BACTERIOLOGY FOR NURSES

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BACTERIOLOGY FOR NURSES

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PREFACE

WHILE bacteriology is one of the most recent subjects introduced into the Training School Curriculum it is by no means of least importance; indeed, so intimately is it related to the other subjects that with the exception of anatomy and materia medica it may be regarded as necessary to a right understanding of them all.

The science of bacteriology has, within recent years, developed so rapidly that it is impossible to more than mention certain phases of the subject in so limited a space. That branch which is of the greatest interest to the student nurse, namely, the study of pathogenic microörganisms, comprises the greater part of the book; at the same time an effort has been made to point out that the ability to produce disease is limited to comparatively few species and that by far the greatest number of these infinitesimal forms of life perform beneficent tasks.

An attempt has been made to present the subject in as clear and interesting a form as possible in order to enable the student to realize the almost incredible force of the microscopic world, a force so powerful both for good and ill, and to place within her reach by increased knowledge the means of combating the baneful effects of those forms with which she is most likely to have to deal.

M. A. SMEETON.

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PART I

CHAPTER I

BACTERIA

CLASSIFICATION — STRUCTURE — COMPOSITION

AMONGST the lowest forms of living things organisms exist which are too small to be seen without the aid of a powerful microscope and of such simple structure that a single cell suffices for all their vital activities. Amongst these unicellular organisms the divergence between animal and plant melts away, and forms are found which possess minor characteristics of both groups. Prior to the seventeenth century little was known of these minute living cells. Conjectures had been made as to the possibility of their existence and the rôle they might be supposed to play in various natural processes and in disease, but they were merely conjectures and no record of trustworthy systematic investigations exists.

Kircher in 1659 observed their presence in putrid meat and milk; later, in 1683, Anton van Leeuwenhoeck, a Dutch microscopist, recording his observations upon tartar scraped from the teeth and mixed with water, wrote, "With the greatest astonishment I saw distributed everywhere through the material I was examining 'animalcules' of the most microscopic size which moved themselves about very energetically." Leeuwenhoeck supplemented his observations with drawings, both of which are remarkably clear and accurate.

During the following one hundred and fifty years little advance was made. Observers, for the most part, were content with simply seeing these minute organisms and marveling at the wonders of nature.

In 1762 Marcus Antonius von Plenciz, a physician of Vienna, published his views on the germ theory of infectious diseases. He insisted that an infectious disease had as its cause its own specific germ and that infective material must contain the living causal agent of the disease.

A decided advance was made by Ehrenberg. In his principal work published in 1838 upon "infusion animals" he described the difference between the larger forms and conferred upon his "animals" some of the names still current in bacteriological nomenclature.

Very soon the question arose as to the origin of these micro-organisms. Were they reproduced from similar preëxisting forms (the so-called vitalistic theory) or were they the result of spontaneous generation due to changes in the material in which they were found. Liebig and his supporters held the view that fermentation and putrefaction were simply chemical processes, and that all albuminoid bodies would if left to themselves disintegrate into smaller molecules. The force of Liebig's authority overshadowed for some time the vitalistic theory until Pasteur (1822–1895) proved that albuminous material had no natural tendency to disintegrate, and that putrefaction and decay did not produce "spontaneous generation of life," but on the contrary were manifestations of the presence of living and growing organisms engaged in satisfying their need of food, and that like all larger animals and plants these organisms come into existence only by means of reproduction.

As a result of the researches of Pasteur the study of the causal relation of bacteria to disease was taken up with renewed vigor. Investigations into the cause of certain infectious diseases in plants and insects placed the doctrine upon a firm foundation; later it was demonstrated that microörganisms were responsible for certain infectious diseases in man and animals also. To Davaine,

a famous French physician, belongs the honor of demonstrating the latter fact. An organism was found in the blood of animals suffering from anthrax by Pollender in 1849 and by Davaine in 1850. It was the latter, however, who demonstrated in 1863 by inoculation experiments that the specific organism was the cause of the anthrax.

The brilliant researches of Pasteur may be regarded as the foundation of the Science of Bacteriology; later investigators have contributed largely to placing it on the basis of an independent position in natural science. A great impetus was given to the study when Robert Koch invented a solid-culture medium by means of which the descendants of a single cell could be studied alone. This made possible the knowledge of such fundamental principles as the mode of development, physiological requirements, and capabilities of each species, a knowledge essential not only to a proper understanding of bacteriology, but also to the practical application of furthering the usefulness of such microörganisms as are of benefit to mankind and of combating those which by their activities produce disease.

Several attempts have been made to provide a satisfactory classification of these microscopic living cells, but as yet no one has succeeded in presenting a really adequate one. This can readily be understood when one realizes the minuteness of their size and the consequent difficulty of determining their relation one to the other.

In addition to the organisms which may be studied by means of the microscope still others exist which are so small as to be invisible with any magnification which we now possess. That they exist is certain because they can be grown in mass on culture media and the cultures when inoculated into susceptible animals produce the characteristic disease; they are so minute that they will pass through the finest porcelain filter. The group is generally spoken of as Ultramicroscopic or Filtrable viruses.

The following broad outline (after Park and Williams) serves to show the relationship of those forms that are of special interest in that they are able to produce disease.

Kingdom	*Subkingdom*	*Classes in which pathogenic species occur*	*Genera in which chief pathogenic species occur*
Plants (Fungi)	Bacteria (Schizomycetes)	Cocci	Micrococcus, Diplococcus, Streptococcus, Staphylococcus, etc.
		Bacilli	Bacillus
		Spirilla	Spirillum (Spirocheta, Treponema)
		Trichobacteria	Leptothrix, Cladothrix, Nocardia, Actinomyces
	Molds (Hyphomycetes)	Mycomycetes	Aspergillus, Penicillium
		Phycomycetes	Mucor
		Unclassified (Fungi Imperfecti)	Trichophyta, Achoria, Microspora, Sporotricha
	Yeasts (Blastomycetes)	Oïdia	Oidium
		Saccharomycetes	Saccharomyces
Unclassified Ultramicroscopic Organisms			
Animals (Protozoa)	Sarcodina	Rhizopodia	Entameba
	Mastigophora	Flagellata	Trypanosoma, Leishmania
	Sporozoa	Telosporidia	Coccidium, Sarcosporidium
		Neosporidia	Nosema, Babesia, Plasmodium
	Infusoria	Ciliata	Balantidium

BACTERIA

Morphological Relations. — Bacteria, the lowest of all the microörganisms known, are generally classed as plants. Their relationship, however, is by no means clearly defined. Like members of the vegetable kingdom certain species have the ability to use as food such simple elements as inorganic carbon and nitrogen; on the other hand certain species show a resemblance to the animal kingdom in requiring complex organic food. The non-motility of some, and the tendency to a thread-like growth, suggests their relationship to plant life; the motility of others and the fact that none possess chlorophyl, the green coloring of plants, suggest a kinship to the animal kingdom. It is best

to think of them as a group of single-celled organisms probably representing primitive forms that existed before differentiation into animal and vegetable kingdoms occurred.

Classification. — Bacteria may be divided into two subgroups, a lower and a simpler form and a higher and more developed one. The members of the lower form are minute masses of protoplasm surrounded by an envelope, each cell a living unit containing all the vital capacities of an independent organism.

Although there are hundreds of different species there are only three general forms, spheres (cocci), rods (bacilli), and spirals (spirilla). The spheres may be large or small, and may group themselves differently; the rods may be long or short, thick or slender, the ends may be rounded or sharply rectangular; the spirals may be flexible or stiff, they may have one, two, or many coils, but still spheres, rods, and spirals comprise all types. So far as is known, it is never possible by any means to permanently change the form of the members of one group to that of another; that is, under suitable conditions cocci always produce cocci, bacilli always produce bacilli, and spirilla always produce spirilla.

The higher bacteria (trichobacteria) show an advance on the lower forms in that they consist of united segments, branched or unbranched, which are surrounded by a sheath and which are more or less interdependent.

Transition forms no doubt exist between the lower and higher forms, and for this reason certain organisms are difficult to classify. The tubercle bacillus, for example, under ordinary conditions is a typical rod but sometimes it produces branching filaments, and for that reason it is classed by some writers with the higher bacteria.

THE LOWER BACTERIA

Terminology. — The terms microbe, microörganism, and germ are frequently used to designate bacteria; they may, however, be applied to any form of microscopic life.

The name bacterium is given to any single member of the group of bacteria, regardless of its own form.

Three terms are in use to designate the spirilla, *i.e.*, vibrio, spirillum, and spirochete. According to Migula the names are made to indicate the possession and arrangement of flagella. Flüge, another systematist, applies the term "vibrio" to all forms that are slightly curved, and "spirillum" and "spirochete" to all wavy forms. The classification of this group is at present an open question.

Size. — In size bacteria vary greatly. The cocci range from [1] 0.5μ ($\frac{1}{50000}$ inch) to 2μ ($\frac{1}{12500}$ inch) in diameter. The smallest bacillus known, the influenza bacillus, has an average size of $1\mu \times 0.2\mu$. The largest bacillus recorded (B. Bütschlii) is 50μ to 60μ long and 4μ to 5μ wide.

Reproduction. — One of the most characteristic features of bacteria is their method of reproduction. They multiply by simple division or fission (hence the term Schizomycetes or fission fungi). This method of multiplication is the distinguishing feature which separates bacteria from yeast, the latter plants multiplying by a process known as budding. When a bacterial cell is about to divide a constriction appears in the middle which gradually becomes more pronounced until the cell is completely divided and two individuals can be recognized. These may become detached at once, or, owing to the slimy envelope which is more or less developed in all bacteria, may remain attached.

Certain cocci divide as described into two individuals which separate at once (micrococci); others dividing in one plane remain attached in pairs (diplococci), or in shorter or longer chains (streptococci); others, dividing in two directions, one at right angles to the other, form groups of four (tetrads); others divide in three directions and form packets in cubes of eight (sarcinæ); others again divide in any axis and form irregular grape-like bunches (staphylococci).

Division among the bacilli and spirilla always takes place at right angles to the long axis of the cell. The cells of the bacilli

[1] 1μ = 1 micron or micromillimetre = $\frac{1}{1000}$ mm., about $\frac{1}{25000}$ inch.

for the most part separate at once; occasionally, however, they are found adhering in pairs (diplobacilli) or chains (streptobacilli). Certain spirilla show a tendency to remain attached. Thus when seen through the microscope a single cell of one of the shorter forms may have the appearance of a comma, a pair the letter S,

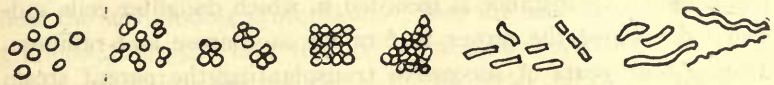


FIG. 1. — Cocci, Bacilli, and Spirilla.

and the union of several elements may appear as a long spiral form (Fig. 1).

Under favorable conditions cell division takes place very rapidly. A cell may reach maturity and divide in from twenty to thirty minutes. It has been estimated that if bacterial multiplication continued unchecked for two days and the division of each cell took place only once an hour the descendants of a single cell would number 281,500,000. It has been further calculated that these 281,500,000 bacteria would form a solid pint and would weigh about a pound. Such multiplication, however, does not actually take place. Bacterial growth may be checked by various factors, such, for example, as unsuitable temperature, lack of food and moisture, the disintegration of food substances into various injurious products such as acids and alkalies, the excretion of the bacteria, or the competition of other organisms.

Involution Forms. — When the bacterial environment has become unfavorable to growth the bacteria may show extremely irregular structures quite different to the original forms. Long thread-like organisms with irregular thickenings may develop which assume a dumb-bell or flask-like shape (Fig. 2). These are termed *involution or degenerate forms*. That they really represent degenerate changes is shown by the fact that when transferred to favorable conditions growth slowly takes place into typical forms again. It sometimes happens, however, that these involution forms lose certain properties which are never regained.

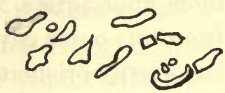


FIG. 2. — Involution Forms.

Mutations. — Another and apparently inexplicable variation sometimes appears which must be distinguished from the above. Bacteria may lose or gain certain properties and the fact may be explained by the minute divergence of successive generations. Sudden changes or *mutations* occur, however, that cannot be thus explained.[1] An instance is recorded in which daughter cells suddenly developed the power to ferment saccharose and raffinose. During four years of successive transplanting the parent strain did not acquire the property and the mutation strain did not lose it.

Structure of Bacterial Cell. — The internal structure of bacteria corresponds in simplicity to their external form. When examined under the microscope in a living condition they appear as minute colorless refractile bodies. In order to study their structure advantage has been taken of their affinity for the various dyes which are used to stain animal cells; in this way several interesting points have been determined. When stained the cell appears finely granular or almost homogeneous. Many theories have been advanced as to the nature of the cell substance or endoplasm. The one most generally accepted is that the cell body consists almost entirely of nuclear material with varying amounts of cytoplasm and that the nuclear material, instead of being gathered in a compact mass or nucleus as in animal cells, is distributed throughout the cell in a finely divided condition.

Encircling the endoplasm is a covering of cytoplasm very similar in composition. The name ectoplasm is generally considered more appropriate for this outer layer than cell membrane; it is from this outer covering that the flagella or organs of locomotion supposedly originate.

In addition to the endoplasm and its covering of ectoplasm, many bacteria, and perhaps all, are provided with a surrounding capsule often of considerable thickness. Organisms in which it is specially conspicuous present a more or less slimy appearance and appear to be embedded in what seems to be a mass of jelly. Such a mass is spoken of as a zoöglœa mass; the individuals are known as

[1] Jordan, Proc. Nat. Acad. of Science, 1915, 1, p. 160.

capsulated bacteria. The capsule is most easily demonstrated in preparations made directly from animal tissues or fluids, where, when stained, it can be seen surrounding the cell like a halo.

Metachromatic Granules. — In certain bacteria granules have been observed which show a greater affinity for nuclear dyes than does the surrounding protoplasm. They are called metachromatic granules from the fact that by appropriate methods they will retain one stain while the rest of the bacterial cell can be made to take another. In young diphtheria bacilli they are often very conspicuous and serve as an aid in diagnosis. Their nature and significance have not yet been determined.

Other granules have been described which have been shown to consist of starch or fat or of other substances; they probably represent material in process of transformation into cell nutrition. In certain bacteria which find their food supply in decaying organic material granules of sulfur have been demonstrated, in others iron granules.

Motility. — Many species of bacteria are capable of independent movement. When seen in a fluid preparation through the microscope their movements may appear of a darting or rolling nature or they may be very sluggish and scarcely perceptible. Bacterial motility is always a real progressive motion and not merely the oscillating vibration exhibited by all finely divided particles suspended in suitable fluid. The latter so-called "Brownian movement" is a purely physical phenomenon which may be shown by dead bacteria and inorganic substances.

The speed with which certain bacteria move has been estimated; the cholera spirillum, for example, may travel for a short distance at the rate of 18 cm., or about 7 inches, per hour. While this does not seem very great it is considerable when one considers the minute size of the organism. Most of the actively motile bacteria are bacilli or spirilla.

The motility of bacteria depends upon their possession of thin hair-like appendages or flagella which are so extremely fine that special staining methods are necessary to demonstrate them. By means of their power of contractility they keep up a lashing

to and fro movement which serves to propel the bacterium through the liquid in which it is growing (Fig. 3).

Fig. 3. — Arrangement of Flagella.

The arrangement of the flagella varies in the different species of bacteria. Following is a classification according to their number and distribution:

Species	Description	Example
Monotricha	A single flagellum at one pole	Cholera spirillum
Amphitricha	A flagellum at each pole	Many spirilla
Lophotricha	A tuft of flagella at one pole	Spirillum undula
Peritricha	Flagella projecting from all parts of the surface	Typhoid bacillus
Atricha	No flagella	All non-motile bacteria

The degree of motility depends upon the species, the age of growth, heat, light, the presence of chemicals, etc. The property by means of which bacteria are aware of the various forces which influence them is known as *taxis*. When they are attracted the phenomenon is spoken of as positive taxis, when they are repelled as negative taxis.

Spore Formation. — Under certain circumstances some species of bacteria produce changes in their protoplasm which result in the formation of bodies known as endospores, and to these new bodies all the vital powers of the original cell are transferred. Its commencement in a bacterium is indicated by the endoplasm becoming turbid and the appearance of minute refractile granules which do not readily take the ordinary stains. By degrees the granules become larger and finally coalesce into a spherical or oval body, always shorter but often broader than the original bacterium. Surrounding the endospore is a dense protective envelope which is supposed to give to the spore its characteristic

property, namely, great resistance to harmful influences such as heat, chemicals, etc.

The spore may be formed in any part of the bacterium but its position is generally constant in the same species. It may lie within the center of the cell without changing the contour of the latter, or it may distend the central part of the cell, giving it a spindle-like shape, or it may be formed at one end, giving the bacterium the appearance of a drumstick.

When conditions again become favorable for growth, the organism assumes its original form. The spore absorbs moisture, becomes swollen, and loses its slimy refractile appearance; later a little bulging is seen on one side of the cell if the spore is central or at the extremity if the spore is polar. This protrusion continues until finally the spore envelope bursts and a rod of soft protoplasm

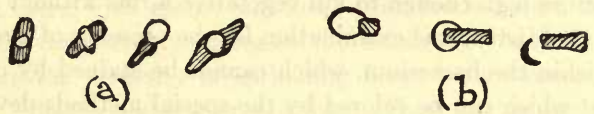


FIG. 4.— (*a*) Position of Spores. (*b*) Germination of Spores.

emerges which then commences to function in the ordinary manner of its species (Fig. 4).

Spore formation must not be regarded as a method of reproduction; it is a *resting* stage which should be contrasted with the *vegetative* stage when active multiplication takes place. It occurs most frequently in bacilli, less often in spirilla and very rarely in cocci. Fortunately there are very few spore-bearing organisms pathogenic for man, a fact which greatly simplifies disinfection and the treatment of infectious diseases.

Two views are advanced regarding the significance of spore formation in bacteria. According to one view it is considered as a period of rejuvenescence and that an alternation between the vegetative and spore stage is necessary in order that the species may maintain its highest vitality. In support of this view there exists the fact that in some cases sporulation will cease at a temperature above or below the optimum, while vegetative growth will have a much longer range. The anthrax bacillus if kept at a

temperature above the limit at which it grows best not only ceases to form spores but it loses its power of sporulation.

The second view is that a bacterium only forms spores when conditions are unfavorable to its life and growth; that it is essentially a process whereby a species may be preserved in a hostile environment until its surroundings again become favorable. The lack of food, the presence of substances excreted by the bacteria themselves, and the products formed by the disintegration of the food material in which they are growing play an important part in making unsuitable surroundings. Species which form spores under these conditions will always change into vegetative forms when placed in a fresh food supply.

