mucosa of the bowel. Here it multiplies and produces the local symptoms of the disease. The infection travels through the lymphatics to the mesenteric lymph-glands, the spleen, liver, and kidneys. The bacillus may also make its way into the blood-current and into the bone-marrow and distant parts of the body. The bacilli have also been found in the rose spots. It is possible to make pure cultures of *Bacillus typhosus* from the blood and from the rose spots.

The *urine* frequently contains the bacilli, especially when the kidneys have been involved. The urine may be the source of infection. Even after convalescence, for as long as two or three weeks, the urine may contain the bacillus. It is fully as important to disinfect the urine thoroughly as it is to disinfect the feces.

The typhoid bacilli can always be found in the *gall-bladder;* occasionally in the lungs, meninges, heart, and testicles, especially when these organs are the seat of typhoid complications. The bacillus has been found in an abscess of the *brain*, and in other suppurative lesions that have developed in the course of or after an attack of typhoid fever.

The *lymphatic hyperplasia* seen so frequently in the internal organs, especially the liver and spleen, is due to the toxin elaborated by the typhoid bacillus. It was at one time assumed that the typhoid bacillus never left its habitat in the intestinal canal, and that the various complications were due to the toxin. This opinion is not held now, as the bacillus has been found and identified in the complicating lesions of typhoid.

Puncture of the spleen has been advocated as a means of

diagnosis in typhoid fever, as the bacilli make their way into this organ very early in the disease. The dangers connected with such a procedure are manifest, and prohibit the encouragement of any such method as a routine practice by the average practitioner.

In a very few instances the bacillus has been found in the *sweat;* and in the *sputum* and the *mucus* obtained from the throat. In typhoidal pneumonia—that is, a pneumonia due to the typhoid bacillus—this organism is constant in the *sputum.*

Mixed and secondary infection: Typhoid fever is rarely a pure infection. *Bacillus typhosus* is most frequently associated with *Bacterium coli communis* and the *streptococcus pyogenes.* The colon bacillus is held responsible for most of the complications of typhoid fever, such as peritonitis, cholangitis, etc. The streptococcus is the cause of otitis media, bronchopneumonia, and empyema. The severe prostration, resembling sepsis, occasionally seen in aggravated cases of this disease is invariably due to a mixed or secondary infection with the streptococcus. Staphylococci and diplococci, especially the pneumococcus, are occasionally associated with the typhoid bacillus. Nearly all the pulmonary complications, however, are due to either the streptococcus or *Bacillus typhosus.* The pneumococcus causes the secondary pneumonia, but not the complicating pneumonia.

Bacteriologic diagnosis: The close resemblance of the typhoid bacillus to the colon bacillus makes its diagnosis a matter of some difficulty. In fact, it can only be done by cultivating the organism and noting the cultural differences between the two. The bacillus is obtained from the feces, urine, blood, etc., and examined microscopically. The typhoid bacillus is more motile and has more flagella than the colon bacillus, but such differences are not very reliable. These two germs also stain alike. Their cultures are alike except on potato, on which the typhoid forms an invisible growth; and the colon bacillus a heavy brownish membrane with slight greenish discoloration of the potato.

Additional *points of differentiation* are the following:

(1) The colon bacillus coagulates milk; the typhoid does not.

(2) The colon bacillus evolves gas, especially in media containing grape-sugar; the typhoid does not.

(3) The colon bacillus gives the indol reaction when grown in Dunham's solution; the typhoid does not.

(4) The colon bacillus is distinctly acid-producing; the typhoid usually produces no acid or only a very slight amount.

Occasionally, however, varieties of either organism are met with that do not answer to these specific reactions, so that the differentiation can never be said to be *absolute*. The Widal test might be of assistance, but even here variations may occur. Pfeiffer's phenomenon is also of value in the differentiation.

When the bacillus is obtained from the *spleen*, the cultures are made from the fluid obtained from this organ. The method of puncture is the same as that used in all exploratory punctures, but the danger from sepsis is much greater, because the typhoid bacillus is capable of causing suppuration. The wound in the spleen may be the starting-point of a fatal septic peritonitis.

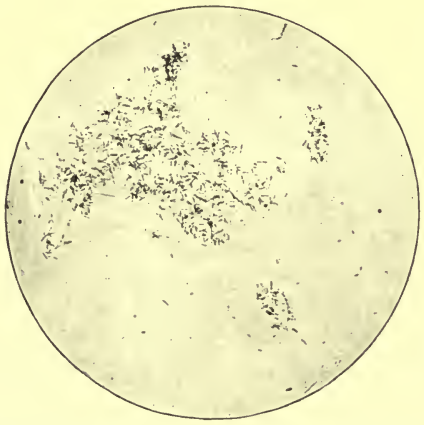
Widal reaction: A most valuable aid in the clinical diagnosis of typhoid fever is the Widal reaction or the agglutination-test. It is not an absolute test, but when carefully and properly performed it is of considerable diagnostic value. The blood-serum of the typhoid patient mixed with a pure culture of the typhoid bacilli yields the agglutination phenomenon of Gruber. The blood is obtained through an aseptic puncture of the tip of a finger or the lobe of the ear, or directly from a vein. If the blood thus obtained is not to be used immediately, it is allowed to coagulate on a sterile piece of isinglass; or in an emergency, on a piece of paper or on blotting-paper. The blood-serum retains its agglutinating power for months, and may, therefore, be sent any distance for diagnosis.

The test, as a rule, is not applicable until after the first week. Fraenkel observed the reaction in one case on the second day. It disappears during convalescence, although in

exceptional instances it has persisted for years. It is important to ascertain whether or not the patient has had a previous attack of typhoid fever, as in that event the reaction would not hold good for the illness which may be present at that particular time.

The Widal test is *performed* as follows (Widal, when he first described his method, advocated the use of fresh blood-serum, but since then Wyatt Johnson, of Montreal, suggested the use of dried serum): The blood is obtained as already described, and is mixed with from 5 to 10 times its

FIG. 128.



Widal reaction: bacilli gathered into one large and two small clumps, the few isolated bacteria being motionless or almost so. (Park.)

bulk of sterile water. If the blood-serum is dried, it is first brought into solution with sterile water. To a drop of this mixture placed on a clean cover-glass is added a platinum needle loopful of an eighteen to twenty-four-hour-old bouillon culture of typhoid bacillus, and the two are thoroughly mixed. The drop is rimmed with vaselin and the cover-glass inverted over a concave slide. The drop is examined with an ordinary high power ($\frac{1}{8}$) lens.

The first change noted in the drop is that the bacilli gradu-

ally lose their motility, and then they are seen to gather in small bunches or clumps (Fig. 128). The more marked this clumping and loss of motility, and the earlier the reaction occurs, the more positive is the diagnosis. Ordinarily this reaction begins to be noticed in about half an hour, but in some instances it does not appear before an hour or two. If the blood contains little agglutinating substance, the reaction does not occur until late and is not characteristic. When blood other than typhoid is mixed with the typhoid culture, the bacilli may lose their motility, but the loss of motility is never complete nor is the clumping perfect. Some experience is necessary before any degree of efficiency is attained in interpreting this phenomenon. The typhoid culture should not be more than twenty-four hours old, and should be virulent. Bouillon cultures should always be used. It is not necessary to sterilize the blood-serum obtained from the patient.

Examination of water for typhoid bacilli: In the examination of water for the *Bacillus typhosus*, its resistance to carbolic acid is a valuable aid in its isolation. From 0.05 to 0.25 per cent. of carbolic acid is added to the suspected water. Gelatin plates are made from this water according to the method of Elsner. As the specimen of water may contain only a few bacteria, it is necessary that many examinations be made. The examination can be facilitated by adding 20° c.c. of a standardized, sterilized, concentrated solution of peptone and sodium chloride to 100 c.c. of carbolized water. The mixture is placed in the incubator, and in about twenty-four hours gelatin plates are made from this mixture. Any growth which takes place is either the typhoid or colon bacillus. The differentiation is made as described above. It is advisable to make several tests at the same time, as that will still further facilitate the work.

Prophylaxis: The prophylaxis in typhoid consists of the strictest antiseptic precautions. All the intestinal discharges and urine must be disinfected at once and thoroughly as described in the chapter on Disinfection. The same is true of soiled linen or clothing. The attendants must be careful not to infect themselves by coming into direct contact with infective material. Reinfection of the patient is avoided by

thorough cleansing of the nates with a bichloride solution after each evacuation of the bowel.

In the *typhoid circular* sent out by the Board of Health of the city of Chicago the following directions are given for avoiding the possibility of infection with *Bacillus typhosus:*

"Boil all drinking-water for twenty-five or thirty minutes.

"Pasteurize all milk and cream, especially for the young. If you do not know how to pasteurize, ask your druggist, or the nearest dispensary, or your family doctor. Five minutes' instruction will teach you, and it costs nothing to speak of.

"Dirty hands may also carry the typhoid poison. Therefore, wash your hands carefully before handling any article of food or drink.

"Food gets poisoned, especially green stuff, by being manured with night-soil; by flies crawling over it after feasting on a typhoid discharge, of which they are especially fond; and often by the filthy dust of the street. Therefore:

"Wash thoroughly all vegetables and fruit intended to be eaten raw. Wash in water that has been boiled and then cooled. Keep flies out of the house as much as possible by screens and fly-paper. Cover all food-supplies so that flies may not have access to them."

In the same leaflets are contained the following instructions for *disinfecting typhoid excreta:*

"If all discharges of every existing case of typhoid fever were instantly disinfected, there would be no more typhoid fever in the world. Therefore:

"If you are so unfortunate as to have a case of typhoid in the family, disinfect every discharge as a duty to your neighbors as well as to prevent others of the family from getting poisoned.

"Sulphate of copper (blue vitriol) is the best typhoid disinfectant, one pound costing ten cents, dissolved in two and a half gallons of water. Keep a pint of this in the vessel for the discharges from both the bowel and the bladder. Stir thoroughly for a few minutes; let stand for fifteen minutes and the poison will be destroyed. Do not allow any discharge from either the bowels or the bladder to be received or disposed of except in this manner."

Furthermore, the people are cautioned against unhygienic surroundings, damp, dirt, garbage, and neglect of personal cleanliness.

Immunization: It is possible to immunize animals to typhoid. Rabbits are injected with gradually increasing doses of either living or dead typhoid bacilli until the desired degree of immunity has been attained. The blood-serum of such rabbits is bactericidal and also slightly antitoxic. Several investigators have observed that the blood-serum of persons convalescent from typhoid fever possesses the same properties as the blood-serum of immunized animals.

It is a matter of record that typhoid fever rarely attacks the same individual more than once, so that the inference can be made that one attack confers immunity. The blood-serum of typhoid patients possesses immunizing properties when injected into animals inoculated with typhoid bacilli.

Preventive inoculation with sterilized cultures of typhoid bacilli appears to be of considerable value. All the reports made show that by far the greater majority of persons inoculated in this manner did not acquire the disease. The inoculation is followed by a febrile reaction, which lasts for about one day, and which produces the agglutinating substance in the blood. The inoculation should be repeated in about two weeks. A dose of 0.75 c.c. of the serum is given at each inoculation.