The tests usually made to determine whether or not spore formation has taken place are: (1) Subjection of the culture to a temperature high enough to kill vegetative forms without injuring spores, (2) Microscopic examination for the presence of a refractile body within the bacterium, which cannot be stained by ordinary dyes but which can be colored by the special methods devised for the staining of spores (page 30).

Chemical Composition. — The chemical composition of bacteria varies somewhat according to the nature of the species and the material upon which they are growing. Ordinarily the bodies of bacteria contain from 80 to 88 per cent of water. Substances of a protein nature similar to the albumins and globulins found in animal and plant tissues are present which probably represent the vitality of the cell. The presence of fat has been demonstrated; also starch-like granules staining blue with iodine have been observed. Sulphur, iron, calcium, potassium, chlorin, magnesium, etc., may also, in small quantities, form part of the bacterial protoplasm.

CHAPTER II

FACTORS INFLUENCING BACTERIAL GROWTH. DISINFECTANTS. RESULTS OF BACTERIAL GROWTH

Habitat. — Bacteria of one species or another exist almost everywhere. Forty different forms have been described as common in soil. They are present in the air in large numbers in populated areas, especially near the surface of the ground. Particles of dust may be laden with them; anything that tends to set the dust in motion considerably increases the number of bacteria in the air, hence the necessity of sprinkling floors before sweeping and "moist" dusting whenever possible. They are present in the mouth, stomach, and intestines of animals and human beings; on the surface of the skin, under the finger nails, and on the hairs. They are present in rivers, and in the ocean. They are especially abundant in all forms of decaying matter. Everywhere, then, in nature there exists this tremendous force with its wonderful power of multiplication, a power which nevertheless is held in control.

FACTORS INFLUENCING BACTERIAL GROWTH

Food. — Perhaps the universality of bacteria is explained by the fact that they can utilize the most diverse substances as food, substances varying from the simplest to the most complex nitrogenous compounds. The presence of nitrogen, however, in some form is indispensable.

A simple classification of bacteria may be made on the basis of their food requirements. Those which supply their nutritional needs while engaged in disintegrating the lifeless remains of plants or animals are known as *strict saprophytes*. On the other hand,

those species which can grow only within or upon a living host (plant or animal) are known as *strict parasites*. A hard and fast line cannot be drawn between these two groups because there exist certain organisms which are ordinarily saprophytes but which may grow in living tissues and cause disease. Such forms are spoken of as *facultative parasites*. Other species exist which grow best as parasites in living tissue but which can be cultivated on non-living material. The pathogenic organisms which can be grown on culture media belong to this class. They are known as *facultative saprophytes*.

Moisture. — Water is essential for bacterial growth. The different species vary in the degree of their need. The cholera spirillum, if deprived of moisture, will die in from two to three hours; the bacillus of diphtheria may live under the same conditions several days. Spores are much more resistant to drying than vegetative forms; they will germinate after remaining in a dry condition for years. It often happens, however, that organisms exposed to such harmful influences, even though they survive, lose some of their original properties.

Osmosis. — A certain degree of dilution is necessary for food substances in solution. When bacteria suddenly find themselves in a concentrated fluid they cannot readily adjust themselves, and if the difference is too sudden or too great death may speedily result. An illustration of this is the keeping qualities of a thick syrup as compared with the rapid fermentation of a dilute sugar solution. The best development of an organism takes place when the osmotic pressure is the same in the surrounding fluid as that within the cell itself. If the fluid is too concentrated water is drawn from the bacterial cell and the protoplasm shrinks from its outer covering; the condition is spoken of as "plasmolysis." If on the other hand the new fluid has a lower pressure the cell absorbs more water and may burst. This latter condition is termed "plasmoptysis."

Oxygen. — The free oxygen of the air is absolutely necessary for the growth of the majority of organisms; there is, however, a small group which cannot live when it is present. Pasteur

was the first to note this extraordinary fact and he suggested the grouping of bacteria into two divisions, viz.: *aërobes* which require the presence of free oxygen, and *anaërobes*, which require the exclusion of free oxygen. Midway between the obligatory aërobes and the obligatory anaërobes, however, there are many organisms which do not belong strictly to one group or the other. Facultative anaërobes grow best in the presence of oxygen but their growth is not checked when the supply is limited. Facultative aërobes on the other hand are anaërobes that can tolerate a certain amount of free oxygen. Anaërobes are not affected by the presence of hydrogen or nitrogen.

Light. — The effect of light upon bacteria is very marked. Bright daylight may inhibit their growth, and many species cannot live when exposed to the full action of the sun's rays. A longer exposure is necessary when they are moist than when they are dry. Typhoid bacilli are killed in about one and a half hours. The bactericidal effect of light is due mainly to the green, violet, and ultraviolet rays. An interesting experiment illustrating the effect of light upon bacteria may be carried out by pouring inoculated media into a Petri dish, the cover of which has been partly shaded by pasting on a strip of black paper. The plate after being exposed to direct sunlight will show colonies only in the shaded portion. A series of plates may be prepared and exposed varying lengths of time, half an hour, one hour, etc. The plates should be kept two or three days at 20° C. to 30° C. to allow the colonies in the shaded portion to develop.

Electricity. — The effects of electricity upon bacteria have not been thoroughly studied. A powerful electric light is supposed to be as fatal as sunlight.

Radium and Röntgen rays have not been shown to have more than a slight inhibitory action.

Temperature. — The range of temperature within which bacterial growth of one species or another may occur lies between 0° C. and 72° C. For every species there is an optimum temperature or a temperature at which its growth is most luxuriant. Each species too has its maximum temperature above which growth

will not take place and its minimum temperature below which it is inactive. Death does not necessarily occur at these limits but reproduction does not take place. The maximum and minimum for each species has a range of from twenty to thirty degrees and its optimum does not extend ordinarily more than five degrees. The maximum for some may be below the minimum for others, for example, for B. phosphorescens the minimum temperature at which growth occurs is 0° C., the optimum 20° C., and the maximum 37° C., while for B. thermophilus, an organism found in fermenting manure, the minimum temperature is 40° C., the optimum about 66° C., and the maximum 72° C. Generally speaking, the optimum temperature for bacteria is the ordinary temperature of their natural habitat. The most favorable temperature then for pathogenic organisms is that of the human body. If grown on culture media at a higher temperature they may lose their virulence.

The vegetative forms of most bacteria are killed by half an hour's exposure in the presence of moisture to a temperature of from 55° C. to 58° C. or by 10 minutes' exposure to a temperature of 60° C. to 80° C. There are no non-spore-bearing forms, except a few cocci, that can live in boiling water even for a few minutes. Most of the pathogenic bacteria, including the cholera spirillum, the typhoid bacillus, and the tubercle bacillus, are destroyed in ten minutes when exposed to moist heat at 60° C. Thus milk properly pasteurized or water brought to the boiling point are rendered harmless so far as these germs are concerned.

Dry heat is much less effective than moist; many pathogenic organisms can withstand in the absence of moisture a temperature of 100° C. for half an hour. Spores are especially resistant to both dry and moist heat. Practically all forms, however, are killed by exposure to dry heat for one hour at 150° C. or to steam under pressure in an autoclave for 15 minutes at 125° C.

Bacteria are affected by low temperatures much less than by high. Many have been subjected to a temperature of liquid air (about −190° C.) without being destroyed.[1] In a culture of

[1] Park and Williams, "Pathogenic Microörganisms," p. 56.

typhoid bacilli exposed to −175° C. for thirty minutes 10 per cent remained alive.

Antibiosis and Symbiosis. — In nature many species of bacteria exist side by side; pure cultures are seldom found outside of the laboratory. In some instances the products of one species are antagonistic to the well-being of another and the weaker is able to multiply very slowly or not at all. Saprophytic forms soon overpower the comparatively less resistant disease-producing forms. Thus infected carcasses eventually become purified by the same processes that destroyed them.

Sometimes, on the contrary, there is a certain amount of coöperation between two species and the presence of one induces the more luxuriant growth of the other. This latter condition is spoken of as symbiosis. Certain anaërobes will grow when air is admitted into the culture tube if cultivated with an aërobe; it is assumed that the aërobe deprives the air of its oxygen content and thus renders conditions suitable for anaërobic growth.

Effects of Chemicals. — Chemical substances vary in their bactericidal powers as do the various bacteria in their degree of resistance. Just what their action really is, in many instances, is not known. In the case of bichloride of mercury or formaldehyde there appears to be a chemical union between the disinfectant and the cell protoplasm. Vegetative forms are affected more quickly than spores.

The following terms are frequently used to express the inimical effect of chemicals or physical forces upon bacteria:

Attenuation. — Function diminished but not impaired.

Antiseptic Action. — Growth arrested but capable of recommencement as soon as surroundings become suitable.

Disinfection. — Destruction of all disease-producing forms and their products.

Sterilization. — Destruction of all forms, pathogenic and non-pathogenic.

Disinfectants which are very effective under certain circumstances may become almost inert under others. Milk of lime is of use only while it remains milk of lime; when the carbon dioxide

of the air has converted it into carbonate of lime it is practically harmless. Bichloride of mercury is a powerful disinfectant under some circumstances but when placed in contact with organic material it forms an albuminate which renders it much less effective. On this account it is not well suited to the disinfection of sputum and feces.

In applying a disinfectant whose strength is known it should always be remembered that it must be present throughout the entire mass in the proportion required. Thus if a disinfectant is active in a 10 per cent solution it cannot be used in that strength to disinfect an equal volume of infected material — the mixture would contain only 5 per cent of the bactericidal substance. An equal volume of a 20 per cent solution would be required to give 10 per cent of the disinfectant in the resulting mixture.

Methods for the standardization of disinfectants have been devised whereby their relative value may be determined. Carbolic acid is used as the standard and the comparative strength of other substances is stated in terms of their coefficiency.

Even after the relative strength of a bactericidal substance is known it should be remembered that the reaction of the solution and the material to be disinfected must be considered. Thus if an alkali such as lime is used to disinfect an acid substance, sufficient lime must be added first to neutralize the acid and then the additional amount required for disinfection must be added.

A great number of more or less effective disinfectants have been put upon the market, the most costly of which are by no means the most reliable. Such well-known chemicals as carbolic acid, bichlorid of mercury, lime, coal tar, creosotes, formalin, etc., give a wide range of choice and in addition their advantages and limitations are well established.

DISINFECTANTS

Carbolic Acid.—A solution of 1 part to 1000 inhibits the growth of bacteria, 1 part to 100 kills vegetative forms in from five to thirty minutes, and 1 part to 20 kills most spores within a few hours and all within a period of from one to four weeks. Carbolic acid

is perhaps one of the most generally used disinfectants because it is so little affected by albuminous substances, it is not readily decomposed, and does not harm fabrics, metals, or wood.

Alcohol. — Ten per cent solution inhibits bacterial growth; absolute alcohol kills vegetative forms within twenty-four hours.

Formalin is a 40 per cent solution of formaldehyde gas; it is supposed to have about one half the germicidal power of carbolic acid; a 2 per cent solution of formalin will kill vegetative forms in from five to thirty minutes.

Formaldehyde is probably of greatest service in its gaseous form as a disinfectant of buildings and furniture. It does not harm delicate fabrics; wood, copper, brass, and silver are not affected by it. Under ordinary circumstances its powers of penetration are not great and it can only be depended on for surface disinfection. Vegetative forms of bacteria exposed directly to the action of concentrated formaldehyde gas are killed at once. It is advisable, however, in disinfecting a room to allow several hours' exposure in order that the gas may reach all corners. It has very little effect upon animals or insects.

Iodoform as such has little effect upon bacteria. When in the presence of pus it is broken down into iodine compounds which act partly by rendering inert the poisons produced by bacteria and partly by destroying the bacteria themselves.

Chloroform will destroy bacteria in the vegetative form in 1 per cent solution. It is not known to have any effect upon spores.

Lysol is a coal-tar product containing about 50 per cent cresols. It is generally used in 1 per cent solution; it has about double the strength of carbolic acid.

Bichloride of Mercury, commonly known as corrosive sublimate, is one of the most potent germicides known. Exposure for half an hour to a solution of 1 part to 2000 parts of water in the absence of organic material, or 1 part to 1000 if it be present, is ample to destroy all vegetative forms. Spores are killed in 1 to 500 solution in one hour. The value of bichloride of mercury is somewhat limited by the fact that it is irritating to the skin, that in the presence of alkaline albuminous substances it forms inert compounds,

and that it has a corrosive effect upon metals. Its use then as a disinfectant for sputa, feces, etc., is not advisable and care should be exercised in using it about household plumbing.

Potassium Permanganate ranks high as a germicide for certain purposes; its greatest usefulness is probably in surgical practice. A 1 to 800 solution will kill vegetative forms. Koch found that a 1 to 20 solution killed spores in one day. Its application is somewhat limited on account of the readiness with which it combines with organic material and thus becomes inert.

Sodium Hydroxide (Caustic Soda). — Vegetative forms of bacteria are killed in a few minutes by a 1 per cent solution. Spores are killed in about three quarters of an hour in a 4 per cent solution.

Sodium Carbonate (Washing Soda). A 5 per cent solution will destroy vegetative forms in a few hours. It has no effect upon spores at ordinary temperatures.

Sodium Bicarbonate has practically no germicidal properties.

CALCIUM COMPOUNDS

Slaked Lime. — Lime is one of the best and cheapest disinfectants known. Slaked lime or calcium hydroxide is prepared by adding one pint of water to two pounds of lime. A 1 per cent solution of this freshly slaked lime will kill all vegetative bacteria within a few hours. A 3 per cent solution kills typhoid bacilli in one hour. Feces or other infected material may be completely disinfected if mixed with equal parts of a 20 per cent solution and allowed to remain in contact for two hours. Freshly slaked lime should always be used; if left exposed to the atmosphere it absorbs more water and carbon dioxide and is converted into calcium carbonate (marble), which is quite inert.

Milk of Lime is slaked lime diluted with four to eight times its volume of water. It should not be used if more than a few days old unless well protected from the air.

Chlorinated Lime, popularly known as **Chloride of Lime** or **Bleaching Powder**, may be used either as a dry powder or in solution. In the dry state it is frequently used in privy vaults

where it also acts as a deodorant and desiccant. Under certain circumstances it is one of the most powerful germicides known. One per cent solution will kill most bacteria in one to five minutes. A 5 per cent solution usually kills all spores within an hour. Unfortunately it bleaches and destroys fabrics.

Recently, chlorinated lime or chlorinated soda has come into prominence as a disinfectant of drinking water. Very minute quantities will render a comparatively clean water sterile.

Labarraque's Solution contains several chlorine compounds, chiefly sodium hypochlorite and sodium chloride. It is a little more expensive and not so efficient as chlorinated lime.

Antiformin consists of a solution of caustic soda and chlorinated lime. It is a strong germicide; a 2 to 5 per cent solution will kill most vegetative forms in five minutes. The tubercle bacillus and other members of the same group, however, are slightly affected by it.

Chlorine Gas is strongly germicidal. Its activity is due to the fact that in the presence of moisture it combines with the hydrogen, thus liberating oxygen which in its nascent state combines with the albuminous substance of the bacterial cell. A 0.2 per cent watery solution kills spores in 5 minutes and the vegetative forms almost immediately.

Iodine has much the same value as chlorine both in its gaseous state and in solution.

Acids are effective germicides. A 1 to 500 solution of sulphuric acid kills most vegetative forms in an hour. Hydrochloric acid is somewhat weaker; acetic, citric, and salicylic acids are much weaker still. A 2 per cent boric acid solution will destroy the less resistant forms of bacteria.

Sulphur Dioxide is a strong insecticide but is of little value as a germicide. The slight action it possesses on bacteria depends on the presence of moisture. It has no effect upon spores.

Application of Disinfectants. — The most effective place to apply disinfectants is as near the seat of the origin of infection as possible. As the excretions from the mouth, nose, and bowels, as well as discharges from eruptions or wounds, are mainly respon-

sible for the spread of disease their immediate disinfection is imperative.

RESULTS OF BACTERIAL GROWTH

Not only are bacteria influenced by their surroundings, but they in turn exert a tremendous influence on these surroundings. The effects of bacterial growth depend largely on the species and on the composition of the substance on which they happen to be growing.

Light. — Twenty different species of bacteria have been described which have the power of emitting light. The phosphorescence sometimes seen on decaying meat and fish is due to the growth of these organisms. They are widely distributed in nature and grow most luxuriantly in media rich in salt, as in sea-water. It is supposed that the phenomenon is caused by a substance, photogen, which is closely combined with the cell substance.

Heat. — The elevation of temperature in substances undergoing bacterial decomposition, such as tobacco, manure, etc., is often attributed to bacteria. It is more generally supposed that the heat is due to chemical reactions.

Pigment. — When bacteria growing on culture media have become sufficiently numerous to be seen with the naked eye, they appear as a more or less moist grayish mass. Certain species, however, have the ability to produce a pigment which may give to this mass a brilliant hue. According to the species the color may be violet, blue, red, green, orange, or yellow. The nature of the pigments or the purpose they serve are not fully understood; they are generally thought to be related to the lipochromes, the coloring matter met with in the yolk of eggs, carrots, etc.

Chemical Effect. — As bacteria consume the material which serves them as food they break up the complex organic molecules into simpler ones and thus entirely change the chemical nature of their surroundings. Three different forms of bacterial activity may occur:

1. The nourishment of the cell protoplasm itself.
2. Excretion from the bacterial cell of waste products.
3. Production of secretions of the nature of ferments or enzymes, which disintegrate the food substance on which they are growing.

The enzymes produced by bacteria are probably responsible for all the processes of disintegration to which they give rise. Two general forms are recognized: *fermentation* and *putrefaction*. The term fermentation is usually applied to the breaking down of carbohydrates into alcohol, acids, carbon dioxide, etc. Putrefactive decomposition is the term applied to the breaking down of nitrogenous substances. It is generally thought that bacterial action on proteins closely resembles that of the digestive enzymes of the animal alimentary tract. The proteins are decomposed into: proteoses, peptones, amino-acids, etc., often with end-products such as skatol, mercaptan, and sulphureted hydrogen. Ordinarily these processes are carried on by aërobic and anaërobic bacteria living in symbiosis.

CHAPTER III

STERILIZATION OF GLASSWARE. PREPARATION OF CULTURE MEDIA

In order to become familiar with the characteristics of any given species of bacteria, it is necessary, first of all, to isolate it from every other form. In nature it is seldom that one species is found growing alone. This does occur in certain diseases, but generally speaking bacteria have to be taken from their natural surroundings and grown on artificial food medium in order to be studied.

The first essential then for bacterial study is to provide conditions whereby one species may grow, without an admixture of other forms. Since bacteria are practically everywhere this can only be accomplished by first destroying all germs in the food medium on which the organism is to be cultivated and on all apparatus likely to come in contact with it. In order to do this one form or another of sterilization is employed, the method used depending largely upon the object to be sterilized; the underlying principle of each, however, is the destruction of bacteria by heat. Hot air or " dry heat " is generally used for glassware and hot water or steam, " moist heat," for culture media.