The blood-serum of typhoid convalescents, as well as sterilized cultures, have been used for **curative purposes** in typhoid fever, but the results have been of such a nature that it is impossible at this time to pass any opinion on the serum treatment of typhoid fever. One investigator used a glycerin extract of the thymus, spleen, bone-marrow, brain, and spinal cord of animals dead of typhoid, and reported extremely gratifying results from its use in eighteen cases. Other clinicians report a decided falling off in the mortality, and also a much shorter duration of the disease.

CHAPTER XII.

ORGANISMS RESEMBLING THE BACILLUS TYPHOSUS.

Bacillus Coli Communis (Bacillus of Escherich).

This organism, when first discovered by Emmerich in 1885, was believed to be the cause of Asiatic cholera; but one year later Escherich demonstrated that it was found in the fecal discharges of healthy persons and animals and in the water and soil contaminated with their discharges. The exact identity of this bacillus has ever since been the subject of much discussion and controversy. It has been described as a non-virulent variety of the typhoid bacillus; and as a germ closely allied to the typhoid bacillus. It has also been stated that the typhoid bacillus was really an involution-form of the colon bacillus.

The colon bacillus very closely resembles the typhoid bacillus in its **morphology** and **biology**:

It is a short, thick rod, with rounded ends, measuring from 1 μ to 3 μ in length, and from 4 μ to 7 μ in width (Fig. 129). Oval forms and thread forms are also met with in culture. It stains like *Bacillus typhosus*, and occasionally exhibits unstained portions resembling spores, but it does not sporulate. It has from eight to ten terminal and lateral flagella and is actively motile. It is a facultative anaërobe, and grows readily on all culture-media at either the room or body temperature.

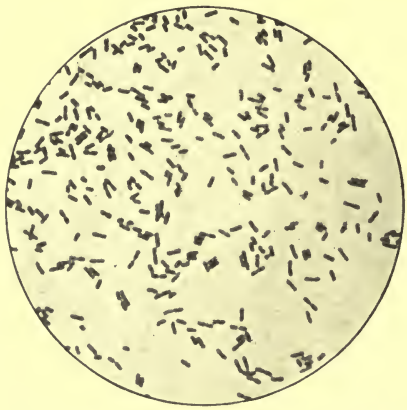
The colonies on the *gelatin plate* are a little larger than those of the typhoid bacillus and they appear earlier. The superficial colonies are small and leaf-shaped. The deep colonies are yellowish-brown in color, round, and finely granular. The medium is not liquefied.

In *gelatin stab* cultures a nail growth is formed without

liquefaction of the gelatin. On *agar-agar* and blood-serum a heavy, grayish-white, moist, translucent film develops along the track of the needle. *Bouillon* is clouded, but as the growth settles to the bottom of the tube the bouillon becomes clear. A surface membrane is formed at times.

In media containing peptone *indol* is formed. Its growth in media containing sugar is accompanied by the evolution of much *gas*, sufficient to break the medium into chunks. The fermentation-tube can be used to demonstrate gas-production.

FIG. 129.



Colon bacilli: twenty-four-hour agar culture. × 1100. (Park.)

Milk is rapidly coagulated. *Litmus* solution is decolorized by the production of large quantities of acid.

On *potato* the growth is quite characteristic. It is very luxuriant, forming a thick moist brown layer which spreads rapidly over the entire surface of the potato. At times the rest of the potato is discolored, turning distinctly green.

The colon bacillus is very resistant to acids and antiseptics. An exposure of ten minutes to a temperature of 60° C. is fatal.

Pathogenesis: Although *Bacillus coli communis* is ordinarily non-pathogenic for man, it is not infrequently found in

abscesses, and especially in suppurations in the vicinity of the intestines. It is believed that in inflammatory conditions of the intestinal tract the organism may become pathogenic. Immediately after death it is found in all the tissues of the body. When injected into the peritoneal cavity of animals, death results in from eight to ten days. Cultures of the colon bacillus obtained from the intestinal discharges of persons suffering from cholera and cholera nostras are much more virulent than those obtained from pus or normal feces. The virulence is increased by rapid passage of the germ through animals, and is diminished by frequent transplantation.

It is possible to *immunize* animals against infection with the colon bacillus by inoculating them with gradually increasing doses of either dead or living cultures.

The colon bacillus may be absorbed from the intestinal canal and produce inflammations in other organs, especially the urinary bladder, the gall-bladder, and all the biliary channels. Multiple abscesses of the liver are not infrequently caused by the colon bacillus. It has often been found in the intestinal discharges of infants suffering with cholera infantum and in dysentery. Other infections in which the colon bacillus has been found in large numbers are meningitis, solitary abscess of the liver, endocarditis, bronchopneumonia, urethritis, and in abscesses of the skin and subcutaneous tissues.

McFarland believes that the colon bacillus is not a single species of bacterium, but merely a name applied to a group of organisms whose appearance is too similar to permit of their differentiation. This group is usually referred to as the **colon group.**

The method of **differentiating** between the colon and typhoid bacilli has been described in the preceding chapter, but it may be well to mention again points of difference:

1. The colon bacillus is shorter, thicker, less motile, and has fewer flagella than the typhoid bacillus.
2. Its growth is more rapid and very luxuriant.
3. On potato it forms a thick brownish membrane which is very visible. The growth of the typhoid bacillus is colorless and usually invisible.

4. Its growth in media containing sugar is accompanied by the evolution of gas and a peculiar odor.

5. Milk is coagulated within from thirty-six to forty-eight hours. The color of litmus-milk is changed to red because of the formation of acid.

6. In nutrient gelatin or agar containing lactose and litmus, and of a slightly alkaline reaction, the colonies of the colon bacillus are red, those of the typhoid bacillus blue.

7. When grown in solutions of peptone the colon bacillus produces indol; the typhoid does not.

8. The colon bacillus grows luxuriantly, whereas the typhoid bacillus does not grow at all, in asparagus solutions.

9. The colon bacillus gives the agglutination reaction with the blood of animals inoculated with this bacillus, but not with typhoid blood. *Bacillus typhosus* agglutinates only with typhoid blood.

Bacillus Enteritidis.

This **organism** was cultivated by Gaertner from the tissues of a cow suffering from an intestinal disease; and from the spleen of a man who was poisoned by eating some of the flesh of the cow.

The **morphology** of **bacillus enteritidis** is almost identical with that of the colon bacillus.

It also resembles it in **culture**, except that the growth on potato is white or yellowish-white. It does not produce indol.

It is **differentiated** from the *colon bacillus* by its ability to cause infection when swallowed and the absence of the indol reaction. It differs from the *typhoid bacillus* in that it coagulates milk, produces acids and gases, and does not agglutinate with typhoid blood.

Bacillus Dysenteriæ (Shiga's Bacillus).

During a recent epidemic of dysentery in Japan, Shiga succeeded in isolating a **characteristic organism** from the intestinal discharges of dysentery patients.

Morphology and biology: The organism belongs to the colon group. It is a short, thick rod, with rounded ends, has no

flagella and is not motile. Polar granules can be made out when the germ is stained with methylene-blue. Gram's stain is not applicable.

Bacillus dysenteriæ develops rapidly at the body temperature, but very slightly at the room temperature. The *culture-medium* should be slightly alkaline, as the bacillus is not in the least resistant to acids.

Both deep and superficial colonies on *gelatin plates* are very small, round and regular, and whitish in color. The medium is not liquefied.

In the *gelatin stab* many minute grayish colonies develop along the track of the needle without any surface growth.

On *agar-agar* large single, bluish-white, regular colonies develop at the end of twenty-four hours. There is no growth on *blood-serum*. On boiled *potato* the growth at first resembles that of the typhoid bacillus, but soon takes on the appearance of the growth of the colon bacillus on potato. *Bouillon* is clouded. *Milk* remains unchanged. The organism does not produce indol, nor does it evolve gas when grown on media containing sugar. Acid-production is very slight.

Bacillus dysenteriæ agglutinates with the blood-serum of persons suffering with or convalescent from dysentery.

Shiga has obtained an **immunizing serum** from horses inoculated with old agar-agar cultures dried in vacuo, and has succeeded in reducing the mortality from 34.7 to 9 per cent. This applies only to cases of epidemic dysentery, and not to tropical dysentery, which is caused by an animal parasite, the *Amœba coli*.

Bacillus Paratyphosus.

It has hitherto always been taken for granted that typhoid fever may differ in its clinical manifestations, at times appearing like a case of mild or abortive typhoid. Recently, however, several investigators have been able to isolate an **organism** from the intestinal discharges and blood of patients apparently suffering from mild forms of typhoid fever, which differs from both the typhoid and colon bacilli, and also from Shiga's bacillus. Nevertheless it is a member of the colon group.

This organism has been designated as the **paratyphoid bacillus**, and is accepted as the specific cause of paratyphoid fever.

Its **morphology** is exactly like that of the typhoid bacillus.

Its **growth** on gelatin, agar, and in bouillon is also the same as that of the typhoid cultures. *Indol-production* is either absent or very slight. *Litmus-milk* remains unchanged. According to some authorities, there is a terminal alkalinity in from one to two weeks. The organism does not agglutinate with typhoid blood, but does with the blood of persons suffering with paratyphoid fever.

The **bacillus** is a very short rod with rounded ends, and has from ten to twelve terminal and lateral flagella. It is actively motile. It is also identical with *Bacillus psittacosis*, which was discovered and isolated by Nocard.

During an epidemic of infectious pneumonia Widal found the paratyphoid bacillus in an abscess in the neighborhood of the thyroid gland. It has also been called the *paracolon bacillus* and *bacillus* 0.

Further investigations are necessary in order to give us a more complete history of this organism.

CHAPTER XIII.

YELLOW FEVER; BUBONIC PLAGUE; INFLUENZA.

Bacillus Icteroides (Bacillus of Yellow Fever).

In 1897 Sanarelli found a bacillus in the tissues and blood of yellow fever patients, which he claimed to be the specific cause of yellow fever. Sternberg, in 1889, isolated an organism from the intestinal contents and the liver of yellow fever cadavers, which he termed *Bacillus X* or *Bacillus cuniculicida Havaniensis*. He was convinced of the identity of his germ with that of Sanarelli's **Bacillus icteroides**. In both instances *Bacillus coli communis* was also present in large numbers.

Morphology and biology: *Bacillus icteroides* is an exceedingly short rod with rounded ends, usually occurring singly, but sometimes associated in pairs (Fig. 130). In culture it has been seen to form short filaments. It very closely resembles the colon bacillus, but is somewhat larger, measuring from 1 μ to 3 μ in length, and from 0.8 μ to 1 μ in width. It is actively motile, but has no flagella. It does not form spores. The anilin dyes stain it rapidly; Gram's method is not applicable.

Bacillus icteroides is a facultative anaërobe, growing on all the various culture-media at either the room or body temperature, but best at the latter.