Cleaning of Glassware. — Before sterilization each article of glassware should be perfectly clean. New glass as a rule only requires washing with soap and water and the adherent dirt removed with a test-tube brush. Old glassware containing cultures should be sterilized either in the autoclave or boiled in 5 per cent solution of washing soda or soapsuds for one hour in a covered boiler. Glassware may be further cleansed if necessary by placing for one hour or more in the following chromic acid mixture:

Bichromate of potassium	6 parts
Water	100 parts
Sulphuric acid	6 parts

Dissolve the bichromate by heating in an agate kettle. Add the sulphuric acid slowly on account of the heat generated; after cooling keep in a glass jar. The mixture may be used more than once.

After thoroughly rinsing and drying, test tubes and flasks are plugged with ordinary non-absorbent cotton. The plugs should not be twisted or creases will form, making possible the entrance of bacteria from the surrounding air. The most satisfactory method is to roll the cotton, which should fit just tight enough to allow one to lift the tube by means of the plug.

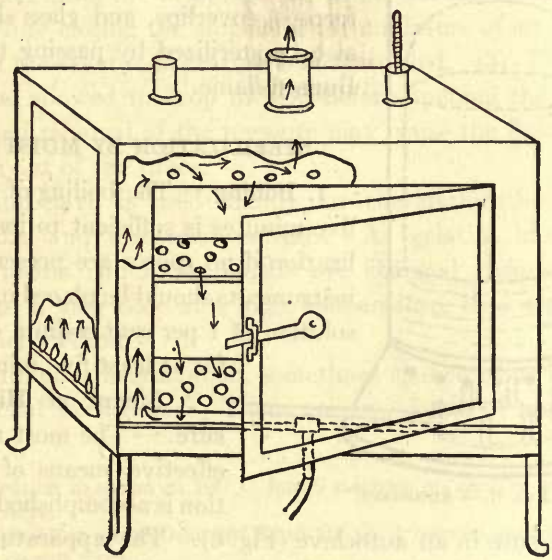


FIG. 5. — Hot Air Sterilizer.

STERILIZATION BY DRY HEAT

1. **Hot Air Chamber.** — This method is used for all forms of glassware. The hot air chamber (Fig. 5) consists of an outer and an inner covering of sheet iron. At the bottom, in the space between the two jackets, several gas jets are arranged. The

air thus heated surrounds the inner chamber and escapes through holes in the top of the outer case. The bulb of the thermometer used to record the temperature should pass about to the center of the inner chamber. Heating for one hour at 150° C. is sufficient to insure sterilization. Articles should be placed in the hot air chamber, without crowding, before heating it and should be allowed to remain there after sterilization is complete until the temperature falls. Sudden heating or cooling may cause the glass to crack.

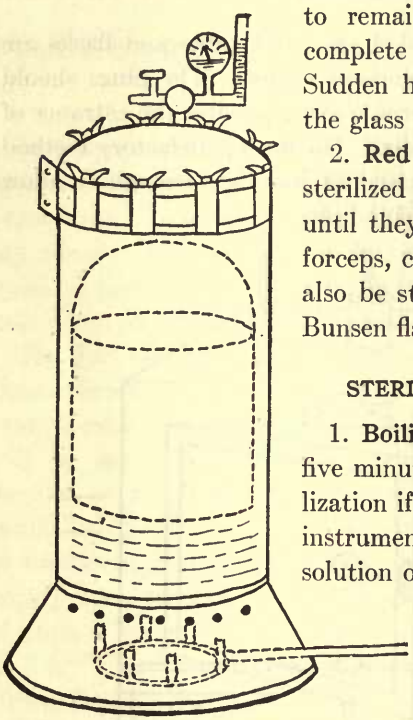


FIG. 6. — Autoclave.

2. **Red Heat.** — Platinum needles are sterilized by flaming in a Bunsen burner until they are red hot. The points of forceps, coverlips, and glass slides may also be sterilized by passing through a Bunsen flame.

STERILIZATION BY MOIST HEAT

1. **Boiling.** — The boiling of water for five minutes is sufficient to insure sterilization if no spores are present. Steel instruments should be placed in a boiling solution of 1 per cent sodium carbonate for at least five minutes.

2. **Steam at High Pressure.** — The most rapid and effective means of sterilization is accomplished by steam under pressure in an autoclave (Fig. 6). The apparatus consists of a metal cylinder supported in an iron case; it is fitted with a pressure gauge, thermometer, and safety valve; its cover can be securely fastened down with nuts and screws. The articles to be sterilized are supported on a perforated diaphragm, and heat to generate the steam is supplied by a large Bunsen burner beneath. The temperature usually employed is 115° C. to 120° C. In order to obtain a temperature of 115° C. a pressure of

about 23 pounds to the square inch is necessary, that is, 8 pounds more than the ordinary atmospheric pressure of 15 pounds. To reach 120° C. the pressure must mount to 30 pounds or 15 pounds plus the atmospheric pressure. Temperature and pressure correspond thus:

Temperature about 115° C. — Increased pressure necessary, 8 lb.
Temperature about 120° C. — Increased pressure necessary, 15 lb.

A temperature of 120° C. maintained for 15 minutes is sufficient to sterilize media in test tubes. Media in bulk is generally allowed 30 minutes.

In using the autoclave care should be taken (1) that baskets of tubes are not placed one on the top of the other or the plugs will become wet from the dripping. (2) All air should be displaced by steam before closing the stopcock. If a mixture of air and steam is present the exact temperature is not recorded. (3) The pressure should be allowed to drop to zero before opening the stopcock; a too rapid removal of the pressure may cause the fluid media to be blown out of the tubes.

The autoclave is used mainly for the sterilization of sugar-free media and discarded cultures. As gelatin, blood serum, and all media containing sugars are changed chemically when heated for a long time at a high temperature, they are sterilized by another method.

Discontinuous Sterilization, sometimes spoken of as *intermittent* or *fractional sterilization*. There are two forms of applying this method:

1. Heating in steam at 100° C. for 20 minutes on each of six successive days.
2. Heating in steam from 56° C. to 70° C., 1 hour on each of three successive days.

The former method is used for gelatin and all sugar media that would be injured by autoclave sterilization; the latter method is employed for media containing blood serum or transudates from body cavities, such as ascitic fluid.

The principle underlying both methods is: all bacteria when free from spores are killed by the temperature of boiling water,

many even at a much lower temperature. Thus heating on the first day will kill all non-spored forms. During the twenty-four hours' interval the media is kept at 22° C. in order that spores if present may develop into vegetative forms and be killed during the second heating. In case any of the spores have been slow in developing and thus escaped the second heating the process is repeated a third time. Generally speaking this method gives good results; it is advisable, however, to prolong the heating of media in flasks to half an hour.

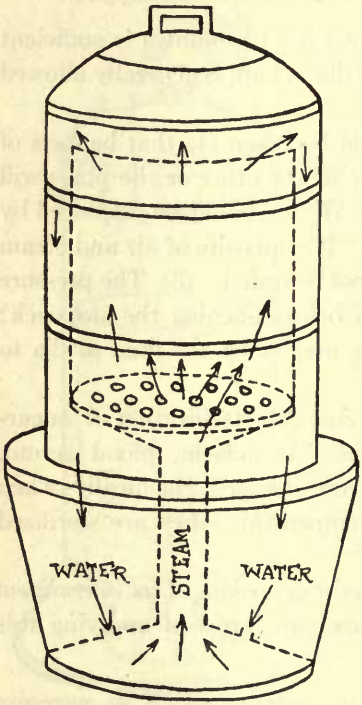


Fig. 7. — Arnold Sterilizer.

The "Arnold" steam sterilizer (Fig. 7) has almost entirely displaced that introduced by Koch for this form of sterilization. It is constructed with a false bottom so that a small quantity of water may be heated to produce steam quickly. A perforated tin diaphragm permits the steam to stream up and surround the objects placed on it. As the steam rises it passes through an opening in the top of the inner jacket and descends as water of condensation to again feed the water below. By this method as by that of autoclave sterilization no evaporation takes place from the media because it is surrounded with an atmosphere already saturated with moisture.

An ordinary kitchen steamer over a pot of boiling water may be used if an Arnold sterilizer is not available.

For the second method, *i.e.* heating at a lower temperature on six consecutive days, an inspissator (Fig. 8) is generally used.

Rubber Stoppers and Tubing should never be heated, they should be cleansed with soap and water and allowed to stand for one hour in a 1 to 1000 solution of bichlorid of mercury, then washed with sterile water before using.

CULTURE MEDIA

One of the points to be observed in the artificial culture of bacteria is that the food medium supplied should resemble as closely as possible that to which the organism is accustomed. Many species grow luxuriantly if only a simple nitrogen and carbon compound and some salt and water are present; others require more complex substances. For certain pathogenic bacteria body fluids such as blood serum and ascitic fluid are used. The fact that certain species will grow abundantly on one kind of medium and not at all on another, or that characteristic growth will occur on certain media, is often an aid in the identification of species.

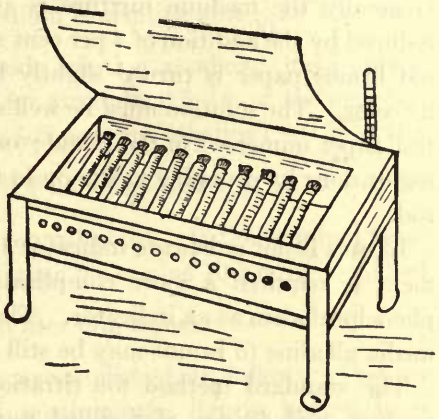


FIG. 8.— Inspissator.

The most commonly used media have as their basis a watery extract of meat to which a small amount of peptone is added. Koch found that by the addition of gelatin to this broth a solid transparent medium would result on which growth from a single organism could be obtained and on which also certain bacterial characteristics became evident. Gelatin, however, has only a limited use; it does not remain solid at the temperature best suited for pathogenic microörganisms; moreover, there are certain species of bacteria which during their growth are able to liquefy it at lower temperatures. To obviate these conditions agar,

a product derived from the stems of seaweed growing in the Chinese seas, has been substituted. Agar is not liquefied by the action of bacteria, does not melt below 98° C., and on cooling solidifies at about 39° C.

Certain technical procedures such as adjusting the reaction, clearing and filtering of media, should be understood before the preparation is attempted.

Titration and Adjustment. — A moderately alkaline reaction to litmus is suitable for the growth of most pathogenic bacteria. Generally the medium mixture is at first too acid; it may be reduced by the addition of 4 per cent sodium hydrate solution until red litmus paper is turned slightly blue and blue litmus retains its color. The solution must be well stirred into the media and the test paper immersed in the liquid; on no account should the testing be done by dropping media on to the paper by means of a glass rod.

Litmus is not a delicate indicator and if a more accurate adjustment is required a more complicated method is adopted, with phenolphthalein as an indicator. The neutral points are different; media alkaline to litmus may be still acid to phenolphthalein.

The standard method for titration is as follows: 5 c.c. of medium and 45 c.c. of distilled water are mixed in a casserole and boiled for one minute, then 1 c.c. of the phenolpthalein solution (0.5 per cent in 50 per cent alcohol) is added. If no color appears the medium is acid, and while hot twentieth normal sodium hydroxide solution $\left(\text{NaOH } \frac{N}{20}\right)$ is run into the casserole from a burette until a faint but distinct color is seen. This color must remain on stirring, otherwise more alkali is needed. From the amount added the acidity of the medium is determined and an estimate made of the amount of normal solution $\left(\text{NaOH } \frac{N}{1}\right)$ to give the required reaction to the bulk of the medium necessary.

For example, if 5 c.c. of medium required 2.4 c.c. of $\frac{N}{20}$ NaOH to neutralize it, 100 c.c. (twenty times as much) would require

2.4 c.c. of $\frac{N}{1}$ NaOH (twenty times as strong); in other words the medium is 2.4 per cent acid to phenolphthalein (+2.4). Assuming the required reaction is +1 per cent we must add 2.4 c.c. minus 1 c.c. or 1.4 c.c. of $\frac{N}{1}$ NaOH to every 100 c.c. of medium, or 14 c.c. to a liter. The plus sign is used to indicate an acid and the minus sign an alkaline reaction to phenolphthalein.

Should, on the other hand, the mixture show a pink color when the indicator is added, the medium is alkaline and $\frac{N}{20}$ HCl is used instead of the sodium hydroxide solution as above, until only a very faint color persists. If, for example, 0.5 c.c. of the acid has been used then the medium is 0.5 per cent alkaline (−0.5%) and it will require 0.5 c.c. of $\frac{N}{1}$ HCl for every 100 c.c., or 5 c.c. for every liter, to bring it to the neutral point. If the required reaction is +1 or 1 per cent acid, then 5 c.c. plus 10 c.c., or 15 c.c., of $\frac{N}{1}$ HCl must be added to each liter of medium.

Clearing Media. — This is accomplished as follows: One or two eggs for each liter of medium are lightly beaten in a pan and mixed with a little water. The medium is cooled to below 60° C. and the eggs are thoroughly stirred into it. The mixture is then heated in the Arnold Sterilizer or autoclave and as the egg albumin coagulates, it enmeshes and carries down with it as a sediment all the fine particles, thus leaving the medium clear.

Filtering Media. — Fluid media may be filtered through paper; for media which will solidify on cooling absorbent cotton is better. In the latter case a spiral of copper wire is placed in the bottom of the funnel and two moderately thick squares of absorbent cotton are so arranged over it that the fibers of one are at right angles to those of the other. Paper or cotton should first be moistened with water so that any fat in the media will not pass through. The first filtrate may not be clear, in which case it should be passed

through the filter a second time. Media which solidify when cool should be filtered in a warm place.

Tubing of Media. — For the most part media are used in test tubes. An improvised apparatus (Fig. 9) may be arranged for filling them as follows: A piece of rubber tubing is attached at one end to a glass funnel and at the other to a glass point; a pinchcock is fixed at the center of the tube. The plug is removed from the tube by taking it between the third and fourth fingers of the right hand and the glass point is placed almost to the bottom of the test tube in order to prevent the medium touching the neck of the tube where the cotton plug might stick. About 7 c.c. of medium is sufficient for each tube. The next step is sterilization by the method most suitable, after which the medium is ready for use.

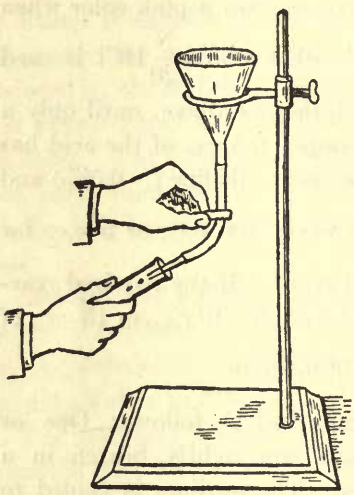


FIG. 9.— Apparatus for tubing Media.

Preparation of Culture Media in Common Use. — The basis of the most commonly used media is an infusion of meat, or meat extract, to which a small amount of peptone and sodium chloride is added.

Meat infusion is prepared as follows: One liter of water is poured over one pound of finely chopped beef or veal. It is heated at a temperature of 50° C. for one hour or it may be allowed to remain twenty-four hours in the refrigerator and not heated. The infusion is then strained through cheesecloth and all the juice thoroughly pressed out of the meat. The fluid contains soluble albumins, extractives, muscle sugar, and salts.

As a substitute for meat infusion Liebig's extract 2 to 3 grams per liter of water may be used.

1. **Nutrient Broth.**

Meat infusion or meat extract	1000 c.c.
Peptone	10 gm.
Sodium chloride	5 gm.

Warm the meat infusion to 50° C., add the peptone and salt and stir until the peptone is dissolved. Add a little sodium hydroxide to reduce the acidity, boil to coagulate the albumin present; ordinarily it is not necessary to clear with eggs. Add water to make up for that lost by evaporation, adjust reaction, filter, and tube or place in flasks for sterilization. Nutrient broth is of service in obtaining the soluble toxins formed by bacteria and in determining motility.

2. **Glucose Broth.** — 1 or 2 per cent glucose is added to nutrient broth. The procedure is the same as in (1) except sterilization should be by the fractional method. Glucose is a reducing agent, consequently no free oxygen can remain in a medium containing it. Glucose broth on this account is used for the cultivation of anaërobic organisms.

3. **Glycerin Broth.** — To broth (1) *after filtration,* 5 to 8 per cent of glycerin is added. This medium is used especially for growing the tubercle bacillus when tuberculin is to be prepared.

4. **Gelatin Medium.**

Meat infusion or extract	1000 c.c.
Peptone	10 gm.
Sodium chloride	5 gm.
Gelatin (gold label)	100 gm.

This is simply the above broth with the addition of gelatin as a solidifying agent. The ingredients are dissolved by warming, the reaction is adjusted, eggs are added to clear the medium (page 19), and it is then heated for 15 minutes. Water is added to make up the original volume; after being thoroughly stirred the medium is filtered through cotton, tubed, and sterilized by fractional sterilization. Characteristic growth takes place on gelatin media which often facilitates identification.

5. **Agar Medium.**

Nutrient broth (1)	1000 c.c.
Shredded agar	15 c.c.

The agar is added to the broth and dissolved by boiling the mixture for thirty to forty-five minutes. Loss by evaporation is made up by the addition of water, the reaction is adjusted, and the media cleared, heated, filtered, tubed, and sterilized in the autoclave. Agar medium serves a great variety of purposes; it is perhaps the most frequently used of all media.

6. **Potato Medium.** — Large potatoes are chosen, thoroughly scrubbed with a brush and then peeled, after which they are kept under running water to prevent discoloration. Cylindrical pieces are removed by means of a large apple corer, and the cylinders in turn are cut in half diagonally.

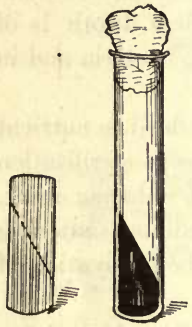


FIG. 10. — Potato Tube.

The reaction of the potato is normally acid. This is corrected by leaving the pieces overnight in running water or by placing them in a 1 per cent solution of sodium carbonate for half an hour. Each cylinder is placed in a large test tube with the slant surface uppermost (Fig. 10). About one c.c. of water may be added to retard drying and the tubes sterilized by the fractional method. Potato is generally chosen as a medium when pigment production is to be studied.

7. **Glycerin Potato** is prepared by covering the potato slices in the tube with a 6 per cent solution of glycerin in water and steaming in the Arnold Sterilizer for half an hour. The glycerin is then poured off and the tubes are sterilized for another half hour. Glycerin potato is sometimes used for the cultivation of the tubercle bacillus.

8. **Peptone Water.**

Water	100 c.c.
Peptone	2 c.c.
Sodium chloride	0.5 c.c.

The peptone and salt are dissolved in the water by heating. As the fluid is generally alkaline it does not need adjusting unless required for special purposes; it may be filtered, tubed, and sterilized at once. Peptone water is used to test for the

formation of indol; it is also used as a basis for other special media.