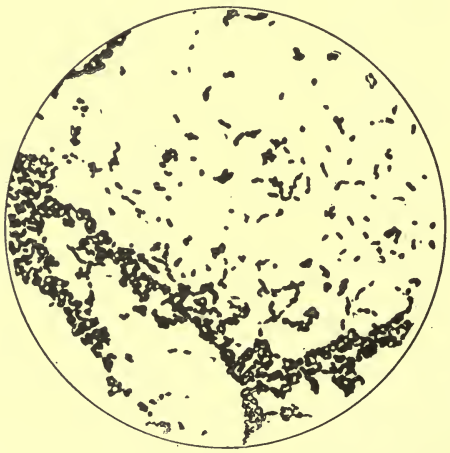
On *gelatin plates* it forms small, rounded, transparent, intensely granular colonies, the centre of which soon becomes very much darker than the periphery. The gelatin is not liquefied. In *bouillon* neither a precipitate nor a surface membrane is formed. On *blood-serum* the growth is almost imperceptible. The growth on *potato* is invisible.

The growth on the *agar-agar* slant is spoken of as being characteristic, providing the temperature does not exceed 22° C. Higher temperatures appear to interfere with the

development on the agar in a distinctive manner. Sanarelli advises an exposure of from twelve to sixteen hours to the incubator temperature, and then an exposure to the room temperature for the same length of time. The colonies look like a drop of milk. They are transparent and bluish, with a dark centre like a nucleus.

Glucose and saccharose are fermented. *Indol* is formed in solutions containing peptone. *Milk* remains unchanged. Bacillus icteroides lives for a long time in sea-water, but dies

FIG. 130.



Bacillus icteroides. (Sanarelli.)

quickly in ordinary water. It succumbs to light, but resists desiccation.

Sternberg's Bacillus X stains with Gram's stain and produces a luxuriant growth on potato ; otherwise it is apparently identical with *Bacillus icteroides.*

Pathogenesis: *Bacillus icteroides* is pathogenic for both man and animals. When injected into the ear vein of an animal, it produces the identical lesions seen in yellow fever cadavers. According to Sanarelli, infection takes place through the

respiratory tract, and not through the gastro-intestinal tract, as is commonly supposed. He suggests that moulds may protect *Bacillus icteroides* and furnish nourishment, especially in damp places like the hold of a ship. The bacillus produces a toxin. Animals immune to yellow fever, or whose susceptibility is not very great, are not affected by this toxin. Sanarelli found that the injection into man of small quantities of a filtered culture of *Bacillus icteroides* was followed by a typical attack of yellow fever.

Sanarelli has also prepared a *curative* serum, which he calls *antiamarylic serum.* It is not an antitoxin, but only a germicide, thus making its administration useless in those cases in which a large amount of toxin has been produced. It must be used early, before any considerable amount of toxin has been elaborated and absorbed. The serum has not, as yet, been tested sufficiently to warrant the expression of a positive opinion as to its curative value.

Infection: Laboratory experiments and clinical researches have positively established the fact that the mosquito, the variety *Anopheles*, is the most important, if not the only source or method by which the infection in yellow fever is conveyed. This mosquito bites the yellow fever patient, and then by again biting a well person carries the infecting germ from the sick to the well. There are two periods when the bite of the mosquito is dangerous: first, shortly after it has bitten a yellow fever patient, when its sucking apparatus still contains the germ-laden blood; second, after the germs have developed sufficiently within the body of the mosquito so that its salivary organs contain the specific germ. In the first case, the course of the disease is a rather mild one, and in the second very severe. One observer asks whether this might not be of some value clinically in producing immunity to yellow fever by a mild attack of the disease. One attack confers positive immunity.

While a number of investigators have confirmed the claims of Sanarelli that **Bacillus icteroides** is the **specific cause** of yellow fever, others are inclined to believe that it is simply one of the many associated organisms so frequently found in the lesions of yellow fever.

F. Novy made a careful analysis of Sanarelli's findings, and came to the conclusion that *Bacillus icteroides* is not the cause of yellow fever. His principal objection is based on the fact that yellow fever is stopped at the appearance of a frost, whereas *Bacillus icteroides* is not injured in the least by cold of even a greater degree than that causing a frost. In the absence of any germ which more nearly complies with the laws of specificity than does *Bacillus icteroides*, we believe that it is proper to regard *Bacillus icteroides* as the specific cause of the disease.

Bubonic Plague (**Bacillus Pestis Bubonicæ; Bacterium Pestis**).

The **specific cause** of bubonic plague was discovered in 1894 by Kitasato and Yersin. The bacillus of plague is found in the pus obtained from the suppurating lymph-glands, in the sputum in cases of bubonic pneumonia, in the feces, and occasionally in the blood and internal organs.

Morphology and biology: Bacterium pestis is a very short and stubby-looking bacillus, with rounded ends. A capsule may at times be demonstrated. The bacillus is extremely variable in culture. At times it appears to be a diplococcus or an oval coccus; at other times short chains are formed. Curved rods, clubs, and odd forms are seen in cultures the vitality of which is almost exhausted (Fig. 131). The plague bacillus does not form spores, although it exhibits polar granules when stained with methylene-blue. It does not stain by Gram's method. It has no flagella and is not motile.

It is strongly aërobic, and grows well on all the usual culture-media at both the room and body temperature.

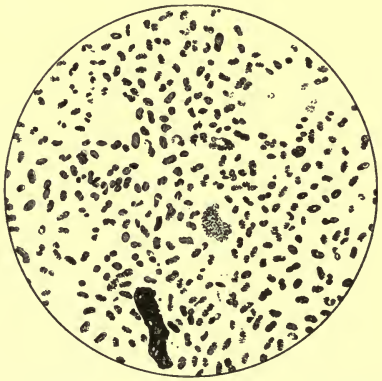
On *gelatin plates* the colonies are granular with a regular border, and brownish in color. The gelatin is not liquefied. In *gelatin stabs* a slight whitish growth is formed on the surface and along the line of inoculation. A grayish-white surface growth is formed on *glycerin-agar*. In *bouillon* a coarse granular deposit forms on the sides of the tube, the fluid remaining clear. On *potato* a thin white pellicle covers the surface of the potato. On *blood-serum* a luxuriant moist whitish growth is formed.

The plague bacillus does not form indol, nor does it ferment. A slight amount of *acid* is formed.

Vitality: A temperature of 55° C. kills the germ in ten minutes; 1 : 1000 bichloride kills immediately; and 1 per cent. carbolic acid or lysol solution in ten minutes. Weak solutions of the mineral acids are rapidly destructive. The bacillus does not resist drying, nor does it survive transplantation to either sterilized or non-sterilized water for any length of time.

Pathogenesis: The plague bacillus is pathogenic for man and for animals, especially the rodent, which is the usual cause

FIG. 131.



Bacillus of bubonic plague. (Yersin.)

of widespread epidemics of plague. The rat acquires the disease by eating plague-infected food or the bodies of animals dead of plague. When the animal sickens, it usually seeks refuge in some dark place, like the cellar of a house or the hold of a ship, where it dies. In this way infection is carried from place to place and over great distances in an incredibly short time. Such houses and ships become known as plague-houses or plague-ships; and the disease may remain limited to its boundaries unless the persons infected with the disease are carried to other places.

Infection: The plague bacillus usually gains entrance to the body through slight injuries of the *skin*, although the organism may be inhaled and lodge in the *lung*. The injury of the skin may be imperceptible and the infection atria may be very numerous. Insect-bites may be the source of infection. At the site of infection a localized suppuration develops, which spreads along the lymphatics to the nearest chain of glands. These glands swell up, and finally suppurate and form buboes, from which the disease has its name of bubonic plague.

In mild cases the infection does not spread further; but in severe cases the lymph-glands in remote parts of the body are also affected. The bacilli may finally make their way into the blood-current, and from there to all parts of the body.

When infection occurs through the *respiratory tract*, a typical pneumonia is produced. The plague bacilli are found in the sputum, and may be associated with the streptococcus and diplococcus. Tonsillar infection has also been observed. When infection occurs in that way, the disease is rapidly fatal.

Prophylaxis: In the prophylaxis of plague general hygienic precautions are of first importance: Properly ventilated dwellings; personal cleanliness; prompt surgical care of any wounds or insect-bites; the careful avoidance of anything suggestive of filth; the immediate disposition of all dead rats, especially in plague-infected localities; and the destruction of as many live rats as possible. The patient should be isolated and watched, so that the infection does not spread to the attendants or members of the family. The bodies of persons dead of plague should be disposed of very promptly after thorough disinfection. Cremation is the best and most thorough method of disposition.

Immunization: Susceptible animals can be immunized with dead cultures of the plague bacillus. Haffkine's protective inoculation against plague consists of the injection of 0.5 to 2.5 c.c. of a devitalized culture of the plague bacillus. The injection is repeated after eight or ten days. Yersin immunizes animals by the intravenous or intraperitoneal injection of dead cultures or by repeated subcutaneous inoculation.

The serum of animals immunized in this way with virulent cultures also protects other susceptible animals. The serum is both antitoxic and bactericidal. The immunity conferred by either of these methods is usually only of short duration —about one month; and is therefore used only as a protective during an epidemic.

The mortality from plague has been reduced considerably by these methods, and better results are to be expected with the perfection of immunization by cultures of the bacillus.

Diagnosis: The plague bacillus can always be obtained in large numbers from the suppurating lymph-glands, from the sputum of plague pneumonia, and by puncture from the swollen but intact lymph-glands. In cases of plague septicæmia the organism is found in the circulating blood and in the organs.

The films are *stained* with methylene-blue, when the characteristic polar staining is seen. The bacilli very often look like diplococci because of the intensity of the staining of these polar bodies.

Cultures are made from material obtained from the lesions, which is spread in a thin layer on gelatin plates. The plague bacillus also *agglutinates* with the blood-serum of persons or animals that have recovered from the disease. This agglutination does not, however, occur before the second week, and is most marked in the second and third weeks.

Influenza (Bacillus Influenzæ—Bacillus of Pfeiffer).

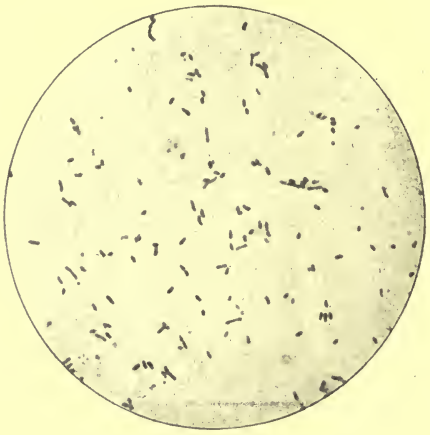
The specificity of this germ was established by Pfeiffer in 1892, when he succeeded in isolating and making pure cultures of **Bacillus influenzæ** from the bronchial secretions of influenza patients. His findings have been confirmed by others, and the organism is accepted as the specific cause of influenza. The peculiar shape of the germ interfered with its earlier discovery.

Morphology and biology: *Bacillus influenzæ* is a short, thick rod, with rounded ends. It is so short and thick that when two germs are placed end to end they resemble *Diplococcus pneumoniæ*. The occurrence usually is single, but may be

in pairs or in short chains of three or four members (Fig. 132). Long chains are occasionally seen in very old cultures. The organism does not form spores, is not motile, and has no flagella.

The anilin dyes *stain* only after a very long exposure of the film to the stain. When stained with Loeffler's alkaline methylene-blue, polar granules can be made out in some of the organisms. Certain it is that *Bacillus influenzæ* stains

FIG. 132.



Influenza bacilli. × 1100. (Park.)

more heavily at its ends than in the middle. Gram's stain decolorizes the germ. *Czenzynke's stain* is the best for staining the bacillus in blood-films. It is made as follows:

Concentrated aqueous solution of methylene-blue,	40 parts;
Solution of eosin (0.5 per cent.) in 70 per cent. alcohol,	20 "
Distilled water,	40 "

The preparation is placed in this solution for from three to six hours, and is then well washed in water, dried, and mounted in balsam. The red corpuscles are stained red, the

leucocytes blue, and the bacillus is also stained blue, appearing as a short, thick rod; or dumb-bell.

Desiccation is rapidly fatal; also a five minutes' exposure to a temperature of 60° C.

Bacillus influenzæ *develops* rapidly at the body temperature, but best at a temperature slightly lower than this. It is strongly aërobic, and will not grow in the total absence of oxygen. It does not form any growth on ordinary media, but develops luxuriantly on blood-serum or on any medium the surface of which has been smeared with blood, hæmoglobin, or leucocytes. It is easily obtained from the sputum or nasal mucus, and also from the throat of persons suffering with influenza.

On media appropriate for its development very small pearly *colonies* appear within forty-eight hours. They are shining, moist and transparent, and may easily escape notice at first. They frequently have the appearance of a small opalescent drop of water. Old colonies turn yellowish brown. The colonies rarely become confluent.

Pathogenesis: The influenza bacillus is apparently pathogenic for man only, as it has so far been impossible to produce the typical disease in animals by inoculation. When inoculated with a large quantity of the culture animals die with symptoms of intense intoxication.

In man **infection** probably takes place through the air-passages, in which the previous condition of the lining mucosa is of importance. The bacillus cannot withstand desiccation, but when contained in a plug of mucus it remains alive for a considerable period of time. The secretions from the mucous membranes are the means of spreading the disease. Coughing or sneezing forces the infected mucus out of the patient's nose or throat, and the disease is thus conveyed directly to other individuals.

It has been suggested that *air-currents* carry the disease for miles from the original seat of the disease. Severe epidemics of the disease, separated perhaps hundreds of miles, have been accounted for in this way. The probability is, however, that if the origin of such epidemics is carefully looked into, it will be found that some individual who is convalescent from influenza was really the carrier of the disease.

The bacilli may remain *latent* in the secretions for months after recovery from the disease has apparently been complete, and under favorable conditions again become virulent.

Immunity: One attack of influenza undoubtedly confers immunity, but unfortunately it is of short duration. In fact, it would appear that when the immunity has disappeared the individual is even more susceptible to the disease than before the first attack. Persons have been attacked several times during the course of the same epidemic. Owing to the insusceptibility of animals to influenza, it has been impossible to immunize them, and thus, perhaps, obtain a serum which could be used for immunizing or curative purposes.

The bacillus is responsible for the lesions, and is always found at the seat of the disease; it does not produce a toxin which is absorbed.

Diagnosis: The bacteriologic diagnosis is readily made from the nasal secretions and the bronchial mucus. A slide can be prepared, stained, and examined immediately. The peculiar appearance of the germ and its behavior to stains are absolutely characteristic. Cultures may be made on blood-serum or other media smeared with blood, hæmoglobin, or sputum, which always contains corpuscles.

It should be borne in mind that the influenza bacillus may be the cause of conditions other than those which are looked upon as distinctive of this disease. During severe epidemics of influenza it is not uncommon to see typical cases of lobar pneumonia which are due to the influenza bacillus and not to the pneumococcus. It is important to determine the cause of such a pneumonia, as it will influence the treatment considerably. The serum treatment of pneumonia could not be used in influenza pneumonia, and the prognosis in the latter is much worse than in a pneumococcus pneumonia.

The influenza bacillus may be confounded with another organism which Pfeiffer found in the sputum of cases of bronchopneumonia, and which he named the *pseudo-influenza bacillus*. This organism differs in culture in that it exhibits a marked tendency to form very long filaments. It is also somewhat longer than the true bacillus. Otherwise the resemblance between the two is very close.

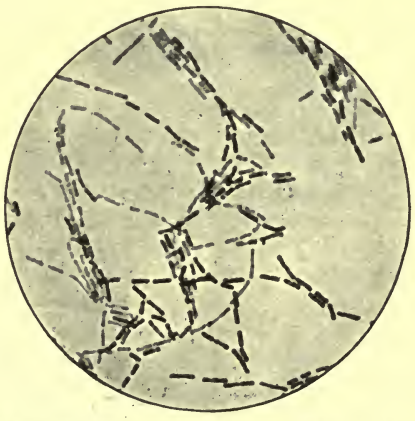
CHAPTER XIV.

ANTHRAX; AND HYDROPHOBIA.

Bacillus Anthracis.

THE specific cause of anthrax was one of the first bacteria which was proved to be an etiologic factor in the production of disease. Bacillus anthracis has served as a basis for most bacteriologic studies which have been made since its discovery. It was detected as early as 1849 in the blood of

FIG. 133.



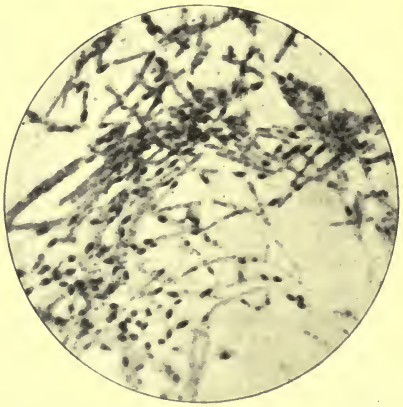
Anthrax bacillus: agar culture. × 900. (Park.)

animals suffering with anthrax, but was not successfully cultivated until 1876. The disease is usually referred to as splenic fever.

Morphology and biology: The anthrax bacillus is a long, slender rod, with squared ends, sometimes slightly concave.

It is from 5 μ to 20 μ long, and from 1 μ to 1.5 μ thick. The organism usually forms very long chains or filaments, which interlace very freely, twisting in and out like a skein of wool (Fig. 133). The individual members of the chain can be identified as a rule. Sometimes one or both ends of the germ are slightly enlarged or swollen, so that the chain presents nodular thickenings at intervals. These nodules and concave ends are seen most frequently in culture specimens. The thin transparent capsule surrounding the germ can be seen when it is stained by Johne's method.

FIG. 134.



Spores heavily stained. Bodies of disintegrating bacilli faintly stained. × 1000. (Park.)

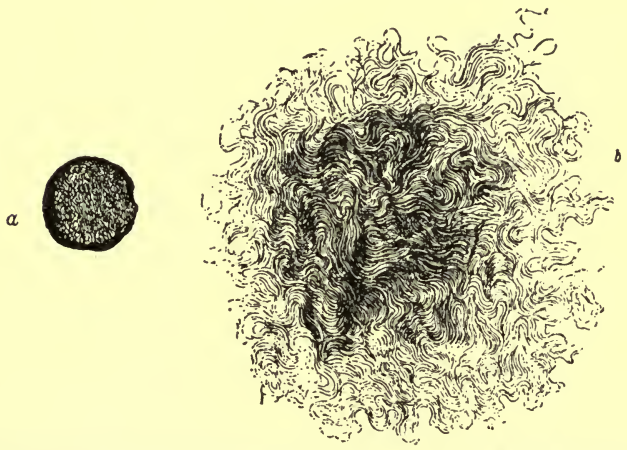
The anthrax bacillus is not motile, and it has no flagella. Spores, large and shining, are formed rapidly in the presence of oxygen. These spores are oval in shape, and each bacillus usually has only one. As the spore increases in size the parent cell is seen to disappear gradually until the spore is finally set free (Fig. 134). The bacillus is strongly aërobic.

Bacillus anthracis is *stained* by the anilin dyes, and also by Gram's method. When contained in tissue it is stained with methylene-blue, the tissue with carmine, or with Gram's stain, or a combination of Gram's and Weigert's stains, picrocar-

mine, or picrocarmine and Gram. The spores are stained as previously described. Owing to their size and affinity for stains, these spores are specially adapted for the study of sporulation. Bacilli have been described that do not sporulate.

The anthrax bacillus is easily cultivated on all kinds of media and between a temperature of 14° and 43° C. The temperature of the body is most conducive to sporulation.

FIG. 135.



Colonies of *Bacillus anthracis* upon gelatin plates. *a*, at the end of twenty-four hours; *b*, at the end of forty-eight hours. × 80. (F. Fluegge.)

The colonies on *gelatin plates* are absolutely characteristic. Within twenty-four hours opaque grayish colonies develop (Fig. 135). The border of the colony is extremely irregular and much lighter in color than the centre. As the colony increases in age the irregularity of the border becomes more marked until the colony finally has the appearance of a badly snarled mass of threads. The gelatin is slightly liquefied. The colonies are examined with a low-power lens or by making a Klatsch preparation. They are very large when fully developed.

A *stab culture in gelatin* develops quite rapidly and luxuriantly on the surface and along the track of the needle. From this whitish linear growth numerous fine, hair-like projections extend out into the medium. Liquefaction begins at the surface, and is complete within a few weeks, when the growth is precipitated.

The growth on *agar-agar plates* is the same as that on gelatin plates, but more distinct. The medium is not liquefied. *Agar stroke* cultures are not at all characteristic. A thin wrinkled layer, with irregular edges, forms along the line of inoculation, and a few fine threads project from the central growth. Sporulation occurs most rapidly on agar-agar. In old cultures the medium is turned a deep brown.

In *bouillon* development is quite rapid, the growth forming in small flaky masses which rapidly settle to the bottom of the tube, leaving the supernatant fluid perfectly clear. Growth on *blood-serum* is very sparse; the medium is slightly liquefied. On *potato* a thick dry white membrane is formed. All the culture-media should be slightly alkaline, as the anthrax bacillus will not grow in the presence of even a very small quantity of free acid.

Vitality: One of the characteristics of the anthrax bacillus, and especially its spores, is the resistance to heat and chemicals. The mature bacilli can withstand a temperature of 60° C. for fifteen minutes. The spores survive live steam at a temperature of 100° C. for from ten to fifteen minutes; compressed steam, for five minutes. A 5 per cent. solution of carbolic acid kills the bacillus in ten seconds, and the spore in from thirty-five to forty days. A 1 : 1000 solution of bichloride destroys the spores in about twenty hours. When kept in sterilized water the spores live for many months, but the bacillus dies in about three days. The bacillus is not able to resist putrefaction, but the spores retain their vitality.

Pathogenesis: Anthrax is a disease of animals, but is met with in man when the individual comes in contact with anthrax infected animals. Shepherds, tanners, and butchers are especially predisposed to the infection because of their occupation, which necessitates handling of the carcass, and

PLATE VI.

a. *b.*

Two cultures of anthrax bacilli prepared from the same material at the same time. (Senn.)

a. With a pressure of $\frac{1}{4}$ cm. of mercury.

b. With atmospheric pressure.

especially the hide. Even tanning of the hides does not lessen the danger of infection. Leather-workers, brush-makers, and others handling the finished product, have been known to become infected with anthrax.