Indicators. — To any of the ordinary media, substances may be added which serve to show any difference in reaction taking place during bacterial growth; in other words, they *indicate* the ability of the microörganism present to produce fermentative or putrefactive changes. *Litmus* is perhaps the most generally used. After filtration of the medium, which should be slightly alkaline, sufficient of a reliable solution such as the " Kubel-Tiemann Solution " is added to give a distinctly bluish tint. A deepening of the blue color will indicate increased alkalinity; a change from blue to a pink color will reveal the presence of an acid. *Neutral red* is an indicator frequently used; in the presence of acid it becomes a deep rose color, in an alkaline medium it is yellow with sometimes a green fluorescence.

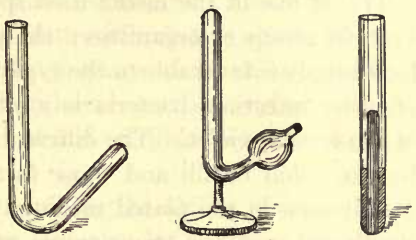


Fig. 11. — Fermentation Tubes.

9. **Milk.** — Fresh milk is placed overnight in the ice box so that the cream may rise; in the morning the milk is siphoned off from beneath the cream and sufficient litmus added to give it a purplish blue color. It will generally be found to be alkaline; if not, a little sodium hydroxide solution should be added to give it the required color. It is then ready for tubing and fractional sterilization. Litmus milk is a convenient medium for observing the ability of certain bacteria to ferment lactose, or to coagulate the soluble albumin present.

10. **Neutral-Red Lactose Peptone Medium.**

Peptone solution (8)	100 c.c.
Lactose	1 gm.
Saturated aqueous solution of neutral red . . .	1 c.c.

The medium is filtered into fermentation tubes (Fig. 11) and sterilized by the fractional method. This medium is used largely for the examination of water and shellfish to determine by the presence of the colon bacillus whether sewage pollution has occurred.

11. **Conradi Drigalsky Medium.**

(*a*) Agar	20 gm.	(*b*) 130 gm. of Kubel and Tiemann's litmus solution	
Sodium chloride	5 gm.	10 c.c. of 1 to 1,000 solution of crystal violet	
Peptone	20 gm.	15 gm. lactose	
Nutrose	10 gm.		
Liebig's Extract	4 gm.		
Water	1000 c.c.		

The ingredients (*a*) should be thoroughly mixed and heated in the autoclave to dissolve. The mixture should then be cooled to below 60° C., cleared, sufficient sodium hydroxide solution added to give a decided alkaline reaction to litmus, and then filtered. Ingredients (*b*) are next added and the medium heated for 10 minutes in the Arnold in order to obtain a thorough mixing. It should then be tubed and sterilized by the fractional method.

This is one of the media used specially for the isolation of the typhoid group of organisms; the principle being that while the food supply is favorable to the typhoid and colon bacilli the growth of other intestinal bacteria is inhibited by the antiseptic action of the crystal violet. The difference between the colonies formed by the colon bacilli and those formed by the typhoid bacilli is readily seen in the plated medium. The B. coli colonies are distinctly red and non-transparent, while those of B. typhosus are smaller, bluish violet in color and of a somewhat glassy appearance.

12. **Loeffler's Serum.** — Three parts of calf or sheep serum is mixed with one part of nutrient broth (1) which has been made neutral to litmus. One per cent dextrose is added and the medium is tubed. In order to coagulate the serum the tubes are placed in an inspissator in a slanting position, or an Arnold sterilizer may be used if the outer cover is replaced by a cloth and the temperature is allowed to rise very gradually to 80° C. After coagulation the medium is sterilized by the fractional method. This medium is especially suitable for the growth of the diphtheria bacillus; it is useful also for other organisms.

13. **Blood Agar.** — One c.c. of fresh or defibrinated blood is added to about 6 c.c. of melted agar cooled to 42° C. to 45° C., well mixed and either slanted in the tube or poured into a Petri dish. The simplest method of preparation is to thoroughly cleanse

a finger and then wash with alcohol, allow the alcohol to evaporate and then with a sterile needle prick the finger. Take up a drop of the blood with a sterile platinum loop and smear it on the surface of an agar slant. Agar poured out in a thin layer in a Petri dish may be smeared with blood in the same way and used for cultures. It is advisable to incubate the blood-smeared medium for 24 hours before inoculating to be sure that it is sterile.

Organisms such as the gonococcus, pneumococcus, and influenza bacillus, which do not grow readily on ordinary agar, are cultivated on this medium.

14. **Hiss Serum Water.** — Beef serum is drawn in a pipette from clotted blood and added to distilled water in the proportion of one part of serum to two or three parts of water. The mixture is heated in the Arnold for 15 minutes at 100° C. to destroy any sugar fermenting enzyme present in the serum, after which sufficient aqueous solution of litmus is added to give a transparent blue color. One per cent of the desired sugar (glucose, saccharose, lactose, etc.) is added and the medium is tubed and sterilized by the fractional method in the Arnold.

This medium is of use in determining the ability of a species to ferment different carbohydrates and also its power to coagulate serum protein.

Method of Obtaining Serum. — Beef or sheep's blood is collected in a sterile jar at the slaughter house. After coagulation the clot should be carefully separated from the sides of the container with a sterile glass rod and the jar placed in the refrigerator for 24 hours. At the end of this time the clear serum can be pipetted or siphoned off with sterile glass tubing and placed in sterile flasks. Serum may be sterilized in its fluid state by exposure to a temperature of 60° C. for one hour upon six consecutive days.

Exudates from the pleural or abdominal cavity may be used instead of beef or sheep serum. The fluid is allowed to flow directly out of the canula into sterile flasks. The instruments should be taken into the ward in sterile water and not in an antiseptic solution. Before using the fluid should be incubated and any flasks showing contamination should be discarded.

CHAPTER IV

MICROSCOPIC EXAMINATION AND STAINING OF BACTERIA

The Microscope. — In order to study the structure and movements of individual bacteria a good microscope is essential (Fig. 12). A complete instrument generally has four oculars or eye pieces *A* numbered from 1 to 4. Number one gives the lowest magnification and number four the highest. At the lower end of the tube *B* there are usually three objectives *C* attached to a revolving nosepiece; the objectives give the main magnification. For the examination of groups of bacteria growing together in solid media, when one wishes to see only the general appearance of the assemblage, the lowest magnification is used, *i.e.* ocular 1 or 2 and objective 4. For unstained preparations, when motility or serum reaction is to be studied, ocular 2 or 3 and objective 7 is employed. When the finer structures of individual organisms are to be noted in stained preparations, ocular 4 and the oil immersion objective $\frac{1}{12}$ should be used; the latter combination gives a magnification of about one thousand diameters. When the oil immersion lens is employed a small drop of oil of the same index of refraction as the glass lens (cedar oil is generally used) is placed on the preparation to be examined. The tube is lowered until the objective is connected with the slide by means of the oil, thus all the rays of light are held together and pass into the tube without the loss through deflection which occurs when air fills the intervening space between the slide and the dry objective. After using, the oil should be gently wiped from the lens with Japanese lens paper or a clean soft linen handkerchief. If absolutely necessary a little zylol may be used but never alcohol; the latter dissolves the material by means of which the lens is fixed in its metal container.

The stage *D* serves as a table on which to place the object to be examined. Immediately beneath the opening in the stage is the Abbé condenser *E*, a system of lenses which serves to condense the rays of light passing from the mirror *F* to the object in such a way as to give the greatest luminosity possible.

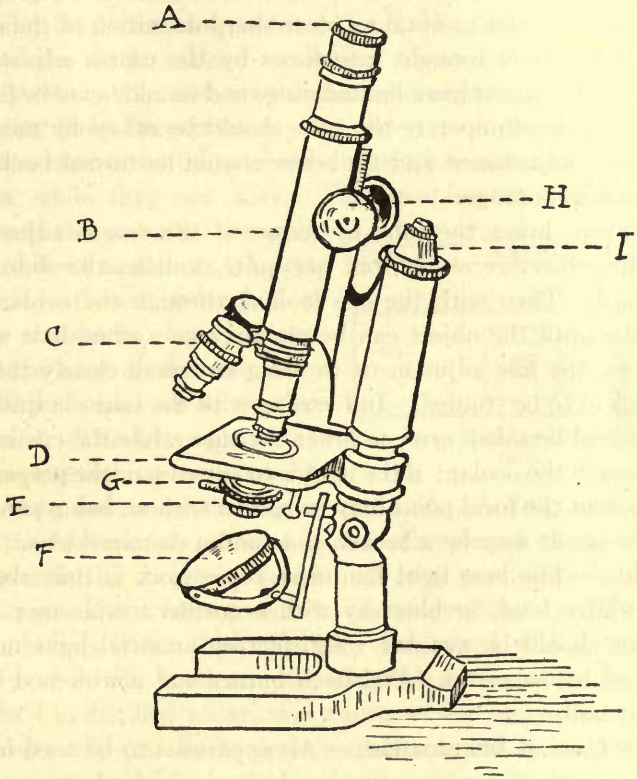


FIG. 12. — Microscope.

The iris diaphragm *G* is placed between the condenser and the mirror and serves the same purpose as the iris of the eye in regulating the amount of light admitted. For unstained preparations, when it is desired to bring out in relief the margins of the organisms to be studied, a small aperture of the diaphragm is used in conjunction with the concave mirror. For stained preparations the

diaphragm should be widely opened and the flat side of the mirror used.

By means of the coarse adjustment *H* the tube of the microscope can be raised or lowered; it is used to bring the object to be studied roughly into focus. The fine adjustment *I* raises or lowers the tube much more slowly and evenly and is used with high-power objectives in order to obtain a clear sharp definition of the object after it has been brought into focus by the coarse adjustment. The fine adjustment has a limited range and should never be forced; when it ceases to operate the tube should be raised by means of the coarse adjustment and the screw should be turned back midway within its range.

To focus, lower the tube by means of the coarse adjustment until the objective nearly, but not quite, touches the slide to be examined. Then with the eye looking through the ocular raise the tube until the object can be plainly seen; when it is well in focus use the fine adjustment to bring out more clearly the part of the field to be studied. In focusing with the coarse adjustment care should be taken *never* to lower the tube while the eye is looking through the ocular; if the light is too intense or the preparation transparent the focal point may be passed without being perceived and the result may be a broken slide and a damaged lens.

Light. — The best light for microscopic work is that obtained from white clouds or blue sky with a northern exposure; direct sunlight should be avoided. Satisfactory artificial light may be obtained by means of a Welsbach burner and a whitened incandescent bulb.

Dark Ground Illumination. — An apparatus to be used in conjunction with the microscope has been devised whereby minute particles are made visible, particles which could not be seen otherwise even with the highest magnification obtainable. The general principle involved is to arrange for light to be thrown obliquely on the object to be examined and to stop the rays passing directly towards the tube of the microscope. An electric arc lamp is used as a source of light; the organisms appear as brightly illumined objects while the fluid which surrounds them forms a dark back-

ground. The method may be employed for the examination of bacteria in general; it is especially useful for the demonstration of spirochetes in secretions.

Double Microscopes have been constructed by means of which a comparative study may be made of two objects at the same time.

Microscopic Examinations. — Bacteria may be studied microscopically, (1) living and unstained in fluids, (2) in stained film preparations, (3) in stained sections of tissue. For such studies perfectly clean slides and coverslips are necessary.

Hanging Drop Preparation. — In order to note motility or watch the method and rate of cell division it is necessary to study bacteria while they are alive. This can be accomplished by means of the so-called hanging drop prepared as follows: A special slide with a circular hollow on one surface is employed and around the edge of the concavity a fine film of cedar oil or vaseline is smeared. This latter serves the purpose of attaching the coverslip to the slide and so preventing evaporation. A drop of fluid in which the bacteria are growing is then transferred to the center of a coverslip by means of a platinum loop previously sterilized by flaming. If the bacteria are to be removed from solid media or are obtained from thick pus they are mixed with a suitable quantity of sterile broth or physiological salt solution (0.9 per cent NaCl in distilled water) and a drop of the suspension placed on the coverslip, or the bacteria may be emulsified in a drop of salt solution directly on the coverslip. The coverslip is then inverted over the slide, gently pressed, and sealed by means of the vaseline (Fig. 13).

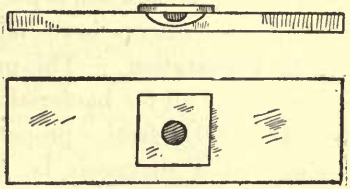


FIG. 13. — Hanging Drop Preparation.

Unstained organisms are difficult to see through the microscope, therefore great care is necessary in focusing. The diaphragm should be partially closed in order to take advantage of the lights and shadows caused by the difference in light transmission in the objects under examination. Since the edge of the hanging drop

is more easily distinguished than the middle it should be found first with the low-power lens and then so arranged that the edge of the drop crosses the center of the field. The high-power objective is then turned into position, the tube lowered until it almost touches the coverslip, and then with the eye at the ocular it should be carefully raised into focus.

Hanging Block. — The manner and rate of cell division can perhaps be best studied by means of the hanging block. The technique is as follows: A thin layer of melted agar is poured into a Petri dish and allowed to harden, after which a small square is removed, seeded with the organisms to be studied and carefully placed on a coverslip, the bacteria being between the agar and the glass. The preparation is placed over a hollow slide and examined under the microscope in the manner described for the hanging drop.

Film Preparation. — This method is perhaps the most frequently employed of all for bacterial examination; by it advantage may be taken of the elective properties of certain bacteria for particular stains and a diagnosis be thus more easily established. The steps in the preparation are as follows: A thin even film of the material to be examined is spread over the surface of a perfectly clean slide; if the material is fluid it is transferred by means of a platinum loop, if taken from solid culture medium a loopful of sterile water is first placed upon the slide and a small particle of a growth thoroughly mixed with it before spreading. The film is allowed to dry in the air. The slide is then held by means of a pair of forceps and passed slowly through the flame of a Bunsen burner three or four times, film side uppermost, in order to fix the preparation on to the slide. Fixing may also be accomplished after the film is dry by immersing in chemicals such as alcohol, formalin, glacial acetic acid, etc., in which case the slide must be immersed in water to remove the excess chemical before the next step. A few drops of the desired stain are then placed on the slide and left there from a few seconds to several minutes according to the dye used. The slide is then washed carefully but thoroughly in water, after which it is dried by gently pressing between layers of filter paper.

The preparation may have a drop of cedar oil placed directly upon it and so be examined under the oil immersion lens; if, however, the slide is to be preserved for future examinations it is better to first place a small drop of Canada balsam on the film and cover it with a coverslip.

Blood Films. — A blood film is prepared by placing a drop of blood near the end of a clean slide and the edge of a second slide is then lowered on to the drop at an angle to the first slide. By capillarity the blood spreads itself along the edge of the second slide, which is gently stroked over the surface of the first, leaving a film the width and thickness of which depends on the angle at which the slide was held (Fig. 14).

FIG. 14. — Method of making Blood Smear.

Another method employed is to place a drop of blood between two coverslips and then draw them apart. If the red blood corpuscles are to be examined, fixing should not be effected by heat; the slide may be placed in a mixture of equal parts of alcohol and ether for half an hour and then washed and dried in the air, or it may be placed in a saturated solution of corrosive sublimate for two or three minutes then washed well in running water and dried. The method of staining depends upon the object for which the examination is to be made.

THE STAINING OF BACTERIA

Staining Principles. — Generally speaking, bacteria react to stains in a manner similar to the nuclei of animal cells; the process is to be regarded as a chemical combination between the dye and the cell substance rather than merely a mechanical saturation. Certain organisms stain readily, others only with great difficulty; the easily stained forms require immersing but a few minutes in a watery dye while those which do not stain so readily require a longer time and in some cases the addition of heat or a mordant. The tubercle bacillus belongs to the latter class. Spores and

flagella are also stained with difficulty. Those organisms that do not stain readily as a rule retain the dye and are not easily decolorized. Two explanations are advanced for this resistance to take on and to part with stains: one hypothesis is that such organisms, or parts, are of different chemical composition; this assumption is probably true in the case of spores and flagella. The other theory supposes the presence of a waxy and therefore difficultly permeable envelope surrounding the bacteria may be the cause. The latter view is probably correct in the case of the tubercle bacilli. The presence of a waxy or fatty material has been demonstrated in certain bacteria; moreover, when this waxy substance has been extracted with ether the dye-resistant qualities of the bacteria have disappeared also. It may be in certain cases that both factors combine to produce the result.

The best bacterial stains are derived from the coal-tar product aniline. Many of the dyes have the constitution of salts and are divided into two groups according as the staining depends on the basic or acid part of the molecule. The basic stains have a special affinity for nuclear material and the acid for cytoplasm. For this reason the basic dyes are especially bacterial stains.

The most frequently used stains are:

Violet — Methyl violet, gentian violet, crystal violet
Blue — Methylene blue, thionin blue
Red — Basic fuchsin
Brown — Bismarck brown

The violet dyes have the most intense action, consequently care should be taken when using them not to overstain the specimen. Methylene blue is perhaps the most generally employed; it gives a good differentiation of structure and it is not easy to overstain with it. Bismarck brown and eosin are weak dyes and are used generally as counterstains.

Saturated Solutions. — It is a convenient arrangement to keep in stock saturated solutions of the dyes most frequently employed and from them to make dilutions for use as required. Since great variations occur between different samples of dyes bearing the same name no definite amount can be quoted as the minimum for

the preparation of a saturated solution. A complete saturation may be obtained by adding the powdered dye to the solvent until no more will enter into solution, a slight residue remaining after repeated shaking on several days being taken as an indication. The following quantities of the most frequently used dyes are approximately sufficient for saturation:

Gentian violet: 1 to 5 per cent in distilled water or 4 to 8 per cent in 96 per cent alcohol

Fuchsin: 1 to 5 per cent in distilled water or 3 per cent in 96 per cent alcohol

Methylene blue: 6 to 7 per cent in distilled water or 7 per cent in 96 per cent alcohol

Stains should always be filtered through paper before use, otherwise sediment may be deposited on the slide which would spoil the preparation.

Mordants and Decolorizing Agents. — Certain organisms are stained with difficulty unless a mordant is employed, which not only increases the intensity of the dye but tends to make the bacterial cell more permeable. Again in films of blood or pus and more especially in sections of tissue, the tissue cells may be so deeply stained as to obscure the bacteria lying within them. To obviate this methods have been devised whereby a mordant may be used to fix the dye in the bacteria while subsequent treatment with a decolorizing agent will remove the dye to a greater or less extent from the tissue cells.

Staining properties may be increased by:

(*a*) The addition of weak solutions of alkalies, such as caustic potash or ammonium carbonate.

(*b*) The addition of carbolic acid, aniline oil, metallic salts, etc.

(*c*) Heat.

(*d*) Prolonged staining.

The decolorizing agents generally employed are weak solutions of acids, alcohol, or a combination of both.

FORMULÆ OF SOME OF THE MOST FREQUENTLY USED STAINS

Lœffler Methylene Blue.