A very forcible *illustration* of the *extreme infectiousness* of the body of an animal dead of anthrax is mentioned by Eineke. An ox died of anthrax. Two persons who ate of the meat of this animal also died of anthrax. The hide of the animal was macerated in a small lake, and was finally worked up by a harness-maker, who was immediately attacked by the disease. Two horses wearing halters made from the hide also fell victims to the disease. Of a herd of sheep bathing in the lake, twenty were attacked by anthrax (Levy and Klemperer).

Portal of infection: The most frequent portal of infection is the *skin*, either through an injury or the bite of an insect which has fed on the anthrax cadaver. If the injury is minute, a "carbuncle" speedily develops at the site of infection. This is usually known as *malignant pustule*, a form of "external anthrax." It is rarely accompanied by a general infection. If the injury is a regular wound, there follows a local, spreading infection known as "anthrax œdema," the other form of "external anthrax," that is always accompanied by a general infection which is usually fatal.

Anthrax infection occasionally occurs through the *gastro-intestinal canal.* Partaking of the food of animals dead of anthrax is invariably followed by a general infection and septicæmia which are fatal. The pasturage of the animal may have become infected with anthrax through the burial of an anthrax cadaver. The spores remain alive in the soil for years, and thus may infect animals grazing on this ground. Pasteur was of the opinion that the earth-worm, feeding on the anthrax cadavers, carried the spores to the surface and deposited them there with their excreta.

Snails, flies, and other insects may also be instrumental in dissemination of the spores. The gastro-intestinal infection in man has been described as an intestinal mycosis. The symptoms caused by such an infection are similar to those of typhoid or dysentery. In some instances the anthrax ba-

cillus passes through the intestinal wall without producing disease.

Gastro-intestinal anthrax infection and wool-sorters' disease are forms of "internal anthrax."

The so-called wool-sorters' disease is an infection with the anthrax bacillus, or its spores, occurring through the *respiratory tract* as a result of the sorting or picking of infected wool.

In all forms of "anthrax" with a general infection, the bacilli are found in the *blood*, and especially in the internal organs, such as the liver, spleen, and lungs. The accumulation of a large number of anthrax bacilli in the capillary vessels is a not infrequent cause of thrombosis and rupture of the vessel. Late in the disease the bacillus is also found in the urine, bile, and feces.

The organisms are not very numerous in a *malignant pustule*. If death occurs it is due to a general infection and septicæmia.

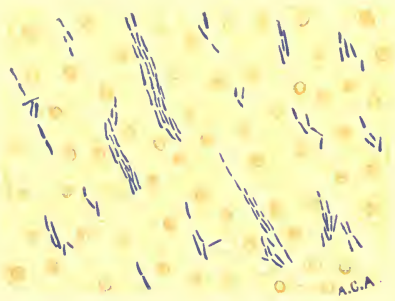
Heredity: Transmission from the mother to the fœtus has been described. Intra-uterine infection was described by Marchand. One observer infected the rabbit's fœtus in utero. If the mothers escaped the disease, they were immune for some time afterward.

Immunity: One attack of anthrax confers a very temporary immunity. It is possible to immunize animals experimentally. Pasteur manufactured two varieties of vaccine from attenuated anthrax bacilli and succeeded in immunizing rabbits. He found that such injections conferred absolute immunity to a subsequent subcutaneous inoculation of the animal with anthrax. But the blood-serum of these animals so immunized did not have the power to confer immunity to other animals.

Prophylaxis: Prophylaxis against anthrax consists in thorough disinfection of the bodies of animals and men dead of the disease; their complete isolation while sick; and careful disinfection of all the excreta and discharges. The best disinfection of the dead body is incineration. This will completely eliminate the possibility of infection from that particular body. Suspicious hides should be thoroughly disinfected before they are handled in any way.

Diagnosis: An examination of the blood or of the material

PLATE VII.



Anthrax bacilli in liver of mouse.

× about 450 diameters. Bacilli stained by Gram's method; tissue stained with Bismarck-brown. (Abbott.)

obtained from a malignant pustule, or the sputum of cases suffering from pulmonary anthrax, will always reveal the characteristic bacilli, perhaps showing spores, and arranged in long chains which may be more or less twisted (see Fig. 125). The characteristic growth on the gelatin plates and the gelatin stab will confirm the diagnosis.

When for sanitary purposes it is necessary to make an examination of a cadaver, a *splenic puncture* is made, and the fluid obtained by this puncture is examined.

Bacillus subtilis and the *bacillus of malignant œdema* may be mistaken for the anthrax bacillus. The former is motile and a strict aërobe and non-pathogenic, whereas the latter is a strict anaërobe, motile, and is decolorized by Gram's stain. It also differs from the anthrax bacillus in its pathogenesis.

Bacillus Anthracoides.

An organism has been described which resembles the anthrax bacillus so closely as to be mistaken for it at times. This organism is called **Bacillus anthracoides.** It is short and thick, but not nearly so long as **Bacillus anthracis.** Neither does it form such long chains. It is encapsulated and forms spores which are stained with difficulty. It is motile, stains easily with the anilin dyes and by Gram's method.

Bacillus of Symptomatic Anthrax.

Symptomatic anthrax, also known as "quarter-evil" or "black leg," is a disease of *cattle.*

It is due to **Bacillus anthracis symptomatici,** an organism which also resembles the anthrax bacillus.

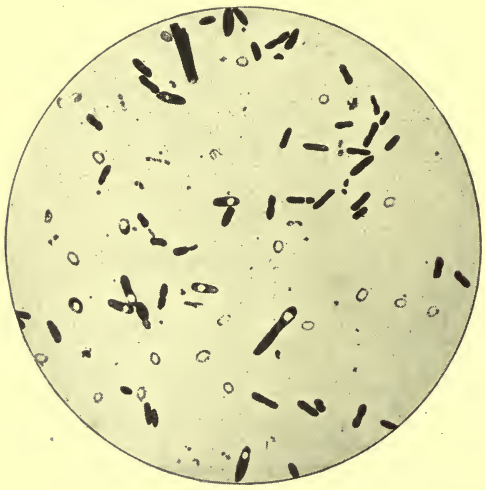
Morphology and biology: The bacillus measures from 3 μ to 5 μ in length, and from 0.5 μ to 0.6 μ in width; it is much smaller than the anthrax germ, has rounded ends, and usually is seen singly or in pairs. It never forms long chains. It is actively motile, and has both lateral and terminal flagella. It forms spores, which are large and centrally located, imparting to the organism a clostridium shape (Fig. 136).

The anilin dyes *stain* it very readily, but it is decolorized by Gram's stain. It is a strict anaërobe, with a temperature

optimum near that of the body. The *spores* are quite resistant to heat, desiccation, and chemicals.

The growth on culture-medium develops as many small white colonies. In *gelatin* a stocking-shaped liquefaction like that formed by the Finkler-Prior bacillus occurs (Fig. 137). All the cultures give off a peculiar odor. Much gas is formed.

FIG. 136.



Bacillus of symptomatic anthrax, containing spores, from an agar-agar-culture. × 1000. (Fraenkel and Pfeiffer.)

In *bouillon* the culture sinks to the bottom. *Milk* remains unchanged.

Pathogenesis: The organism is pathogenic for cattle and sheep only. No cases of infection in man have been noted. The infection in animals usually occurs through a deep wound of the skin, through which the bacillus gains entrance to the subcutaneous tissues. The organism is also found in the soil, and it is possible that it is ingested by the animal while feeding.

One attack of the disease confers absolute **immunity**. Artificial immunity can be produced in small animals, like mice

and rabbits, by intravenous inoculation; or the inoculation may be made in a little pocket at the root of the tail. For the production of immunity in *cattle*, a dry powder made from the muscles of animals dead with the disease has been used with much success.

Fig. 137.

Colonies of the bacillus of symptomatic anthrax, in deep gelatin culture. (After Fraenkel and Pfeiffer.)

Hydrophobia (Rabies; Lyssa).

Although the specific cause of rabies is as yet unknown, its clinical history corresponds with the history of the other infectious diseases so closely that no other inference is possible than that the disease is caused by some **micro-organism.** The exciting cause, then, is unknown. All we know of this disease and of its action and manifestations and results we owe to the painstaking and untiring work of the great Pasteur. Even the treatment which is of the most avail originated with him.

All warm-blooded animals are **susceptible** to rabies. It is communicated to man by direct inoculation.

Judging from the very rapid action of the infectious material and the fact that the principal manifestations of the disease are at points far distant from the site of inoculation, we can come to only one conclusion—that the disease is a **toxæmia.** It can be classed with tetanus and diphtheria.

The most common **source of infection** is a rabid animal, especially the dog, which by its bite conveys the disease to man.

Hydrophobia is a common disease in most countries of Europe, especially France, Russia, and Belgium. It is rather infrequent in this country; in Australia the disease is entirely unknown.

In order that **infection** can take place, it is necessary that there be an injury, and that the *saliva* of the affected animal

come into direct contact with the wound.. Whatever the poison may be, it is evidently, elaborated in the salivary glands, the parotid gland being the one most involved The secretions of the lachrymal, adrenal, and mammary glands, and the pancreas, may also contain the virus. The brain and spinal cord, especially the medulla oblongata, are highly virulent. The virus has never been found in the *blood*, *urine*, or *aqueous humor* of the *eye*.

As in the case of tetanus, the virus of rabies has a **selective action** on the nerve tissues in general and the central nervous system in particular.

The **symptoms** are not manifested immediately after the injury, but after a definite period of incubation, during which the causative germ is elaborating its toxin.

The **period of incubation** and the severity of the disease are dependent upon several factors. The usual period of incubation is from twenty to sixty days; but several cases have been reported in which the period of incubation lasted for several months. The *factors* which influence the incubative period are, first, the character and location of the wound; second, the amount of virus introduced into the body; third, the virulence of the poison.

The period of incubation is very *short* when the wound is a lacerated one, especially of the face; or when it is located in some portion of the body that is well supplied with nerves, such as the finger-tips; or where a large nerve-trunk is very near to the injury, as at the elbow-joint.

Slight or clean-cut wounds and wounds of the back are usually followed by *long* periods of incubation; and, in general, deep wounds are followed by shorter periods of incubation than are superficial wounds.

These facts would naturally lead to the conclusion that the germ causing this disease is undoubtedly an *anaërobic organism*, and that may account for the fact that it has not as yet been discovered, for it may have to be grown in a certain atmosphere.

Infection: It has been determined *experimentally* that subcutaneous injection of the virus is not productive of the disease unless the virus is injected deeply into a muscle or near

a large nerve-trunk or into a peripheral nerve. Rabies has, however, followed absorption of the virus by an intact mucous membrane, such as that of the nose, or the conjunctiva, or the genitalia, or the digestive tract. Intravenous injection always is followed by positive results. Intra-uterine infection is also possible. The nervous system of an animal dead of rabies contains a sufficient quantity of virus to produce the disease when these tissues are injected into other animals.

Vitality: The virus is extremely resistant to chemicals and temperature. An exposure of one hour to a temperature of 50° C. is necessary to destroy the virus. A 5 per cent. solution of carbolic acid is destructive in fifty minutes. The same is true of a 1:1000 bichloride solution and a 5 per cent. potassium permanganate solution. The desiccated spinal cord of an animal dead of hydrophobia retains its virulence for two weeks.

Immunization: The treatment of rabies, aside from the treatment of recent and single wounds, which is surgical, is limited almost entirely to preventive inoculation with hydrophobic serum. Pasteur began by inoculating animals with preparations of spinal cords possessing only a slight degree of virulence, and gradually increased the virulence of the injection until he succeeded in producing immunity, which protected the animal against a quantity of virus that would invariably kill an unprotected animal.

In order that the treatment may be a success, it is absolutely necessary that the patient come under the observation of a physician as soon after the injury as possible. Usually people are inclined to temporize and see whether or not anything will happen to the victim. After the symptoms of the disease have appeared it is too late.

The *serum* which is used for "curative" purposes consists of an emulsion of the rabbit's spinal cord which has been dried over caustic potash for from seven to ten days. The first injection, which is made subcutaneously, consists of about two grams of this emulsion. This dose is repeated every day for twenty-five days. For each successive injection an emulsion is used which is made from a cord which has not been dried so long, and which contains a more viru-

lent virus. The last injection consists of an emulsion made from a spinal cord which was dried for only three days. The treatment is really a process of immunization carried out during the period of incubation.

The *emulsion* is made by rubbing up 1 centimeter of dried spinal cord with four or five times its bulk of bouillon until a perfect emulsion results. The injection is made with a syringe sufficiently large to contain the required amount of the serum. No two injections should be made in the same place. Needless to say that everything should first be rendered sterile in order to prevent the occurrence of sepsis. The injections are usually made into the hypochondriac region.

A number of methods of immunization against rabies have been proposed by different clinicians, but the results obtained by Pasteur's method have thus far not been surpassed. During the year 1897, 1521 cases were immunized at the Pasteur Institute in Paris in the manner described, and only one of these died. In all the other years that the emulsion has been used the results have been equally good, and ought to convince the most skeptical that this is the only method of treatment of rabies which is productive of favorable results.

CHAPTER XV.

BACILLUS OF MALIGNANT ŒDEMA; BACILLUS AËROGENES CAPSULATUS; BACILLUS PROTEUS VULGARIS.

THE **bacillus of malignant œdema** is identical with the *Vibrion septique* of Pasteur. It is a large slender bacillus,

FIG. 138.

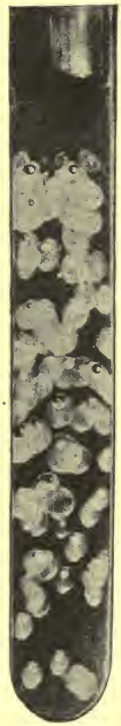
Bacillus of malignant œdema, from the body-juice of a guinea-pig inoculated with garden earth. × 1000. (Fraenkel and Pfeiffer.)

with rounded ends. It possesses both terminal and lateral flagella, and is actively motile. It is usually found in pairs, but in culture it forms long chains and filaments (Fig. 138). It is very difficult to cultivate the germ. When grown at the temperature of the body, it forms a large oval spore which is situated either in the middle or at one end of the

germ. It is decolorized by the Gram method, but stains well with the anilin dyes.

The bacillus of malignant œdema is easily *obtained* from the œdematous tissues of animals suffering with the disease, or from contaminated garden earth. The bacillus of tetanus is frequently associated with it. It is an obligative anaërobe.

Fig. 139.



Colonies of the bacillus of malignant œdema in deep gelatin culture. (After Fraenkel and Pfeiffer.)

The *colonies* usually develop in the depth of the medium, and are solid-looking masses, white in color. The colonies have a very irregular edge like those of the hay or potato bacilli, and appear to be filled with a mass of threads (Fig. 139). *Gelatin* is liquefied. In glucose-gelatin *gas* is evolved, and there is a distinct odor. *Blood-serum* is usually rapidly liquefied. *Bouillon* is rendered turbid, with the formation of CO_2 and hydrogen.

The organism is invariably present in *putrefactive processes*, in garden earth, in manure, and in dust, and is always associated with the tetanus bacillus and several saprophytic germs. Only a very few cases of malignant œdema have been reported as occurring in man. As in the case of the tetanus bacillus, a penetrating wound is necessary before infection will occur. In fact, the same conditions which obtain in infection with the tetanus bacillus also hold good in infection with the bacillus of malignant œdema. Animals are readily immunized to infection.

Bacillus aërogenes capsulatus: This organism was discovered by Welch in the blood-vessels of a patient suffering with an aortic aneurism. It is a straight or slightly curved rod, of variable length and thickness, with either rounded or squared ends. It is encapsulated and forms long chains of filaments. It is non-motile and does not form spores.

The *Bacillus aërogenes capsulatus* is an obligative anaërobe, and grows best at the temperature of the body. It stains well with the anilin dyes, and also by Gram's method.

Nutrient *gelatin* is peptonized but not liquefied. In *agar-agar* grayish-white irregular round colonies are formed, which have fine, hair-like projections. *Milk* is coagulated and *litmus*-milk is decolorized. All the cultures are accompanied by abundant evolution of gas.

The bacillus is not very resistant to heat or to chemicals. A ten minutes' exposure to a temperature of 58° C. is fatal.

FIG. 140.

Proteus vulgaris. × 285. (Hauser.)

The organism is found in the soil and the intestines, and occasionally upon the skin.

Pathogenesis: The organism is not pathogenic, but when associated with other germs it may be the cause of death. It finds a ready lodgement in old blood-clots, especially in aneurisms. After death, when the blood is no longer oxygenated, the germ grows very rapidly in the tissues with an enormous production of gas.

In man *infection* follows an injury, especially when dirt has been ground into the wound. The gas produced by the

organism is inflammable. At the autopsy gas bubbles are found in most of the internal organs; and also the bacillus in pure culture.

Bacillus proteus vulgaris: The proteus bacilli are minute rods, usually arranged in pairs and occasionally forming chains or filaments. They possess many flagella and are exceedingly motile. The organisms can be *stained* with carbol-fuchsin, but not with the anilin dyes nor by Gram's method; they do not form spores; and grow equally well at either the room or body temperature.

On *gelatin plates* small yellowish round colonies are formed, which have an irregular margin and many fine, hair-like projections (Fig. 140). The gelatin is rapidly liquefied, with the formation of free islands of the growth. This peculiar appearance of the culture has given to this organism the name *Bacillus figurans*. The growth on either potato, agar, bouillon, or gelatin is not characteristic. All the cultures give off an extremely disagreeable odor.

The proteus bacilli are present in all *putrefactive processes*, and especially when these are situated in the gastro-intestinal canal. Febrile icterus, or *Weil's disease*, is said to be caused by a proteus infection.

Plates are made from the pus obtained from putrid phlegmons, and also from the urine obtained from cases of Weil's disease.

CHAPTER XVI.

MALTA FEVER; MUMPS; RELAPSING FEVER; WHOOPING-COUGH.

Micrococcus Melitensis.

In 1887 Bruce succeeded in isolating a **micrococcus** from the spleen, liver, and kidneys of persons suffering from Malta fever. The injection of pure cultures of this organism into animals produced the disease, thus establishing its specificity. It also agglutinated with the serum of Malta fever patients.

Morphology and biology: *Micrococcus melitensis* measures about 3 μ in diameter and usually occurs singly. Chains are never formed. It stains well with an aqueous solution of gentian-violet, but not by Gram's method. It exhibits Brownian movement, but not actual motility.

The organism grows well on *agar-agar* at the body temperature, forming very minute round translucent *colonies*, which do not become confluent. The growth is not apparent until after forty-eight hours. In the *gelatin stab* and *agar stroke* the development is the same as on the plate, but the colonies gradually increase in size until they finally form a rosette-like mass. The track of the needle shows a brownish growth with serrated edges. If the tube containing the growth is examined by transmitted light, the centre of the colonies composing the growth is yellowish in color and the periphery bluish-white. By reflected light the entire colony is milky in appearance.

The growth in *gelatin* is always imperceptible. The medium is not liquefied. There is no growth on *potato*.

The natural **habitat** of this germ and the method of **infection** are unknown. It is always found in the organs of Malta fever cadavers, and can also be obtained in pure cult-

ure by splenic puncture. It is pathogenic for the usual laboratory animals.

Mumps—Epidemic Parotitis.

Pasteur discovered a **bacillus** in the blood of persons suffering from epidemic parotitis. Micrococci and other bacilli have been found in the blood, urine, and saliva. The difficulty which naturally attends the study of these organisms, because of their location in the parotid gland and their contamination, as they pass through the mouth, with both staphylococci and streptococci, is probably one reason why the specific cause of this disease has not been discovered.

A **diplococcus** which grows in pairs and fours and in zoöglœa masses has also been described.

Pasteur obtained his **Bacillus parotitis** directly from Stenson's duct after thoroughly disinfecting the mouth, and also by withdrawing a few drops of fluid from the inflamed parotid gland with a hypodermic syringe.

The bacillus grows very slowly, which is characteristic, forming small white colonies on *gelatin*. The medium is gradually liquefied. On *potato* it begins as a thin white streak which slowly spreads over the surface of the potato. The growth on *blood-serum* is more rapid but not characteristic. *Milk* is coagulated; *litmus*-milk is decolorized by the production of acid.

This germ has never been found in the mouth of *healthy persons*. Injections of pure cultures into animals have not as yet produced the disease.

The **diplococcus**, which has been found in the blood and feces, was contained in the cells like a gonococcus; but it is considerably smaller than the gonococcus.

This diplococcus *stains* readily with the anilin dyes, but not by Gram's method.

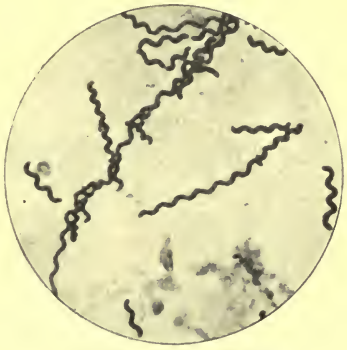
When *cultivated*, it forms very minute transparent colonies which remain discrete. On slightly alkaline media the growth is more rapid. Ascitic fluid is also available as a culture-medium. Milk is coagulated. It has not been possible to establish its specificity.

Relapsing Fever.

The exciting cause of relapsing fever is the **spirochæte** of **Obermeier**, or the spirochæte of relapsing fever. This organism is seen usually as a very long, wavy thread varying in length from 16 μ to 40 μ. It is flagellated and actively motile (Figs. 141 and 142). Sporulation has not been observed. It has not been cultivated as yet.

The spirochæte is readily *stained* with the aqueous solutions of the anilin dyes, but is decolorized by Gram's stain. Guenther's method of staining is quite useful: The fixed cover-

FIG. 141.



Very large spirilla. (Park.)

glass is immersed in 5 per cent. acetic acid for ten seconds, in order to extract the hæmoglobin from the red corpuscles; it is then stained with anilin gentian-violet or fuchsin.

Pathogenesis: The organism is always found during the paroxysm in the blood of patients sick with relapsing fever. It disappears in the interval. It has also been found in leeches that have gorged themselves with the blood of such fever patients.