Saturated alcoholic solution of methylene blue	30 c.c.
1-10,000 solution of caustic potash in distilled water	100 c.c.

Films may be stained with the above preparation for from two to five minutes without being overstained. It is a useful stain for structural differentiation and is generally employed in routine examination for the diphtheria bacillus.

Neisser's Stain. — Two solutions are used:

(*a*)	5 per cent alcoholic solution of methylene blue	20 c.c.
	Glacial acetic acid	50 c.c.
	Distilled water	950 c.c.
(*b*)	Bismarck brown	2 gm.
	Distilled water	1000 c.c.

Films are stained in (*a*) for three to five seconds, washed in water and stained in (*b*) for five seconds, dried and examined.

The stain was originally introduced by Neisser as an aid in the identification of the diphtheria bacillus. If the film is made from a twenty-four hour culture grown on serum medium the bacilli frequently show when stained by this method deep blue granules with surrounding protoplasm of a faint brown.

Ziehl-Neelsen's Carbol Fuchsin Stain.

Basic fuchsin	1 gm.
Absolute alcohol	10 c.c.
5 per cent solution of carbolic acid	100 c.c.

After fixing the film may be placed in the stain, heated until steam rises and allowed to remain there for five minutes, or it may be allowed to remain in the cold stain from twelve to twenty-four hours. The excess of stain is then washed off with water and the film placed in a decolorizing solution; 3 per cent hydrochloric acid in 80 per cent alcohol gives good results as a decolorizing agent. After a few seconds remove the film and wash in water; if only the faintest pink color persist decolorization has been sufficient; if

on the other hand a distinctly red color remains the process should be repeated until the proper tint is obtained. The slide is next immersed in a 10 per cent watery solution of methylene blue, washed in water, and dried.

The above method is used for the group of organisms known as "acid-fast," amongst which are the tubercle bacillus, the leprosy bacillus, the smegma bacillus, the hay bacillus, and a number of others. These organisms require a powerful dye containing a mordant, and the staining process must be continued a long time or its action aided by the application of heat. When once stained, however, they resist the decolorizing action of strong acids; for this reason they are spoken of as "acid-fast." Stained by the above method they appear under the microscope to be bright red while any other organisms present take the counterstain and appear blue.

Spore Stain. Moeller's Method. — The films are immersed in chloroform for two minutes, washed in water, then covered with 5 per cent chromic acid one minute and again washed in water. They are next placed in carbol fuchsin and the solution is heated until it commences to steam. After remaining in the hot solution for three minutes the slides are removed, washed in water, and decolorized in 5 per cent sulphuric acid for five to ten seconds, then washed in water and counterstained in 10 per cent aqueous methylene blue one minute. By this method the spores appear red while the remainder of the bacterial cell is stained blue. If bacilli containing spores are stained with a watery solution of any of the aniline dyes the spores remain unstained and appear as clear spaces surrounded by stained protoplasms.

Capsule Stain. Hiss' Method. — Films are made in the usual way and fixed by heat. The slide is then covered with a 5 per cent watery solution of fuchsin or gentian violet and heated over a Bunsen flame until it steams. The dye is washed off with a 20 per cent aqueous copper sulphate solution, after which it is dried between layers of filter paper without further washing in water. By this method the capsule appears as a faint blue halo surrounding a dark purple or red cell body.

Flagella Stain. Van Ermengen's Method. — Three solutions are necessary:

(1) Twenty per cent tannic acid solution	60 c.c.
Two per cent osmic acid solution	30 c.c.
Glacial acetic acid	4 to 5 drops

The fixed film is placed in this solution for one hour at room temperature or for five minutes at 100° C. It is then washed in water and afterwards in absolute alcohol, followed by immersion for one to three seconds in

(2) Silver nitrate 3 to 5 per cent solution. Without washing the slide is transferred to

(3) Gallic acid	5 gm.
Tannic acid	3 gm.
Fused potassium acetate	10 gm.
Distilled water	350 c.c.

The slide should be moved gently to and fro in this solution for a few minutes, then returned to the silver nitrate until the film turns black. It is then thoroughly washed in water and dried.

The staining of flagella is one of the most difficult of bacteriological procedures. In order to get good results the slide must be scrupulously clean, the film should be made from a young ten to eighteen hour agar culture and should be spread as carefully and with as little manipulation as possible.

Indian Ink Method for the Examination of Spirochetes. — An emulsion of good quality Indian ink is sterilized by steaming and allowed to settle for a few days. One drop of the sediment and one drop of water are thoroughly mixed with a loopful of the material to be examined on a clean slide. The film is dried in the air and examined with the oil immersion lens. If spirochetes are present they stand out unstained surrounded by the dark Indian ink.

Wright's Stain. — This is one of several modifications of the polychrome Romanowsky stains used chiefly for staining animal cells and also for bacteria that stain faintly by ordinary methods. It can be purchased ready for use or it may be prepared as follows: 1 per cent methylene blue and 0.5 per cent sodium carbonate are

mixed and steamed in the sterilizer for one hour. When cold add 0.1 per cent aqueous solution of eosin in the proportion of 5 parts eosin solution to 6 parts methylene blue solution. The mixture becomes a purple color and a granular sediment appears. It is then filtered and the precipitate remaining on the filter paper is pressed dry. A saturated solution is made of the dried precipitate in methyl alcohol; this saturated solution is then filtered and diluted by the addition of 10 c.c. of methyl alcohol to 40 c.c. of the stain.

In using the stain a few drops are placed on a fixed film for one minute, then the same quantity of water is dropped on to the slide by means of a medicine dropper and the mixture of stain and water is allowed to remain two to three minutes. The slide is then washed in water and dried.

The stain gives particularly good results in the examination of blood films. Erythrocytes appear yellow or pink; the nuclei of leucocytes various shades of purple and the cytoplasm a light blue color; blood plaques dark blue; bacteria blue. Malarial parasites stain characteristically: the chromatin mass appears a garnet red and the surrounding protoplasm a robin's egg blue.

Gram's Stain. — The fixed film is covered with a fresh solution of aniline gentian violet made as follows: One c.c. of anilin oil is added to 10 c.c. of water and shaken until thoroughly emulsified, after which it is filtered through wet filter paper. One part of saturated alcoholic gentian violet is then added to nine parts of the filtrate. The slide is allowed to remain in the above dye for five minutes, after which it is immersed in the following iodine solution for 2 to 3 minutes.

Iodine	1 gm.
Potassium iodide	2 gm.
Distilled water	300 c.c.

The film is then decolorized with 97 per cent alcohol about one minute or until no more stain can be washed out of the preparation, after which it is washed in water and counterstained with eosin for thirty seconds.

This method of staining is frequently used in bacterial differen-

tiation. By its means organisms are divided into two classes: those which retain the initial stain are spoken of as Gram positive and those which are decolorized and take the counterstain as Gram negative. Most bacteria fall decidedly into one class or the other; borderline cases do occur, however, and a few species show a tendency to change from Gram positive to Gram negative in old cultures.

CLASSIFICATION OF THE PRINCIPAL PATHOGENIC BACTERIA ACCORDING TO THEIR REACTION TO GRAM'S STAIN

POSITIVE	NEGATIVE
(Retain the violet stain)	(Take the counterstain)
Cocci	*Cocci*
M. tetragenus	M. catarrhalis
Pneumococcus group	M. gonorrheæ
Staphylococcus group	M. meningitidis
Streptococcus group	M. melitensis
Bacilli	*Bacilli*
B. ærogenes capsulatus	B. acidi lacti
B. anthracis	B. coli group
B. botulinus	B. dysenteriæ group
B. diphtheriæ group	B. enteritidis group
B. tetani	B. influenzæ group
B. tuberculosis and other acid-fast bacilli	B. Koch-Weeks
	B. lactis ærogenes
	B. maligni edematis
	B. mallei
	B. Morax Axenfeld
	B. mucosus capsulatus
	B. pertussis group
	B. pestis
	B. proteus
	B. pyocyaneus
	B. typhosus group
	Spirillum
	S. choleræ

CHAPTER V

CULTIVATION AND IDENTIFICATION OF BACTERIA

General Laboratory Rules. — A jar containing 1 in 20 carbolic acid solution should be always at hand in which to place all glassware that has been used for infective material; after several hours such articles may be cleansed by boiling in soapsuds. Old used cultures may be sterilized in the Arnold for three or four hours or for a shorter period in the autoclave. It is well to have within easy reach a basin of mercuric chloride 1 in 1000 or carbolic acid 1 in 40 in which the worker's hands may be disinfected in case of accidental contamination. Any infective material spilled on the table should be covered with carbolic solution and carefully wiped off with cotton held by forceps. Unnecessary movement in the laboratory should be avoided in order that the air may be kept as quiet as possible. Hands should always be well washed before leaving the laboratory and food should not be eaten there. Labels should never be moistened with the tongue and nothing should be placed in the mouth that has touched any surface in the laboratory.

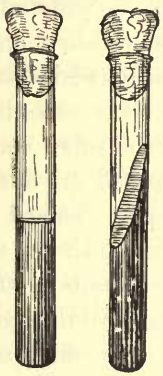


Fig. 15. — *A*. Tubed Agar for Stab Culture. *B*. Agar Slant.

Cultivation of Microörganisms. — In order to learn the special characteristics of an organism it must be studied apart from all other forms on artificial culture media such as already described. A large surface for growth is obtained by filling tubes with solid media such as agar or gelatin about one sixth full, and after sterilization, while still liquid, placing them in a slanting position so that when solidified they will give an oblique surface of three to four inches (Fig. 15). Care should be taken that the medium does not extend to the cotton plug.

A tube of media in which bacteria are growing is spoken of as a "culture." When only one species is present it is said to be a "pure culture." If a small portion of an already existing growth is transferred to a tube of fresh medium the resulting culture is spoken of as a "transplant" or "subculture." To transfer bacteria from one tube to another a platinum wire or loop is used.

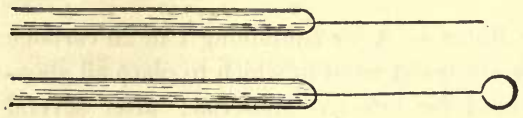


Fig. 16.—Platinum Wire and Loop.

It should be thin yet sufficiently stiff and about two and a half inches long. The wire is attached to an aluminum holder or fused into the end of a glass rod about seven or eight inches long. For making stab cultures or for "fishing" a straight "needle" or wire is used; for transferring growth from a fluid medium a wire turned at the end to form a loop is employed (Fig. 16). The platinum needle or loop should *always* be sterilized in the flame of a Bunsen burner immediately before and after using.

A subculture is made as follows: The culture and the tube to be inoculated are held in a slanting position between the thumb and the first and second fingers of the left hand; the plugs are gently twisted around once or twice to make sure they are not sticking to the tubes and if they have been exposed to dust they should be slightly singed in the gas flame. The platinum wire, held between the thumb and the first and second fingers of the right hand, somewhat in the manner of holding a pen, is sterilized by heating red hot in a flame. The plugs are then removed, one is grasped between the middle and third fingers and the other between the third and fourth fingers. When cool enough the wire is carefully passed into the culture tube and without touching the side a small amount of bacterial growth is removed and immediately transferred to the tube which is to be inoculated (Fig. 17). If the tube contains

Fig. 17.—Method of Inoculating.

slanted medium the growth is deposited by lightly smearing the surface from the lower to the upper portion of the slant. A "stab" culture is made by plunging the needle down the center of medium that has not been slanted or the two methods may be combined on an agar slant; growth may be smeared on the surface and the needle plunged into the medium before it is withdrawn. Surface growth and deep growth may thus be observed in the same tube. If the organisms are transplanted from one fluid medium to another a loopful is removed and gently rubbed off against the glass in the upper portion of the fresh medium. When the growth forms a pellicle it is sometimes necessary to transfer a portion of the pellicle and so place it that it rests on the surface of the fresh medium, or growth will not take place. After the medium is inoculated the platinum needle is removed, the plugs replaced, and the wire immediately heated red hot in the flame; at the same time several inches of the glass rod should be passed through the flame also.

Plating. — If pathological material or such substances as milk or water be placed in culture medium many different kinds of organisms develop at the same time. Since it is impossible to study the characteristics of each species unless they can be separated, a method devised by Koch of plating in solid media is employed whereby individual bacteria are held apart and the descendants of each are soon sufficiently numerous to appear as a colony visible to the naked eye. By a procedure known as **colony fishing** the members of a single species can be transferred to fresh sterile media and a pure culture thus obtained.

A thin layer of medium presenting a moderately large surface is obtained by the use of the so-called **Petri dish.** Each Petri dish consists of two circular glass plates, the larger forming a loosely fitting lid for the smaller.

The method of making a **pour plate** for the isolation of bacteria is as follows: The solid medium is liquefied and then cooled to a temperature of 42° C. A loopful of the material to be examined is placed in a tube and thoroughly mixed with the medium by rolling the tube between the hands, care being taken not to shake

the tube and produce air bubbles. Three loopfuls of this mixture are transferred to a second tube and the mixing process repeated; five loopfuls from the second tube are carried to a third and mixed. The necks of the tubes are then flamed and the contents of each poured into a Petri dish previously marked with the number one, two, or three according to the dilution it is to receive. In pouring the inoculated medium into the Petri dish the cover should be raised along one margin just high enough to permit the entrance of the neck of the tube, care being taken that the sides of the tube do not touch either the top or bottom part of the dish (Fig. 18). The cover is immediately replaced and the dish gently rotated if necessary to insure the even distribution of the medium over the bottom of the dish before it solidifies.

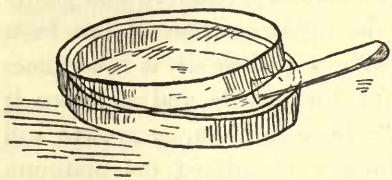


FIG. 18. — Method of pouring Media into a Petri Dish.

It is advisable to make a stained film preparation of the material to be examined before plating as above, in order to have some idea of the number of organisms present. If they are relatively few, more material should be inoculated into the first tube; if numerous, then a further dilution will be necessary.

When agar has been used for plating, the Petri dishes are inverted as soon as the medium has solidified and placed in the incubator at a temperature of 37° C.; gelatin plates are kept in a dark place at room temperature. Colonies will develop in from one to three days according to the species, and in the second or third dilution they will usually be found sufficiently far apart to be "fished."

Surface Streaking. — When it is desired to isolate bacteria which are particularly sensitive to their surroundings, such as the gonococcus, and which require special medium, **surface streaking** instead of the **dilution method** is employed. The technique is as follows: Pour plates are made of suitable medium and when hardened the material containing the organisms to be isolated is smeared over the surface of several plates in succession. If the organisms are removed from the nose or throat by means of a swab, the swab

is gently stroked over the medium in plate one, and then, without turning, the same portion of the swab is smeared over a second and a third plate. If the material to be examined is liquid a small quantity is deposited on the surface of the medium by means of a platinum loop and the loop is then stroked lightly over the medium in several plates without recharging.

A method of plating is frequently used by means of which the dilutions are carried out in definite proportions so that the number of bacteria present may be estimated.

The procedure is as follows: To 9 c.c. of sterile water 1 c.c. of the material to be examined is added and the resulting mixture is thoroughly shaken to separate the organisms. One c.c. of this 1 in 10 dilution is added to 9 c.c. of sterile water, producing a 1 in 100 dilution. After thorough shaking the process may be repeated, giving as a result a 1 in 1000 dilution, and so on until the desired limit has been reached. One c.c. of each dilution is placed by means of a sterile pipette in a Petri dish previously numbered, and melted agar cooled to 40° C. is poured into the center of it. Mixing is accomplished by gently rotating the plates before the medium solidifies, care being taken that the medium remains flat on the bottom and is not smeared over the sides. After growth has occurred the colonies are counted and the number of bacteria present in the material examined estimated.

For example, if on the Petri dish containing the 1 in 100 dilution 180 colonies appear that number is multiplied by 100, and it is assumed that approximately 18,000 organisms were present in 1 c.c. of the material examined. The dilution showing between one hundred and two hundred colonies is chosen as the most representative for counting. Higher numbers are difficult to count; also crowding may have checked the development of some of the organisms. Counting may be facilitated by dividing the Petri dish into sections by lines made with a colored pencil or by placing the dish on a Wolffhügel counting plate (Fig. 19). Whenever possible all the colonies should be counted; if they are too numerous and a counting plate is used, squares from representative parts of the dish are counted and the total number of colonies estimated.

As sixty-three squares would cover the standard Petri dish the number of colonies in one square multiplied by sixty-three would give the total number present if they were evenly distributed throughout the medium. Since this rarely occurs it is best to count ten representative squares and multiply the resulting num-

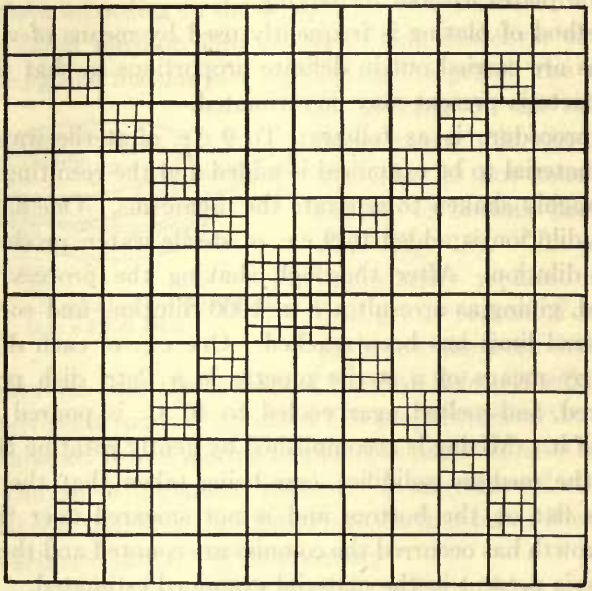


FIG. 19.—Wolffhügel Counting Plate.

ber by 6.3. This is in turn multiplied by the dilution used to give the number of bacteria present in the material examined. Briefly it may be expressed thus:

Number of colonies in 10 squares × 6.3 × dilution
= Colonies developed from 1 c.c. of material plated.

The above method is very satisfactory when only one species of bacteria is present and the medium and surrounding conditions are known to be suitable to their growth. If on the other hand the material contains a number of different species, it is not likely that the optimum conditions for one will be the optimum conditions for all, and many organisms may not develop into colonies

at all. In the latter case the method does not give absolutely accurate information, nevertheless it is the best known for the routine examination of water and milk.

Fishing. — When the object of plating is to obtain a pure culture rather than the enumeration of the organisms present, a colony is fished from the Petri dish to a tube of fresh culture medium. Fishing is best accomplished by using the microscope; otherwise a minute colony of another species unseen by the naked eye may be touched and the resulting subculture contaminated. The lower part of the Petri dish is placed on the stage of the microscope and the low-power lens focused over the colony chosen. The sterilized platinum needle is held in the right hand and introduced between the objective and the colony. When the point of the needle is visible through the microscope it is gently lowered until it is seen to touch the colony and to carry away a small portion of it. The needle is then withdrawn without being permitted to touch anything in passing and the organisms clinging to it are transferred to the medium desired. Fishing requires practice; only by careful manipulation can the subculture be obtained pure.