These organisms appear in the blood just before the onset of the paroxysm, during which they multiply rapidly, and disappear just before the crisis. The spirochæte is strictly a blood parasite.

The disease is contagious, but the method of infection is not known. It is possible to produce the disease experimentally in animals by inoculating them with the blood of relapsing fever patients. It has been suggested that insects play an important role in the transmission of the disease, but such a method of infection has not been demonstrated. Intra-uterine transmission has been observed. The inability to cultivate this organism has interfered with all attempts to learn something of the nature of the disease; the method of

FIG. 142.

Spirillum Obermeieri in blood of man. × 1000. (Fraenkel and Pfeiffer.)

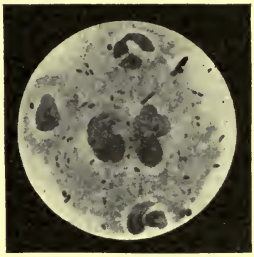
infection; whether the symptoms are due to the germ or to a toxæmia; finally what dispositien is made of the germ when the disease has run its course. The germ may be disposed of by the phagocytes or by an insusceptibility of the blood, due to its saturation with some substance which is fatal to the spirochæte.

Whooping-cough.

Various observers have from time to time found a number of *organisms* in the sputum of children suffering from whoop-

ing-cough. The most frequent of these organisms is a very **small bacillus** about the size of the influenza bacillus (Fig. 143). It also grows somewhat like the influenza bacillus.

FIG. 143.



Whooping-cough bacillus.

The data at our disposal do not warrant any positive statements as to the specificity of this germ.

Infection in whooping-cough probably always occurs through the respiratory tract, the sputum acting as the infecting medium. One attack usually confers immunity.

CHAPTER XVII.

ACUTE EXANTHEMATA.

THE specific causes of the various exanthematous diseases are unknown. That these diseases are due to bacteria cannot be doubted. They are highly contagious, and run a course which corresponds to the life-cycle of an organism. By exercising the usual precautions observed in all diseases having a bacterial cause, the spread of the exanthemata is prohibited. All cases have their origin in some other case, so that the exciting cause cannot be otherwise than bacterial. Furthermore, one attack confers an immunity which under ordinary conditions is permanent.

Measles: In measles the most frequently occurring organism has been a coccus obtained from the blood of patients sick with measles. Its specificity has not been proved, however. The contagium is contained in the nasal mucus, the conjunctival secretion, the sputum, and the blood. Contact with the sick is necessary before infection can occur. Measles is rarely conveyed by clothing or carried any great distance. Children are more susceptible to the disease than adults, but when adults are infected the disease usually runs a very severe course. One attack confers immunity.

Scarlet fever: The virus of scarlet fever is much more virulent than that of measles.

Infection may occur even months after recovery, from handling the clothing or anything else that the patient may have used during his illness. Direct contact with the contagious element is necessary. Infection usually occurs through the respiratory tract. The *scales* appear to play a very important role in conveying the disease, but just how is still a matter of conjecture.

Streptococci and *diplococci* of various kinds have been found in the blood and scales of scarlet fever patients. The

poison is apparently excreted by the kidneys, as these organs are frequently and extensively involved, especially in severe cases.

Class, a few years ago, described a diplococcus which he believed to be the specific cause of scarlet fever. He named it *Diplococcus scarlatinæ*. Proof positive as to its relationship to the disease is wanting, however. Class grew this germ on a mixture of agar-agar and garden earth. It could not be cultivated in any medium which did not contain earth.

One attack of scarlet fever confers **immunity**, although second attacks have been recorded. Scarlatina, like measles, is a disease of youth, and when it occurs in adults it is as an aggravated form of the disease.

Smallpox: The exciting cause of smallpox is also unknown. The virus is known to be contained in the pustules, in the desquamating skin, in the sputum, and in the nasal secretions of smallpox patients. The virus retains its virulence for many months.

Infection is conveyed through the air and by bed-clothing, linen, and other articles that may have been contaminated by the virus; but the *nature* of the infection and the *method* of conveyance have not been determined. The skin and the respiratory tract would naturally suggest themselves as portals of infection, and yet the first symptoms of smallpox, as in measles and scarlatina, are not local but general manifestations indicative of an intoxication. One case of smallpox, unless isolated, may form the nidus of a practically unlimited epidemic. The same is true of scarlet fever. Isolation and protection of the well effectually check the spread of the disease. It is possible that the virus may be inhaled, but, with rare exceptions, smallpox lesions do not develop in the respiratory tract. The skin is the usual seat of the exanthematous eruption, and perhaps the infection enters by that channel. *Microscopic examination* has shown that the tissue-cells in the infected areas contain from one to four spheroidal or slightly irregular bodies which vary in size, the largest being of the size of a cell-nucleus. With nuclear stains they stain more faintly than the nucleus. They are homogeneous. When the hæmatoxylin and eosin double stain is used, the

nucleus of the cell is stained a dark purple, the cell-bodies pink, and the peculiar bodies a light uniform purple.

Immunity: One attack of smallpox confers an immunity of from ten to twenty years' duration, although not infrequently the immunity persists for a lifetime. A mild attack of the disease will confer immunity against severe attacks. This fact forms the basis of vaccination, which is now practised almost universally.

The **virus used for vaccination**, or **vaccine**, is obtained from the pustules of cowpox. The contents of the pock are rubbed up with glycerin. Human lymph was also used at one time, but its use has been abandoned for the bovine lymph, because of the possibility of its being contaminated with tuberculosis and syphilis. The bovine vaccine is also more easily obtained. The vaccine is placed on the market either in the form of an ivory point which is coated on both sides with the dried vaccine; or in capillary tubes. Good vaccine will keep in sterilized capillary tubes for three months, although some tubes deteriorate before that time.

Vaccine always contains *bacteria*, very few of which are pathogenic, however. These organisms usually disappear from the lymph within three or four months. For this reason it is not well to use too fresh a lymph, because it may contain staphylococci, which will induce severe local suppuration around the vaccinated area, or even a fatal pyæmia.

Recent investigations by Funck show that vaccinia is caused by **Sporidium vaccinale**, a parasitic protozoön. Animals inoculated with this organism developed both vaccinia and variola. These organisms were found in the lymph, in the smallpox vesicle, and also in sterile glycerinated vaccine.

When examined in the hanging drop two forms of this organism are seen. One, a cyst-form filled with spores, and, second, free spores. The spore-cysts are round or oval, and about 25 μ in diameter. The spores are irregular in outline, highly refractive and motile, measuring from 1 to 3 μ in diameter. Larger bodies, which look like epithelial cells filled with the organism, are also seen occasionally.

Sporidium vaccinale can be obtained in *pure culture* by plating in agar a few drops of sterile glycerinated vaccine.

These findings have been confirmed, but the bacterial origin of vaccine and smallpox is doubted by many investigators.

Vaccination: It is advisable to vaccinate at least once in every seven years, and oftener in case of an epidemic of smallpox. Unless smallpox is epidemic, it is not necessary to vaccinate an infant at the time of its birth. Neither is it desirable to vaccinate babies during the first or even second summer of their existence. On the whole, it is better not to vaccinate the infant until it has passed through the periods of stress which it must encounter during its first years of life. In case of a smallpox epidemic, however, the child should be vaccinated immediately.

The usual *site* for vaccination in boys is the skin over the insertion of the deltoid muscle of the left arm. In girls, for æsthetic reasons, the skin on the back of the calf, or on the inner side of the thigh near the knee, is chosen. Other portions of the body may be selected. Some one has proposed to vaccinate over the epigastrium, because that part of the body is more easily protected from injury.

In *performing* a vaccination, we should keep in mind that it is a surgical operation, and that the strictest antiseptic precautions should be observed. Everything—the site of vaccination, the hands of the operator, and the instrument used —should be thoroughly cleansed and disinfected. Unfortunately, vaccination is too often looked upon as a trivial procedure. It is done in a hurry, and without regard for either asepsis or antisepsis. Most, if not all, of the disastrous results which follow vaccination can be ascribed to improper methods and carelessness. It is customary to blame the vaccine, but more often it is the fault of the vaccinator.

After everything is ready the *skin is scarified.* This scarification may be done with the vaccine point (a barbarous method); a sterilized needle; or a special scarifier; but the best results are obtained with a sharp scalpel.

The scalpel can be sterilized or disinfected each time it is used, and it does not cause so much pain as either the needle or the other scarifiers. The vaccination can be done more accurately, because the pressure on the scalpel can be regu-

lated by the vaccinator. The sharp edge of the scalpel is passed gently to and fro over the skin until the tops of the papilla in the skin begin to appear. The scarification is covered with the oozing serum, but no blood is drawn. The method is absolutely painless—in fact, it is accompanied by a rather pleasant sensation. It is not necessary to make a deep gash or a number of scratches that draw blood, because those vaccinations usually do not take. No blood should ever be drawn, as it hinders absorption of the lymph, *i. e.*, vaccine. The flow of blood does not permit of entrance of the lymph, and when the blood coagulates it forms an effectual barrier to the entrance of the lymph into the lymph-channels.

The scarification, *when properly performed,* is then covered with a very thin layer of serum, and into this the ivory point is gently rubbed, after having first been dipped into warm water, until all the lymph on the point is rubbed off.

If the capillary tube is used, the ends are broken and the contents of the tube blown on the scarified area and rubbed in with the scalpel; or even this is not necessary, as the glycerinized lymph is rapidly absorbed. The serum remains on the wound for a long time afterward, even after all the lymph has been taken up by the lymph-vessels: so that when the moisture persists the scarification can be covered with a piece of sterile absorbent cotton; or a vaccination shield can be applied, and over this a dressing. The wound should be protected from infection, and for this reason it is well to keep a protective dressing on until it has healed.

It is not necessary to scarify a *large area,* because a vaccination will take just as well in a small spot as in one the size of a silver dollar. It is not necessary to have the "taking" of the vaccination accompanied by terrific suffering because of a large abraded surface. A scarification about one-quarter of an inch square suffices.

In from one to three days a small red *papule* appears. This is followed by a vesicle, and this by a pustule which is surrounded by a bright-red areola. All the stages of a typical variolous eruption are represented in this vaccination area. Ordinarily only slight *constitutional symptoms* follow a vaccination, but occasionally there may be high temperature,

chills, headache, malaise, and all the symptoms of a mild attack of variola. If the fever is very high and the axillary lymph-glands much enlarged and tender, it means that infection with the pus germs has occurred. Very sore and inflamed arms are not a part of a typical vaccination.

PART IV.

MICRO-ORGANISMS PATHOGENIC FOR ANIMALS ONLY.

Chicken Cholera (Bacillus Gallinarum).

Bacillus gallinarum, the specific cause of chicken cholera, is a very short and thick bacillus, with rounded ends, occurring singly and in short chains (Fig. 144). Polar staining is

FIG. 144.

Bacillus of chicken cholera, from the heart's blood of a pigeon. × 1000. (Fraenkel and Pfeiffer.)

very marked, giving the organism the appearance of a diplococcus. It does not form spores, neither is it motile. Gram's stain is not applicable. Heat and drying are rapidly fatal. It is strongly aërobic.