Incubation. — The temperature at which the inoculated media should be kept depends largely upon the organisms to be grown. Many saprophytes are accustomed to the temperature of an ordinary room and consequently no special apparatus is necessary for their cultivation. Pathogenic organisms, on the other hand, require the body temperature 37.5° C. for their best growth.

In order to maintain a constant temperature at any required degree an incubator is employed. Different makes vary somewhat in detail but all are constructed on much the same principle. Generally speaking an incubator is a double-walled copper chamber with double doors; the space within the two walls is filled with water, which being a poor conductor of heat prevents rapid changes of temperature taking place within the chamber as a result of changes on the exterior. It may be heated by electricity, gas, or oil; a thermo-regulator is usually attached to automatically control the flame.

Anaërobic Methods. — Special methods have been devised for the cultivation of anaërobic organisms, the principle of each being the removal of free oxygen from the container in which the bacteria are growing. The earliest methods depended on the removal of oxygen by mechanical means; later, other methods were employed whereby the oxygen might be displaced by an inert gas such as hydrogen or absorbed by an alkaline solution of pyrogallol.

Mechanical Exclusion of Air. — Tubes of glucose agar or glucose gelatin are heated and then rapidly placed in ice water to prevent reabsorption of oxygen while the medium is hardening. The tubes are inoculated by deep stabs after which the surface may be covered with a layer of albolene; or tight-fitting corks covered with sealing wax or paraffin may be used to replace the cotton plugs.

Displacement of Air by Hydrogen. — This method consists in passing a stream of hydrogen through an airtight chamber in which the tubes or plates of inoculated media have been placed. Hydrogen may be generated from a mixture of zinc and sulphuric acid in a Kipp's generator which is connected with a Novy jar containing the cultures. Hydrogen is allowed to flow through the jar about ten minutes and the stopcocks are then closed.

Chemical Absorption of Oxygen. — Any vessel with a tight cover such as a Novy jar or a Mason fruit jar may be employed. Dry pyrogallic acid is placed in the bottom of the jar and a small quantity of 5 per cent sodium hydroxide is poured over it. The inoculated tubes are put in place and the jar immediately closed. If plated cultures are being cultivated the plates must be raised above the pyrogallic mixture. The method may be applied to individual tubes; the inoculated tube is placed in a larger one in the bottom of which pyrogallic acid and sodium hydroxide solution have been placed. The larger tube is closed with a tight-fitting rubber stopper. Still another method is to place the pyrogallic mixture in a tumbler and invert the inoculated tube of solid medium into it. A layer of oil is then run over the surface of the pyrogallic acid to prevent the access of atmospheric oxygen. As the acid absorbs oxygen it becomes first pale yellow, finally deepening to

a dark brown. When the color ceases to darken it may be assumed that all the surrounding oxygen has been absorbed.

IDENTIFICATION OF SPECIES

When it is desired to identify an unknown organism already isolated in pure culture a study is made of its general characteristics.

A. Morphology, method of grouping, motility, spore formation, and staining reactions.

B. Cultural reactions.

C. Effect on animals.

A. **Morphology and Staining** reaction may be determined by film preparations and **motility** by means of a hanging drop made from a twenty-four-hour broth culture. To test for **spore formation** a film may be made from a forty-eight-hour culture and stained by the method already described, or the culture may be heated to 75° C. for half an hour, after which a subculture should be made and incubated. No vegetative forms will be found to resist that temperature.

B. **Cultural Reactions.** — Ordinarily, young cultures of twenty-four hours' growth should be observed and the following points noted:

(1) *Growth on agar plates.* — Size of colony, outline, transparency, texture, color. (The colonies should be observed with a hand lens or under the low-power objective of the microscope.)

(2) *Surface growth on agar slant at 37° C. and 22° C.* — Scanty or abundant, smooth or irregular, moist or dry, slimy or brittle.

(3) *Growth in stab culture.* — Most abundant at the top or bottom, extension of growth into medium (an indication of motility). Growth a continuous line or beaded.

(4) *Growth in broth.* — Cloudy at upper or lower level or throughout, pellicle or sediment formation.

(5) *Productive pigment.* — Potato medium is best for this purpose; if it is not available a little of the growth may be taken from an agar slant with a platinum loop and spread on white paper, when the color will, if present, stand out against the white background.

(6) *Food requirements.* — Growth on simple media or necessity for media containing sugar or body fluids.

(7) *Temperature.* — Minimum — optimum — maximum.

(8) *Oxygen requirement.* — Growth only in the presence of free oxygen on the surface. Growth only in the absence of free oxygen at the bottom of stab culture.

(9) *Proteolytic action.* — Liquefaction of gelatin; indol formation. For the latter test peptone water medium without the addition of sugar is employed. The culture is incubated for from four to six days, after which 1 c.c. of a 10 per cent solution of sulphuric acid and 1 c.c. of a 1 in 10,000 solution of sodium sulphite is added. At the point where the acid comes in contact with the medium a pink color appears in the presence of indol.

(10) *Fermentation of sugars.* — The tests usually employed are for the detection of acid and gas production. The different species of bacteria vary greatly in their ability to break down the various sugars; one species may have the power to produce acid from one kind of sugar, acid and gas from another, and yet have absolutely no effect upon a third. The medium employed is generally peptone solution to which has been added 1 per cent of the sugar chosen and an indicator such as litmus or neutral red. Hiss's serum water is frequently used for the detection of acid; the cleavage of the carbohydrate is indicated not only by a changed color of the indicator but also by the coagulation of the serum.

The test for acid and gas production may be carried on at the same time in a specially devised fermentation

tube (Fig. 11). The tube is filled beyond the bend with the colored sugar medium and sterilized by the discontinuous method. After inoculation it is incubated, and if the organism is capable of producing gas from the sugar present the gas will be found to have accumulated in the closed arm and the medium displaced into the bulb. If after twenty-four to forty-eight hours the column of gas no longer increases the tube may be removed and the amount noted. The gas produced is mainly carbon dioxide and hydrogen. A rough estimate of the percentage of each may be made by first marking the tube at the line of displaced medium, then filling the bulb with a solution of caustic soda. The cotton plug is replaced by a rubber stopper and the tube is inverted several times; on placing the tube in an upright position the remaining gas will again collect in the closed arm and that absorbed (CO_2) may be roughly estimated. The gas still remaining will be hydrogen; if the tube be inverted so that it is forced into the bulb the fact may be ascertained by exploding it with a lighted match.

C. **Animal Inoculation.** — Certain organisms produce characteristic lesions in definite animal species, which are often of great value in identifying the organisms in question. Moreover, the inoculation of susceptible animals with contaminated material often facilitates the recovery of pathogenic bacteria in pure culture from the lesions produced.

The animals most frequently used for experimental purposes are mice, rats, guinea pigs, and rabbits, the choice depending mainly on the purpose to be served. Inoculations are made as a rule by means of a sterile hypodermic syringe and needle. The material used may be a discharge such as pus, the juice of organs, or a culture of bacteria; if the latter has been growing on solid medium several loopfuls are emulsified in a small quantity of sterile broth or physiological salt solution.

The method of inoculation may be

(1) *Cutaneous.* — The hair is removed by shaving from a small area of the abdomen or back and the skin thoroughly cleansed. A few parallel scratches are made just deep enough to draw blood and the infective material rubbed in with a sterile spatula or platinum loop.

(2) *Intracutaneous.* — The hair is cut or shaved from the part to be inoculated and the skin thoroughly cleansed. A little of the skin is pinched up between the thumb and forefinger of the left hand, the needle inserted in a slanting direction, and the inoculation made. The puncture may be sealed with collodion or painted with iodine.

(3) *Subcutaneous.* — The procedure is the same as (2) save that the hypodermic needle penetrates beneath the skin. When mice or rats are injected by this method, the inoculation is usually made near the base of the tail.

(4) *Intraperitoneal.* — The hair is cut over the lower part of the abdomen and the skin cleansed. The entire thickness of the abdominal wall is pinched up between the thumb and forefinger of the left hand, the hypodermic needle inserted and gently moved about to be sure that the intestine has not been perforated, and the injection made. On withdrawing the needle the puncture point is painted with iodine.

(5) *Intravenous.* — The larger animals are usually employed for this method. If a rabbit is to be inoculated a vein on the outer margin of the ear is chosen, the hair clipped, the skin cleansed, and the animal is held head downwards for a few seconds so that the head may fill with blood and the vein become distended. The hypodermic needle is held almost parallel to the ear and then inserted into the vein. If when the fluid is injected a slight increase in the lumen of the vein is noted it will be evident that the injection has been made into the vein; if on the other hand a small round swelling occurs the fluid is in the surrounding tissue and has not entered the vein.

(6) Inoculations are occasionally made into the anterior chamber of the eye, the pleura, and the cranium. In certain experiments animals are made to inhale dust or infected spray, in others the infective material may be given in food or passed into the intestines by means of a rubber tube.

Autopsy on Dead Animal. — The autopsy should be made as soon as possible after death. If an interval occurs the animal should be kept at a temperature between 1° C. and 4° C. For small animals, the procedure is as follows: The body is wiped with a 1 in 20 carbolic acid solution and stretched out back downwards on a shallow metal trough having a perforation at each end through which a tape or cord may be passed. Two sets of sterile forceps, scissors, and scalpels and several sterile Petri dishes should be ready. The skin in the median line of the pelvic region is lifted with forceps, and with the scissors an incision is made through the skin, only upwards to the neck. From the ends of this median incision the skin is cut outward toward each leg and drawn back with forceps. If the injection has been subcutaneous the neighboring lymph nodes are examined, and if abnormal they are removed with sterile scissors and forceps and placed in a Petri dish to await further examination. An incision is then made through the abdominal wall, the diaphragm is freed, and the thorax is opened by cutting through the ribs on both sides of the sternum. Films and cultures are made from any exudates and from diseased organs. To obtain heart blood the tip of the left ventricle is seared with a red-hot spatula, an incision is made through the seared area, and the blood removed with a capillary pipette or a platinum loop.

When the examination is finished the body should be immediately burned. The instruments should be boiled in a 3 per cent sodium carbonate solution for half an hour, and the dissecting trough may be allowed to stand twenty-four hours in a 1 in 20 carbolic acid solution.

CHAPTER VI

BACTERIA IN NATURAL PROCESSES AND INDUSTRIES

WHEN or how life first began on the globe no one has as yet been able to discover. Nevertheless, it is true that it has existed through countless ages with no apparent decrease of vigor! The question arises as to what has become of the waste products of life during these millions of years and from what limitless store was the necessary food supply derived. The condition of the world is hardly conceivable if in the past ages the dead bodies of plants and animals had simply accumulated on the surface of the ground. By their very bulk they would have so covered the earth as to afford no room for the further growth of plants or animals. Nor could the soil furnish a food supply large enough for the countless millions who have inhabited the earth and the probable millions yet to come without being constantly replenished from an inexhaustible store. The task seems tremendous, yet a large and important part of it is performed by bacteria.

First of all, bacteria act as scavengers in keeping the ground in a proper condition for the growth of plants and animals. When a tree dies and falls to the ground an innumerable host of microorganisms at once begin their work of transformation; the wood becomes softened and finally crumbles into a powdery mass which sinks into the soil and disappears from view. The body of a dead animal undergoes a similar change; the tissues decay rapidly, and even the bones are eventually disintegrated and sink into the soil, leaving no visible trace. The process of decomposition is fundamentally the same whether the object be the carcass of an animal, an insect, or a tree, provided the necessary conditions for bacterial growth are present.

What has become of what was once an animal or a tree? The answer is the explanation of nature's perpetual youth: no part of that disintegrating mass is lost; all is again utilized in one form or another. The greater part is transformed by bacteria into substances that can be used by plants as food. The fact that Nature works in a cycle, using the same material over and over again, first by the plant and then by the animal and then again by the plant, explains her seemingly limitless supply.

A well-known phase of the interdependence of plants and animals is the fact that animals during respiration take in oxygen but exhale it again in combination with carbon; on the other hand, plants draw into their leaves carbon dioxide, retain the carbon, and exhale the oxygen.

Nitrogen Cycle. — A similar but more complex cycle occurs with all the other elements of plant and animal life. Substances are

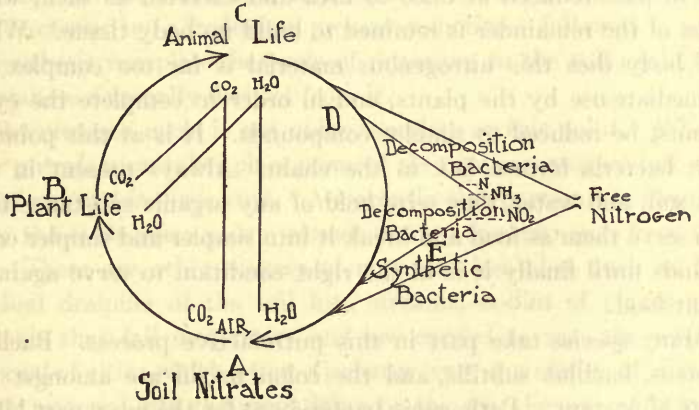


FIG. 20.— Nitrogen Cycle.

carried through a series of changes and in their transition furnish the necessary energy for the life work of the individual whether plant or animal, and finally they are returned to approximately the same form again, to start once more on their journey.

The food cycle is partially represented in Fig. 20. The air furnishes the plant with carbon dioxide and water and the soil *A* with the remaining ingredients. Of the latter the nitrogen com-

pounds in the form of nitrates ($-NO_3$) are the most important. Potassium, phosphorus, and certain other elements furnish a part of the plant food, but they are of minor importance and for the sake of simplicity may be left out of consideration here. The plant takes from the soil and the air the material it requires, and by means of the energy furnished it by the sun's rays it builds these simple substances into more complex ones such as sugars, starches, proteins, and fats. This brings us to step B. From these products of plant life animals obtain their food. Standing at the top of the circle *C*, they are incapable of utilizing the simpler compounds, but are wholly dependent on the products manufactured by the plants. The complex food then is eaten by animals and becomes part of their bodies. As a result of muscular activity part of the carbon and oxygen is speedily converted into carbon dioxide and exhaled into the air. The nitrogen compounds are in part reduced at once to urea and excreted as such, while most of the remainder is retained to build up body tissue. When the body dies this nitrogenous material is far too complex for immediate use by the plants, and in order to complete the cycle it must be reduced to simpler compounds. It is at this point *D* that bacteria form a link in the chain. Always present in the air, soil, and water, they seize hold of any organic substance that can serve them as food and break it into simpler and simpler compounds until finally it is in the right condition to serve again as plant food.

Many species take part in this putrefactive process. Bacillus proteus, bacillus subtilis, and the colon bacilli are amongst the most important. Pathogenic bacteria are for the most part killed during the early stages of decomposition.

The breaking down of the highly complex protein substances into simpler and more stable compounds is spoken of as *mineralization* or *denitrification*. Sometimes it happens that the process is carried so far that the products are too simple even for plant food. From decaying animal bodies part of the carbon is returned to the air as carbon dioxide and part combines with the alkali in the soil to form carbonates. The nitrogen compounds that

may have been reduced to free nitrogen (N), to ammonia (NH_3), or to nitrites ($-NO_2$), must be built up into nitrates ($-NO_3$) before the plants can use them.

Here it is that other species of bacteria *E* intervene, and this time their work is one of construction rather than destruction. In the soil everywhere there exists a class of bacteria, known as *nitrifying* bacteria, which have the power of uniting oxygen with these simple compounds. There are apparently two distinct steps in the process. In the first stage ammonia is oxidized to nitrous acid or to nitrites by the *nitrosobacteria* or *nitrosomonas*. These nitrites are somewhat unstable and are quickly oxidized into nitrates by still another species known as *nitrobacter*. Whether in the form of nitric acid or nitrates the nitrogen is now ready to be absorbed by the roots of the plant and to start once more on its journey around the food cycle. Thus plants by a constructive process form the connecting link between the soil and animal life, and bacteria, by a reducing process sometimes followed by one of synthesis, complete the cycle of returning to the soil again the substances originally derived from it.

The nitrogen cycle is not quite complete at this point. Whenever putrefaction takes place some of the nitrogen escapes into the air as gas and is dissipated. Apparently this portion has escaped from the cycle since plants cannot extract free nitrogen from the air. There are other sources of apparent loss also, such as the gradual draining of the soil into streams, bodies of plants and animals that fall into rivers and are carried to sea, the sewage disposal of cities which often discharge vast quantities of nitrogenous material into the great lakes or the sea, the use of nitrogenous compounds as explosives. It would seem in this way that large amounts of nitrogen must be irretrievably lost from the cycle. Fortunately, however, other bacteria are unceasingly at work to redeem this supply which has apparently flown off at a tangent. It has been found that soil entirely free from plants, but containing certain species of bacteria, will slowly but surely gain in the amount of nitrogen that it contains. That the compounds are manufactured by bacteria is certain because they do

not accumulate unless bacteria are present. A rather strange fact is that this fixation of nitrogen is not accomplished by any one species alone, but only takes place when two or three are acting together. The name *azobacter* is generally applied to the group.

A second method by which bacteria aid in reclaiming this dispersed nitrogen is in combination with some of the higher plants, chiefly beans, peas, and clover. When growing in soil that contains little or no nitrogen, these plants will, during their growth, be found to have accumulated a considerable store of combined nitrogen in their tissues. It is evident that the only possible source of supply is the nitrogen of the air that permeates the soil. When a plant gains its nitrogen in this manner it develops upon its roots little protuberances known as root nodules or tubercles, which when examined microscopically are found to be nests of bacteria. By what process the plant and the bacteria growing together succeed in extracting the nitrogen from the air is not known. The plant continues to increase the store of nitrogen in its roots, stem, and leaves probably during the whole of its normal growth. Finally it dies, and, falling upon the ground becomes buried. It is immediately seized by the decomposition bacteria and the destructive changes already described begin. The eventual result is, that which seemed lost nitrogen is once more converted into nitrates and again forms part of the cycle.

Bacteria, then, play a threefold rôle in the cycle: (1) they reduce complex nitrogenous substances to simpler and more stable ones such as may be used by plants for food; (2) they build up those that are too simple into suitable compounds; (3) they *fix* the nitrogen of the air by an unknown process, but by one that makes it again available for the nourishment of plants.

It is important to note that almost the entire cycle takes place upon the surface or in the upper layers of the soil. A few feet below the surface there are very few bacteria; consequently a carcass buried deep or sewage placed too low are not acted upon so completely. At a depth of six feet very few organisms are found.

It is extremely difficult to determine the exact number of bac-

teria in any portion of the soil; many of them are anaërobes and many require special media for their growth. Of such as can be grown on ordinary media there have been found approximately 100,000 per gram in an uncultivated soil, 1,500,000 per gram in a garden soil, and 115,000,000 per gram in soil mixed with sewage. The actual numbers must be infinitely greater.