All the cultures of this germ are absolutely devoid of any characteristic. The culture is white in color, and develops

rapidly and luxuriantly at the incubator temperature on all the ordinary media except potato, on which the growth is almost invisible.

The bacillus is **pathogenic** for chickens, geese, mice, pigeons, rabbits, etc. The injection of pure cultures produces a fatal septicæmia with pronounced intestinal symptoms. The bacillus is found in all the organs of the affected animal, but chiefly in the intestine. Chickens inoculated with attenuated cultures are made immune to infection with virulent organisms. One peculiarity of this organism is that when injected into different species of animals different diseases are produced in each species, such as rabbit septicæmia, swine plague, etc.

Bacillus Suipestifer (Hog Cholera).

This **organism** belongs to the same group as the colon bacillus. It is the specific cause of hog cholera, a disease which is both common and fatal. It is a short, thick rod, with rounded ends, flagellated and exceedingly motile. It does not form spores. It is stained by the anilin dyes, but not by Gram.

The organism is easily cultivated on all the ordinary culture-media at the temperature of the body. The cultures as well as the germ bear some resemblance to *Bacillus typhosus*, but the bacillus of hog cholera produces both gases and acids. It is exceedingly resistant to both heat and chemicals.

The organism is markedly **pathogenic** for animals; and is found in the *intestine* of animals so diseased.

The injection of gradually increasing doses of pure cultures of *Bacillus suipestifer* into cows **immunizes** them and causes the formation of an antitoxin in the blood of the cow which is capable of protecting guinea-pigs from the disease.

Bacillus Suisepticus (Swine Plague).

This **organism** resembles the bacillus of hog cholera; and the two diseases are not infrequently associated. The one may be mistaken for the other. The disease is rapidly fatal. *Bacillus suisepticus* is an exceedingly short, thick rod, which

may be taken for a diplococcus. It is not motile, has no flagella, and does not form spores. It stains well with the usual dyes, and sometimes exhibits slight polar staining. Gram's stain is not applicable. It is feebly resistant to heat and desiccation. It is a facultative anaërobe.

Its growth in **culture-media** resembles the cultures of the hog-cholera bacillus. No growth forms on potato unless it is alkaline, when a very thin grayish film is seen to develop. It produces a very slight amount of acid, not sufficient to coagulate milk nor to discolor litmus solution.

It is **pathogenic** for animals only.

Bacillus Typhi Murium.

This is a very small, **short germ**, which in culture often forms very long filaments. It varies greatly in thickness. It is not motile, although it possesses numerous flagella. It does not form spores; stains well with Loeffler's alkaline methylene-blue; it is a facultative anaërobe.

Plate **colonies** on *gelatin* are at first grayish, but soon turn a yellowish-brown. In the *gelatin stab* a grayish-white growth is formed on the surface of the medium, with an almost imperceptible growth along the track of the needle. When grown in *milk*, acid is produced, but the milk is not coagulated.

The germ is intensely **pathogenic** for mice. It can be isolated from the blood and lymph-channels. It has been used with remarkable success in freeing infested houses from mice by saturating bread with a bouillon culture of the bacillus. The bread is pushed into the mouse-holes at a time of the year when food is not plentiful. The mice which are infected in this way die rapidly, and their dead bodies are eaten by other mice, which also succumb to the disease. In this way the premises can be rid of the pests in a very short time, usually in about ten or twelve days.

Bacillus Murisepticus (Mouse Septicæmia).

Bacillus murisepticus resembles an organism found in the lesions of swine erysipelas so closely that these two germs are considered by many as identical. They are very small, about

1 μ long and 0.2 μ wide. Flagellation, motility, and sporulation are doubtful. The bacillus is strongly inclined to be a strict anaërobe. It can be cultivated at either the body or

FIG. 145.

Colony of the bacillus of mouse septicæmia. × 80. (Fluegge.)

room temperature. It stains with Gram and the usual anilin dyes. It is killed by a temperature of 52° C. in fifteen minutes.

The colonies on *gelatin plates* resemble the lacunæ in bone with their contents and processes (Fig. 145). The colony is grayish in color, irregular, and has many fine, wavy, branched projections. The gelatin is gradually softened and evaporated, the colonies coalescing to form a grayish film.

FIG. 146.

Bacillus of mouse septicæmia; gelatin puncture; culture three and a half days old. (Guenther.)

In *gelatin stabs* development takes place along the entire needle-track, with little or no surface growth. Usually the top of the growth is slightly beneath the surface of the medium. The growth in the gelatin tube is peculiar. It resembles a column of discs, each disc being separated from the other by a layer of cloudy fluid (Fig. 146). The gelatin is not liquefied. The germ does not grow on *potato*. On *agar-agar* or *blood-serum* the growth is devoid of any characteristic.

The organism is **pathogenic** for mice and swine. The bacilli are found in all the organs, especially the spleen and lungs. Many of the germs are enclosed by the leucocytes.

It is possible to produce temporary **immunity** by injecting the blood-serum of rabbits immunized with pure cultures of the bacillus.

APPENDIX.

Student's Individual Bacteriology Outfit.

½ gross slides.
½ ounce cover-glasses.
1 slide box.
1 pointed forceps.
1 Stewart forceps.
1 inoculating needle.
6 dozen test-tubes, 6 × ⅝ inch.
2 dozen test-tubes, 5 × 1 inch (for potato).
6 Petri dishes.
1 1000 c.c. flask.
1 glass stirring rod.
1 large glass funnel (ribbed).
1 large bowl.
1 Bunsen burner.
1 iron tripod.
1 piece of wire gauze, 6 × 6 inches.
1 wooden filtering-stand.
1 cork-borer, ⅝ inch.
1 wire test-tube basket, 5 × 10 inches.
1 test-tube rack—24 tubes.
1 potato knife.
1 test-tube brush.
Cotton batting.
Towel and cheese-cloth.
1 pint of alcohol in glass-stoppered bottle.
1 pint of sterilized water in glass-stoppered bottle.
1 ounce of carbol-fuchsin in dropping-bottle.
1 ounce of Loeffler's alkaline methylene-blue in dropping-bottle.

1 ounce of anilin gentian-violet in dropping-bottle.
1 ounce of Gram's solution in dropping-bottle.
1 ounce of 30 per cent. nitric acid.
1 ounce of xylol balsam.
1 dozen of 13-inch filter-paper.
1 package of blue litmus-paper.
1 slide with concave centre.
3 medicine-droppers.
1 package of labels (fifty).
1 tube of vaselin.

Syllabus of Laboratory Work in Bacteriology.

Explanation: The experiments presented in this syllabus cover the entire requirements of the first three months' laboratory work in bacteriology. Each student is required to conduct each experiment personally, and will receive credit for the experiment upon presentation of the completed work. The experiments are to be conducted in the order in which they are placed; but it is not required that each be completely finished before the next is begun. However, the work of Part I. must be completed entirely before that of Part II. can be undertaken. The details of the experiments and the methods will receive explanation in the didactic course. Specimens for study and material for making media will be supplied from the preparation-room upon application. Each student is expected to make up sufficient culture-media from time to time to continue the various experiments, and it is therefore taken for granted that members of the class will keep themselves at all times supplied with sufficient media.

Part I. Technique.

EXPERIMENT 1: *Sterilization by Dry Heat:* Wash and dry one wire basket full of test-tubes and all of the Petri saucers in the outfit. Plug the test-tubes with cotton, fit the dishes together, and sterilize all by dry heat for one hour at 165° C.

EXPERIMENT 2: *Culture-media:* (*a*) *Sterile Potato:* Prepare four Petri saucers with slices of potato; also prepare

twelve test-tubes with blocks of potato. Sterilize all by steam for one hour at 100° C.

(*b*) *Preparation of Sterile Beef-tea:* Prepare 1000 c.c. of beef-tea culture-fluid. Fill one dozen sterile test-tubes, with one inch of the beef-tea in each, and sterilize them in the steam sterilizer for one hour.

Formula for nutrient beef-tea:

Beef-extract,	2 grams;
Peptone,	10 "
Salt,	5 "
Water,	1000 c.c.

(*c*) *Preparation of Nutrient Gelatin:* Prepare 1000 c.c. of nutrient gelatin. Fill into sterile tubes as in the case of the beef-tea and sterilize by the fractional method. Solidify with tube in upright position.

Formula for nutrient gelatin:

Beef-extract,	2 grams;
Peptone,	10 "
Salt,	5 "
Gelatin,	100 "
Water,	1000 c.c.

(*d*) *Preparation of Nutrient Agar:* Prepare 1000 c.c. of nutrient agar. Fill into test-tubes as in the case of gelatin and sterilize. Solidify with tube in the inclined position. The formula for agar is like that of gelatin, excepting that 10 grams of agar are substituted for the gelatin.

EXPERIMENT 3: *Demonstration of Sterility, Infection, and Contamination:* Select three of the Petri saucers containing sterile potato. Expose one of them to the air for five minutes, causing it to be contaminated. Another, infect by rubbing the finger over the laboratory desk and then across the potato. The third allow to remain closed, so that it will remain sterile. Label each dish properly. Notice the result in the next few days.

EXPERIMENT 4: *Study of a Mould:* Select a well-developed

mould colony and examine its structure with different powers of the microscope. Make a set of drawings showing the details of structure and the mode of reproduction in the specimen that you have.

EXPERIMENT 5: (*a*) Determine the motility of the various growths of bacteria on potato in the Petri dishes.

(*b*) Make four permanent slides from different appearing growths, stain with methylene-blue and examine microscopically. Make a drawing of each, showing their structure.

EXPERIMENT 6: *Staining of Spores:* Select a culture of bacteria in which spore-formation can be seen, and double stain the spores and bacteria (carbol-fuchsin and methyl-blue).

EXPERIMENT 7: *Colony Culture:* (*a*) Inoculate a tube of beef-tea with a portion of each of the different growths on potato. (*b*) After forty-eight hours make a plate culture on agar and an agar roll culture, inoculating each of these from the beef-tea culture previously made.

EXPERIMENT 8: *Tube Culture:* Make stab and streak cultures on gelatin, agar, potato, and bouillon of various different appearing colonies in the plate and roll cultures. Label each.

EXPERIMENT 9: (*a*) Make slides of each bacterium and compare them with the slides previously made from the original growths on potato. (*b*) Make drawings and describe fully the appearance and growths of each bacterium on the various media and in the colony.

EXPERIMENT 10: Inoculate an agar tube with a drop of tap-water; make plate culture. Cultivate each bacterium present, make slides, drawings, and describe fully as in previous experiments.

EXPERIMENT 11: Study of most common non-pathogenic bacteria, following out the directions laid down in experiments as above.

Part II. Study of Pathogenic Organisms.

FORM TO BE FILLED OUT FOR EACH CULTURE.[1]

Microscopic Appearance: 1. Species; 2. Arrangement; 3. Motility; 4. Spores; 5. Flagella; 6. Capsule.	
Colony on Agar Plate or Roll.	
Colony on Gelatin Plate or Roll.	
Growths in Tubes: 1. Bouillon; 2. Gelatin; 3. Agar; 4. Potato.	
Special Cultures. *Remarks.*	

[1] This form to be accompanied by a drawing of bacterium, plate colony, and tube cultures.

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