Bacteriological Examination. — For the examination of surface soil a specimen may be taken with a sterile spoon or tube. When taken from a lower level a special instrument is generally used. The usual form is that of a drill with a hollow chamber just above the point. A sliding door to the chamber is so arranged that it can be opened or closed by a mechanism controlled at the handle. The chamber is first sterilized and then the drill is forced into the ground to the desired depth; the door of the chamber is opened and by a twisting movement the soil is forced into the chamber; the door is then closed and the drill removed.

Before removing the soil the chamber is weighed, then a small amount about the size of a bean is dropped into a flask containing a liter of sterile water and the chamber is again weighed to ascertain the quantity removed. The moisture in the flask is then vigorously shaken to insure an even distribution of the organisms, and the examination is made in the same manner as that described for water. Quantities as small as 0.1 c.c. and 1 c.c. should be plated and cultivated both aërobically and anaërobically.

Almost all the bacteria in the soil are saprophytes. The organisms pathogenic for man do not find conditions favorable for development; for the most part the temperature is too low, and, further, they are so crowded out by the saprophytes that they die in the struggle for existence.

Pathogenic Bacteria Associated with the Soil. Tetanus bacilli. — Spores of the tetanus bacilli frequently occur in the soil, although it is improbable that the organisms ever multiply there. Wound infections usually occur as a result of contact with the soil of the object inflicting the wound.

Anthrax Bacillus. — The anthrax bacillus, like that producing tetanus, would probably not continue to exist in the soil were it

not for its resistant spores. Its presence there is a greater menace to animals than to man.

Malignant Edema. — Wound infections with the bacillus of malignant edema occur which give rise to extensive hemorrhagic edema. The bacillus is an anaërobe and occurs in the upper layer of the soil.

Welch's Gas Bacillus or B. aërogenes capsulatus. — This organism occurs in the intestines of man and animals and in the soil. When introduced into wounds it may produce suppuration and a great amount of gas. In the majority of cases it leads to no harm, yet in a small percentage it causes one of the most rapidly fatal infections known.

Typhoid Bacillus. — Typhoid bacilli may find their way into the soil with human excreta. Multiplication, however, rarely takes place there. As a rule they do not live more than a month unless the ground is frozen, in which case their life may be prolonged to several months. The chief danger so far as typhoid bacilli are concerned is the washing by heavy rains of human excreta that has been deposited on the ground into a stream used for drinking purposes, or drainage through the soil into a near-by well.

Cholera Spirillum. — Cholera spirilla too may be deposited upon the ground in human feces; they live only a very short time there and are not likely to regain entrance into the human body except by means of drinking water.

BACTERIA IN THE INDUSTRIES

The ability of bacteria to produce decomposition is the basis of several industries. Certain of these depend upon the result of bacterial fermentation, others exist to prevent it. Many of them were in operation long before their intimate relation to bacterial activity was known. In some cases the original methods are still employed, though modified somewhat by the use of pure cultures or an increased knowledge of antiseptics and germicides.

Preservation of Food. — The ceaseless energy of bacteria and

their presence everywhere makes it impossible to preserve meat and fruit for more than a few days without applying special methods. This fact, coupled with the necessity for conserving a supply for the months when such foods are scarce, has given rise to one of the most important industries. Canning of meats and fruit is simply preserving them from the attack of micro-organisms; heating kills all the bacteria present, and hermetically sealing prevents others from gaining access.

The process of canning was practised as early as 1804. Monsieur Appert of Paris found that food in sealed vessels would keep indefinitely if, after being sealed, the containers were kept for one hour in boiling water. His method is still employed except that after an interval of a day a second heating is now given to destroy forms that might have been in a spore stage on the first day. Canning is really a practical application of fractional sterilization.

Drying is one of the oldest and simplest methods of preserving food from bacterial attack. Exposure to sun and air deprives the substances of their moisture and consequently renders them unsuitable for bacterial growth. Smoke-dried meats in addition to losing their moisture are impregnated with antiseptic substances such as creosote, which are present in varying amounts in wood smoke. It has been found, however, that smoking cannot be depended upon to destroy disease-producing organisms in contaminated meats.

The addition of chemical substances that prevent the growth of bacteria has long been practiced; vinegar is a familiar example. Meat placed in brine and fruit in a thick sugar sirup are preserved, because the density of the solution being greater than that of the bacterial cell, water is drawn from the microörganism rather than supplied to it. Strongly bactericidal substances are sometimes used, such as borax, salicylic acid, and formaldehyde. They may accomplish the purpose for which they were added, but except in very small amounts they are injurious to the consumer.

The cold-storage method of food preservation has as its principle the inhibition of bacterial growth by a low temperature. Bacterial multiplication ceases at a few degrees above freezing

point; hence refrigeration is an excellent preservative. Bacterial activity is thus a reason for the existence of the ice industry and the manufacture of refrigerators.

Occasionally bacteria are intentionally allowed to decompose food up to a certain limit. The so-called gamy flavor of meat is due to the first stage of decomposition. Sauerkraut is another example of food expressly allowed to ferment. The special flavors produced by bacteria in the preparation of butter and cheese are other instances.

Vinegar Making. — The first step in the process of vinegar making is brought about by yeast cells. By their ability to ferment grape sugar they produce alcohol. The next stage is accomplished by bacteria which cause the alcohol to unite with oxygen, thus producing acetic acid or vinegar. Oxidation of alcohol into vinegar can be brought about by a chemical process, but it is impracticable on a large scale.

One of the usual methods employed is to add to a weak solution of cider a small quantity of vinegar. After a short time a thick, felted scum forms on the surface of the alcohol. The scum is a mass of bacteria spoken of as the "mother of vinegar," which in some way causes the oxygen of the air to unite with the alcohol. After the amount of acetic acid reaches a certain percentage bacterial action stops and no more acid is produced, even though there be alcohol remaining. It was at first thought that only one species of bacteria was able to produce this fermentation. Later study has shown that several different kinds have the power; most of them have a common characteristic in that they grow in long filaments without any trace of division.

A rapid method of vinegar manufacture is carried on by filling high cylinders about three fourths full with wood shavings which have been soaked in warm vinegar. Weak alcohol is then poured in, and as it slowly passes over the shavings it is oxidized into acetic acid.

Occasionally the fermentation does not proceed in a satisfactory manner; other species find their way into the fermenting liquid and produce undesirable substances which give it a totally dif-

ferent flavor. By degrees a more scientific method is being adopted. It is gradually becoming the custom to heat the alcohol and then add the desired bacteria in pure culture.

Maceration Industries. — The separation of linen from the flax stem is a process usually brought about by bacterial activity. The valuable linen fibers and the coarser wood are so bound together by a cementing substance that it is seldom possible to separate them by mechanical means. In order to decompose this binding material several methods may be employed, the principle of each being to subject the stems to suitable heat and moisture to encourage bacterial growth. A fermentation is thus started which softens the gummy substance holding the fibers together and permits their separation. This "water-retting" process is supposedly brought about by anaërobic bacteria.

The same principle is applied in the manufacture of jute and hemp and in the preparation of cocoanut fiber. In the tanning of leather, the preparation of sponges, and the curing of tobacco bacteria also play a large part.

CHAPTER VII

BACTERIOLOGICAL EXAMINATION OF WATER AND SEWAGE

NATURAL waters are usually considered in three classes according to their location; namely, rain water, ground water, and surface water.

Practically all, from whatever source, contain bacteria, the number and kind varying under different conditions. Rain water contains comparatively few excepting the first shower, which washes the air and brings down most of the floating dust particles and bacteria in its fall.

Ground waters, which include springs, shallow wells, and deep artesian wells, rank next from the standpoint of bacteriological purity. The water as it percolates through the soil gradually leaves behind its bacterial content in the upper layers of the ground and finally emerges in a spring or well almost germ free. This is especially true of artesian wells and springs. Shallow wells are more liable to variation. Unclean surroundings, privy vaults, or barns placed in such a position that drainage may take place in the direction of the well may lead to contamination of the water and a consequent increase in the number of bacteria.

Surface waters include streams, rivers, ponds, and lakes, and these of all natural supplies contain the most bacteria on account of the exposure to contamination to which they are subjected. During heavy rains soil washed down from the banks of rivers or streams supply an additional number of bacteria; wind currents and waves stirring up the bottom mud may bring up bacteria that have been sedimented; sewage and trade wastes from near-by towns may add enormously to the bacterial content.

Relative Purity. — An arbitrary standard of relative purity is almost impossible to fix. Several hundred bacteria per cubic centimeter might be normal in a river water, whereas the same number found in well water would immediately arouse suspicion. According to certain authorities water containing less than 100 bacteria per c.c. is presumably uncontaminated by surface drainage, one with 500 bacteria per c.c. is open to suspicion, one with 1000 per c.c. is presumably contaminated by sewage or surface drainage. A practical classification from a sanitary point of view is as follows: (1) *good,* as determined by bacteriological and chemical analyses, physical inspection, and a sanitary survey of the watershed; (2) *contaminated,* if organic waste of either animal or vegetable origin be present (a contaminated water is suspicious but not necessarily dangerous); (3) *infected,* if the water contains specific organisms causing disease.

Significance of the Presence of Colon Bacilli. — Enumeration of bacteria gives an approximate idea of the degree of pollution with organic material, but it gives no idea of the kind of bacteria present. Unfortunately there is no reliable method whereby pathogenic organisms such as the typhoid and dysentery bacillus can be isolated from water with any degree of certainty, even though it is actually known that the organisms are or have been present because of cases of disease which have developed from drinking it. The bacteria may be present in such small numbers that though an ordinary tumbler of water might contain sufficient to cause infection it would be a rare chance if one or two of them should be in the small quantity taken for examination. Another likely reason for failure is the fact that they do not live many days in water. It is known, however, that certain organisms, such as the colon bacilli, which are normally present in the intestines, have a longer life in water than those which are present only in diseased conditions. Moreover, it is practically sure that all the pathogenic organisms which give rise to water-borne infections find their way into the water supply by means of intestinal discharges from human beings. Under these conditions it is practically safe to assume that the absence of the colon bacilli in water

means the absence of pathogenic bacteria except in such extremely rare cases as contamination by urine. The presence of the colon bacillus does not necessarily signify danger, but it does mean pollution with fecal discharges. Deep well water should be condemned if any colon bacilli are found in it. On the other hand, surface water may contain one colon bacillus per c.c. without the presence of pathogenic organisms being suspected, particularly if it is known to drain an inhabited area. The fact that the colon bacillus is found in the feces of animals makes it difficult to determine whether pollution is of animal or human origin. A fresh hillside stream may contain colon bacilli brought to it by rain washings from manured fields through which it passes or by a stray horse or cow. Any water, however, containing ten colon bacilli per c.c. is usually considered as decidedly polluted and unsafe for human consumption.

Generally speaking, no single test should be relied on alone, although the colon test surpasses all others in delicacy in determining pollution. An inspection of the surroundings, a chemical and bacteriological examination, are all necessary for complete information.

Bacteriological Analysis. — In the bacteriological examination of water two lines of inquiry are generally followed. First, the approximate number of bacteria per c.c. is estimated, and second, the presence or absence of the colon bacillus is determined, and if present in what number per c.c. it occurs.

As in the case of soil, so also in a given sample of water, it is impossible to determine the exact number of living organisms present. This fact, however, does not prevent a numerical estimation from being of value. An approximate idea of the number present can be obtained, and also certain inferences of importance can be drawn by comparing the results obtained by different methods. For example, if a moderate number of bacteria develop at 20° C. and very few at 37° C. the water may, from a sanitary standpoint, be comparatively pure. If, however, the condition is reversed and many colonies appear upon agar plates incubated at 37° C. and few at 20° C., then the majority of the organisms present may be

regarded as accustomed to the same temperature found within the animal body and the water consequently as suspicious.

Collecting Samples. — Care must be taken that a sample is representative. If taken from a tap or pump the water in the pipe must first be run off to obviate any effect the metal may have had; if from a lake or pond the surface scum or bottom mud should be avoided or both may be examined separately; if from a brook the specimen should be taken some distance from the bank.

The container must of course be sterile and the test made as soon as possible, for an increase or a decrease in the number of bacteria begins immediately. If a short delay is unavoidable the sample should be kept on ice at a temperature of 5° C.

Technique for Quantitative Analysis. — The sample is vigorously shaken about twenty-five times and then by means of a sterile graduated pipette 1 c.c., 0.1 c.c., and 0.01 c.c. are placed respectively in three sterile Petri dishes previously marked with the amount to be received. Immediately a tube of liquid agar cooled to 40° C. is poured into each dish and the water and agar are thoroughly mixed by a rotary movement of the dish before the agar solidifies. The test should be made in duplicate, one set of plates being placed in the incubator at 37° C. for twenty-four hours and the other kept in the dark at room temperature for forty-eight hours. Usually the number of colonies developing at room temperature is far greater than that developing at 37° C. The colonies are counted in the manner already described.

Presumptive Test for B. Coli. — To determine the presence of the colon bacillus a medium such as the Conradi-Drigalsky is employed, and the plating procedure already indicated is done in triplicate, to the third series of plates being added the colored medium instead of the plain agar. After twenty-four hours' incubation if colon bacilli are present in the amount of water tested red characteristic colonies will appear which stand out well against the blue background.

A second test is made by inoculating fermentation tubes containing colored lactose peptone water with varying amounts of water: 10 c.c., 1 c.c., and 0.1 c.c. After incubation at 37° C. for

forty-eight hours those tubes showing a color change and the production of gas are presumed to contain the colon bacillus. If, for example, gas appears in the tubes containing 10 c.c. and 1 c.c. of water and not in the tube containing 0.1 c.c. it is assumed that the water contained at least one colon bacillus per c.c. The interpretation of the results by the plate method is evident. The number of red colonies developing on the plate containing 1 c.c. of water would give the number of B. coli present per c.c.; if these should be too numerous to count or if the color of the entire medium be changed, then the colonies on the plate containing 0.1 c.c. of water should be counted and the number multiplied by ten to give the total number per c.c.

It should be remembered that the above are only *presumptive* or *partial* tests for the colon bacillus, and although fairly reliable and of value for routine examination other tests are necessary before an organism can with certainty be said to be the colon bacillus.

Determination Test. — In order to determine beyond doubt that B. coli has caused the fermentation of lactose in the tubes a loopful of the culture is streaked on plates containing solidified Conradi medium, and after twenty-four hours' incubation a typical red colony is fished and incubated in a tube of broth. At the end of from twelve to twenty-four hours' incubation about 0.1 c.c. of the broth culture is pipetted into the following media, each of which gives a characteristic reaction when used for the cultivation of the colon bacillus: *gelatin*, absence of liquefaction; *neutral red lactose peptone water*, production of acid and gas; *milk*, production of acid and coagulation of the protein; *peptone solution*, production of indol.

Sewage Streptococci. — In addition to the many different bacilli normally present in the intestines there are also certain cocci often spoken of as sewage streptococci. They, too, produce pink colonies on Conradi medium. They are much smaller, however, than those of the colon bacillus and can easily be differentiated. They are not hardy and quickly die in water; thus their presence represents recent pollution.

Isolation of the Typhoid Bacillus. — So many difficulties attend the search for the typhoid bacillus in water that it is rarely attempted except in experimental research. Under ordinary circumstances the organisms do not multiply in water; they rarely live longer than seven days in cold water and even a shorter period when it is warm.

Several methods have been devised for the isolation of the typhoid bacillus, one of which is the addition of large quantities of water to the same volume of double-strength broth containing a substance known to inhibit the growth of saprophytic organisms without having an injurious effect on the typhoid bacillus. After twenty-four hours pour plates are made, employing one of the special media, such as the Conradi-Drigalsky. It is exceedingly rare, however, that the organism is isolated. Water is more often condemned on circumstantial evidence than on the actual finding of the typhoid bacillus.

Cholera Spirillum. — The isolation of the cholera spirillum from water is somewhat less difficult. It occurs in much greater numbers in the excreta of cholera patients than does the typhoid bacillus in the feces of those suffering from typhoid fever. Koch, the discoverer of the cholera spirillum, suggested a practical method which has proved of value. The water to be examined is itself converted into medium by dissolving in each liter ten grams of peptone and sufficient sodium carbonate to make it slightly alkaline. The mixture is incubated at 37° C. from sixteen to twenty hours, after which gelatin or agar plates are streaked with the surface growth. If cholera spirilla are present characteristic colonies with irregular margins will develop which will agglutinate with specific cholera serum.

Purification of Water. — Nature has various methods of her own for the purification of water. Enormous quantities of sea water and marsh water are being constantly evaporated and then returned in the form of rain in a practically pure state. Streams tend to become purer in their flow; organic matter is gradually oxidized, thus diminishing the bacterial food supply. Microscopic animals such as protozoa feed upon bacteria, and they in turn serve as

food for rotifers and crustacea. Dilution plays an important rôle in that a small amount of infection in a lake or river is soon so diluted as to practically become lost. A slow-moving river is purified much in the same way that snow clears the air: the particles of mud which are constantly settling enmesh the bacteria in their fall and carry them down to the bottom, where they soon die.

Boiled Water. — So far as water-borne infections are concerned boiling renders water safe. Typhoid and dysentery bacilli and cholera spirilla are killed even at a lower temperature. Holding for twenty minutes at 60° C. or a few minutes at 70° C. is sufficient to destroy them.

The principal methods employed for the purification of water on a large scale are (1) storage, (2) filtration, (3) addition of a chemical. In some cities two or even all the methods are combined.

Storage. — Several of Nature's methods are applied in this form of purification; namely, time, oxidation, dilution, sedimentation, etc. The growth of algæ and decomposition of organic matter sometimes gives to water stored in an open reservoir a disagreeable taste and odor. That may be obviated, however, by the use of a closed reservoir.

Filtration. — Two forms of filters are in general use for public water supplies: *slow sand filters* and *mechanical filters*.

A slow sand filter consists of a large shallow reservoir with underdrain pipes and containing five or six feet of filtering material of graded size, beginning at the bottom with broken stone or gravel and finishing with an upper layer of fine sand. The water passes through the filter very slowly from above downwards, and in its passage almost all the bacteria and fine particles are strained out. The process is not merely a simple straining; its efficiency is due rather to bacterial activity. The spaces between the finest sand are enormous compared to the size of bacteria, and yet 99 per cent of bacteria do not pass beyond the upper layer. What really happens is that the microörganisms resting upon the surface grow and form gelatinous masses which adhere to the particles of sand and gradually close up the interstices. This continuous carpet-

like mass effectively holds back the bacteria. Thus their removal is largely a biological process due to the bacteria themselves.

The action of a *mechanical filter* is strictly a straining. A chemical coagulant is added, generally sulphate of aluminium, and the water is passed rapidly through a layer of sand. The coagulant clears the water much as the white of an egg clears coffee. Bacteria are enmeshed and deposited on the surface of the sand, forming thus an artificial inorganic carpet in place of the natural organic one of the slow sand filter bed. Mechanical filtration is a comparatively quick process and especially suitable for turbid waters containing much clay; its action is somewhat less uniform than slow sand filtration. It removes from 95 per cent to 99 per cent of bacteria.

Household filters of the ordinary type cannot be relied upon to make infected water safe. They are serviceable in rendering a turbid water clear, but they should not be depended upon for more than that.

Addition of Chemicals. — Ozone is an effective purifier of water, but its use is limited in that it does not clarify, and the expense of producing it is comparatively large.

Chlorinated Lime, Chloride of Lime or Bleaching Powder. — The germicidal action is due to liberated chlorine which acts on the water, setting free nascent oxygen. So effective is chlorinated lime that one part per million parts of water will destroy 99 per cent of the bacteria in water containing little organic material. It does not clarify water nor remove discoloration, but it is a cheap, efficient, and harmless method and is widely used.

Copper Sulphate. — It was first claimed that the addition of copper sulphate in small amounts to water would destroy both the algæ which produce objectionable tastes and odors and the pathogenic microbes. Later it was found that while even in great dilution it destroys algæ and many microörganisms it has little effect upon typhoid and dysentery bacilli. It is generally used in the proportion of one tenth to one quarter part per million parts of water. The copper combines with the bodies of the organisms and both settle to the bottom as a sediment.

Ultraviolet Rays. — Exposure of a clear water to ultraviolet rays has a speedy effect on bacteria. Most of the vegetative forms are killed in from ten to twenty seconds; a longer time, however, is necessary if the water is turbid. After preliminary rough filtration to remove coarse particles the water supply of Marseilles passes a quartz tube mercury arc lamp three times. It is claimed that between 98 and 99 per cent of the bacteria present are destroyed during the process.

Sewage Purification. — The rôle which bacteria play in the disintegration and consequent purification of sewage is an important one, even though the details of the process are somewhat obscure.

The treatment of sewage may consist of one, two, or all of the following processes: (1) screening or removing the larger substances that might injure filters, etc. Screens may vary all the way from gratings of iron bars to fine ones of wire cloth. The material screened is pressed and burned under a boiler or buried in land and the effluent passed into (2) a sedimentation tank. Several different types of tanks have been devised for the purpose, in all of which the underlying principle is the disintegration by bacteria of the organic material present. From the sedimentation tank the sewage is carried to (3) a filter bed, usually composed of some form of porous material such as coke or brick, through which it percolates to underdrains below. The final process (4) is the removal or destruction of bacteria in the effluent. Chlorinated lime is one of the best substances for the purpose; about fifty pounds per million gallons for good effluents will destroy from 95 to 99 per cent of the bacteria.

Methods of bacteriological examination to determine the efficiency of a purification plant are the same as those described for the examination of water except that smaller quantities are used. In a sewage effluent as in water the absence of the colon bacillus is regarded as an indication of its harmlessness.

CHAPTER VIII

MILK

BECAUSE milk is one of the most valuable articles of diet, and above all because it is an indispensable food for infants and young children, its purity from a bacteriological standpoint is of the greatest importance. As a rule, however, it contains more bacteria than any other article of food; frequently many more than are found in sewage. For the most part the bacteria which find their way into milk are saprophytes, but even so, their presence in large numbers is by no means desirable in a food for infants.

When first secreted in the udder of a normal cow milk is practically germ free. It is impossible, however, by ordinary dairying methods to obtain it in such a pure condition. Bacteria from the air and surroundings find their way into the milk ducts, and as a little milk always remains there from the previous milking they find exactly the conditions they require: food, moisture, and a suitable temperature, and they begin to multiply rapidly. By the next milking they are abundant, and the first milk drawn washes them into the milk pail, where they continue to grow. The milk receives an additional supply from all the objects with which it comes in contact. The hands of the milker, the air through which it passes, and the pail into which it falls add their quota. The hairs of the cow and particles of manure which may drop into the pail furnish more. Generally the farmer makes an attempt to remove the coarser dirt by straining the milk through a cloth. That process, however, does not affect the bacteria present.

Even a moderate degree of cleanliness has an appreciable effect upon the bacterial content. A clean barn in which to milk, clean pails with small openings, clean hands, and a clean condition of the

cow's udder and flanks will mean a considerably lower bacterial count. Milk obtained by the cleanest methods may contain only a few hundred bacteria per c.c. when drawn; collected with less care it may contain several thousand, and, unless promptly cooled, the number soon mounts to millions. An excessive number of bacteria, therefore, is an indication that milk is dirty or old or that care has not been taken to keep it cool. The following table illustrates these points well.

TABLE I [1]

Milk collected under the best conditions possible. Bacterial content at commencement of test 3000 per c.c.

KEPT AT	24 HRS.	48 HRS.	96 HRS.	168 HRS.
0° C.	2,400	2,100	1,850	1,400
4° C.	2,500	3,600	218,000	4,209,000
10° C.	11,500	540,000	300,000,000	1,000,000,000
20° C.	450,000	500,000,000	—	—

TABLE II

Milk collected under ordinary conditions. Bacterial content at commencement of test 30,000 per c.c.

KEPT AT	24 HRS.	48 HRS.	96 HRS.	168 HRS.
0° C.	30,000	27,000	24,000	19,000
4° C.	38,000	56,000	4,300,000	38,000,000
10° C.	89,000	1,940,000	1,000,000,000	—
20° C.	4,000,000	1,000,000,000	—	—

Germicidal Property. — Freshly drawn milk appears to have a slight germicidal action. If samples are examined every hour the colonies at first decrease in number. Soon, however, this property disappears and there follows a continuous and sometimes rapid increase. At temperatures under 10° C. the effect may be

[1] Adapted from Park and Williams, "Pathogenic Microörganisms," 1917, p. 634.

marked for from eight to twelve hours; at higher temperatures it is scarcely perceptible. It is supposed by some authorities to be an agglutinative rather than a germicidal property; that is, the bacteria may not actually be destroyed but gathered together in clusters that will not readily separate. Thus a colony may result from a group of bacteria instead of a single individual and a wrong impression of decrease in number may be obtained. In any case the action is comparatively feeble and is soon lost.

Estimation of Bacterial Content. — The statement made concerning the bacteriological examination of soil and water is equally applicable to the examination of milk; by no known method can the exact number present in a given sample be determined. Some species grow slowly or not at all on culture media; some are aërobes, others anaërobes; some require body temperature, others grow best at a lower temperature. Clusters of bacteria may remain attached even after vigorous shaking and develop as one colony instead of several. Again, bacteria are not equally distributed in the milk and the sample may not be representative. As the cream globules rise they carry along with them numbers of the bacteria present, until finally four or five times as many organisms may be found in the cream and upper layer as in the lower portion. Unless the milk is thoroughly mixed before the sample is taken this also may constitute a source of error.

The method usually employed to estimate the number of bacteria present in a cubic centimeter of milk is the plating method already described. The direct microscopic examination of a film of milk has been advocated in order to eliminate the above sources of error. A square centimeter is ruled on a glass slide and 0.01 c.c. of milk accurately measured and evenly spread over it. The film is dried in the air, fixed with methyl alcohol, the fat dissolved with xylol, and finally it is lightly stained with methylene blue. The oil immersion lens is employed for the examination, and the tube of the microscope is so arranged that the microscopic field covers $\frac{1}{50}$ sq. mm. The average number of bacteria found in each field is multiplied by 5000 to give the number of bacteria contained in 0.01 c.c. of milk. When the results of the two methods

are compared it is generally found that the microscopic method gives a much higher count than the plate method. If, however, the clumps of bacteria seen in the former are given only the value of one the two counts closely agree. Microscopic examination of pasteurized milk is not practical, since it offers no means of distinguishing between living and dead bacteria.

Milk Standards. — Several cities have endeavored to obtain a purer milk supply by admitting for sale only the milk that reaches the standard they have set. The requirements are based on farm conditions and chemical and bacteriological analyses. The number of bacteria permissible in the milk sold in New York City is as follows:

Grade A[1] must not contain more than 60,000 bacteria per c.c. It may be raw or pasteurized; the raw is obtained from cows that have successfully passed the tuberculin test.

Grade B is all pasteurized. Before pasteurization it may contain 1,500,000 and after pasteurization 50,000 per c.c.

Grade C is all pasteurized. It may contain any number within reason before pasteurization and not more than 100,000 per c.c. afterwards. It is to be used for cooking purposes only.

Sour Milk. — Under ordinary circumstances milk, after a short period, becomes sour. The milk sugar, lactose, is fermented and lactic acid is produced; curdling results as a precipitation of the casein from solution by the acid.

When it was first discovered that the souring of milk was due to bacteria it was thought that only one species was responsible for the change, and an organism isolated was named Bacillus acidi lacti because it had the ability to decompose lactose into lactic acid. Later it was discovered that more than a hundred other species have the same power in a varying degree. The amount of acid produced depends largely upon the bacteria pro-

[1] Park and Williams, "Pathogenic Microörganisms," 1918, p. 642.

ducing it. As soon as the amount becomes injurious to the organism growth ceases and no more acid is formed.

Sour milk obtained from clean milk is considered beneficial as a food. In certain parts of Asia and eastern Europe it forms part of the staple diet. Within recent years similar sour milk products have been manufactured commercially on a large scale in western countries. It has long been recognized that a mutual antagonism exists between the acid-producing bacteria and those causing putrefactive changes, and on this basis attempts to combat such changes occurring in the intestines, which lead to so-called "auto-intoxication," were early made by adding to the diet acid-forming bacteria together with carbohydrates. At first the results were only moderately successful. Then it occurred to Metchnikoff that probably the organisms used had not the capacity to produce acid in large enough quantities. In his search for a more powerful acid producer his attention was attracted to Bacillus bulgaricus, an organism isolated from milk in 1905 by Massol and Cohendy, said to produce as much as twenty-five grams of lactic acid per liter of milk in addition to smaller quantities of other acids. The fact that it does not attack proteins and that it is not pathogenic makes it particularly suitable for its therapeutic rôle.

Putrid Milk. — When boiled milk is allowed to stand at room temperature it sometimes becomes bitter and has an alkaline reaction. A spore-bearing group of bacteria and also certain anaërobes are responsible for this change; they decompose the protein into injurious substances resembling "ptomains."

Ropy Milk. — Bacteria which produce this condition are widely distributed in nature. In Europe Bacillus lactis viscosus is considered the main agent. A micrococcus, two forms of streptococci, and certain of the lactic acid bacilli are also able to bring about the same condition. In certain European countries ropy milk is considered a delicacy. It is not injurious provided the sliminess is not the result of a mucopurulent discharge from the udder of the cow.

Colored Milk. — A red color in milk may be due to blood if the udder is diseased; it may also appear if bacteria giving a red

pigment, such as B. prodigiosus or B. erythrogenes be present. Blue milk is usually due to the presence of B. cyanogenes. Milk colored by bacteria is apparently harmless.

Pathogenic Organisms in Milk. — There are two sources from which disease-producing bacteria may gain access to milk: from the cow, or from some human case. The latter is the much more common. Bacteria causing typhoid fever, diphtheria, or scarlet fever may find their way into the milk from carriers, convalescents, or persons suffering from a mild form of the disease who are engaged in handling the milk. Or infection may come in a less direct way: contaminated water may be used for rinsing milk pails or flies may convey the bacteria from excreta improperly disposed of.

Of the diseases transmitted to man from the cow bovine tuberculosis and septic sore throat occur the most frequently. The micrococcus causing Malta fever is usually conveyed by goats' milk, although cows are said to be susceptible to the disease. Cases of foot-and-mouth disease transmitted by milk are extremely rare.

Just how the bovine tubercle bacilli find their way into the milk supply has long been a question of intense interest. In the case of a tuberculous udder it is a simple matter. It has been suggested that cows suffering from pulmonary tuberculosis may cough up bacilli, swallow them, and then pass them in their feces. As enormous numbers have sometimes been found in feces, and as practically all market milk has been contaminated with manure rubbed off from the flanks of the cow, it is reasonable to assume that occasionally tubercle bacilli gain access to the milk in this manner.

For many years it has been thought that bacteria never pass through the mammary gland unless there is a local lesion. Recent experiments, however, tend to prove that in case of generalized tuberculosis of the cow tubercle bacilli may pass into the milk without any evidence of the udder being diseased.

To what extent milk contaminated with bovine tubercle bacilli is responsible for human tuberculosis is still an undecided question. It is stated that in districts where the milk from tuberculous cows is consumed the children are frequently found to suffer from

diseases of the joints and cervical glands and that tubercle bacilli of the bovine type have been obtained from these lesions. This may be explained by the fact that children drink more milk than adults and that they are more susceptible to the bovine type. Certain authorities hold the view that pulmonary tuberculosis in adults may be accounted for by an infection contracted in childhood due to milk. That, however, is by no means a general opinion.

The mode of access of the tubercle bacilli from human cases of the disease is quite easily conceived. A milker suffering from tuberculosis of the lungs, whose fingers have come in contact with his sputum, might readily wash off enormous numbers of bacilli into the milk pail, or droplets expelled from the mouth while talking or coughing might carry their quota.

Septic Sore Throat. — Many epidemics of septic sore throat have occurred directly traceable to the milk supply, and several varieties of streptococci have been isolated as the causal agents. It is assumed that the majority of outbreaks are due to organisms derived indirectly from human sources. The streptococci which produce garget in cows do not produce sore throats in human beings, and conversely, the species of streptococci which produce tonsillitis in man are only slightly pathogenic for cows. Experiments have shown that the streptococci giving rise to septic sore throat may find their way into the milk ducts when the teats have been wiped with an infected cloth; thus they may become implanted in the udder of the cow and continue to grow for several weeks without giving rise to any form of disease. In this way the cow may be a " carrier " of the human variety of streptococci.

Foot and Mouth Disease has been reported to have been transmitted to man through dairy products coming from diseased cows. In many the disease is very mild and seldom fatal.

Infantile Diarrhea. — Whether the majority of cases of infantile diarrhea are due to the bacterial content of dirty, stale milk or to its changed chemical content is not yet decided. While no single organism has been isolated that can truly be said to produce the malady, it remains true that in localities where fresh, clean

milk is supplied cases of infantile diarrhea are of much less frequent occurrence.

Typhoid Fever. — The typhoid bacillus is the cause of more milk-borne epidemics than any other organism; yet it is seldom that it can be isolated. Generally, however, examination of the feces of the employees handling the milk reveals a convalescent case or a "carrier." In some cases infection has been traced to the use of contaminated water for washing the milk utensils. In Washington during the four years 1907–1910 10 per cent of all the cases of typhoid fever were traced to milk.[1]

Scarlet Fever. — Although the organism causing scarlet fever is as yet unknown many epidemics have occurred, presumably due to milk infected from human sources.

Diphtheria. — Milk-borne epidemics of diphtheria are less frequent than those of typhoid or scarlet fever. The source is generally a convalescent case or a carrier.

General Character of Milk-borne Epidemics. — An explosive onset and a gradual decline generally indicate a contaminated milk or water supply. If the bacteria in the milk are comparatively few the disease may only appear in a few susceptible persons who drink it; if the organisms are numerous the infection may be carried along the entire milk route. At first the disease appears only amongst those who have partaken of the infected milk; later, secondary cases may appear.

Sterilization of Milk. — With the realization of the possibility of disease-producing organisms being present in milk the question arises as to the best method of destroying them and rendering the milk safe as a food. Sterilization by heat is the only practical method. Boiling, however, is objected to by pediatricians on the ground that cases of scurvy and rickets are likely to develop in infants fed exclusively on boiled milk. Fortunately these dangers can be obviated if instead of being boiled the milk is pasteurized.

Pasteurization. — The process devised by Pasteur for preserving wines without loss of their original flavor is found to be equally well adapted for the treatment of milk. As the object of pasteur-

[1] Rosenau, "Preventive Medicine and Hygiene," p. 574.

ization is the destruction of the pathogenic organisms without so changing the food constituents that it is less suitable for infant feeding, the temperature used and the length of time of exposure depend on these two points. The lowest temperature, therefore, that will kill non-spore-bearing bacteria in a reasonable length of time is the one chosen. An exposure to 60° C. for twenty minutes or to 70° C. for five minutes has been found to be efficient. It is advisable in commercial practice where milk is pasteurized in large quantities to increase the temperature a few degrees and prolong the heating ten to fifteen minutes.

There are three methods in general use:

I. The flash method consists in heating the milk to 81° C. and chilling it at once. It is the quickest and cheapest method, but the least reliable.

II. The holding method consists in heating the milk to 65° C., then holding it at that temperature from thirty to forty-five minutes. Specially devised tanks have been constructed as "holders" that give excellent results. The method has proved most satisfactory for commercial purposes.

III. Pasteurization in the bottle is the ideal method. All danger of recontamination is thus eliminated. The bottles are tightly stoppered, immersed in a water bath, brought to the required temperature, and held there a sufficient length of time.

Whichever method of pasteurization is employed rapid cooling is of great importance.

The following experiment well illustrates the result of pasteurization so far as diminution in the number of bacteria is concerned.[1] The milk was heated to 70° C. for a half and for one minute.

SAMPLE I

Raw milk	600,000 bacteria per c.c.
½ minute pasteurization	2,000 bacteria per c.c.
1 minute pasteurization	1,000 bacteria per c.c.

[1] Adapted from Park and Williams, "Pathogenic Microörganisms," p. 635.

SAMPLE II

Raw milk	5,400,000 bacteria per c.c.
½ minute pasteurization	7,400 bacteria per c.c.
1 minute pasteurization	600 bacteria per c.c.

The pasteurization of dirty milk containing many millions of bacteria is a rather questionable procedure. The number of microörganisms may be considerably reduced, but all deleterious qualities will not be entirely removed.

A striking illustration of the beneficial effect of the pasteurization of milk of an average quality is given below.

When the children in a New York institution (Randall's Island) were given milk from a selected herd pastured on the island the death rate was as follows: [1]

	CHILDREN TREATED	NUMBER OF DEATHS	PERCENTAGE
During the years 1895 to 1897 inclusive	3,609	1,509	48.81

A pasteurizing plant was installed in the early part of 1898. No other change in diet or hygiene was made.

	CHILDREN TREATED	NUMBER OF DEATHS	PERCENTAGE
During the years 1898 to 1904 inclusive	6,200	1,349	21.75

Butter. — Cream from which butter is to be made is usually allowed to sour or " ripen "; that is, it is purposely allowed to stand in a container two or three days in order that the bacteria present may develop a characteristic flavor and aroma. The process is one of decomposition, but up to a certain point it gives pleasurable and profitable results; beyond that point it is undesirable and offensive. Ordinarily it is stopped at the right moment. The method usually employed is that of allowing the cream to ripen under the influence of any species of bacteria that happen to be present. Occasionally, however, by this " hit or miss " procedure

[1] Adapted from Jordan, "General Bacteriology," 1917, p. 561.

organisms that produce disagreeable flavors gain ascendancy and the subsequent butter is of a low grade.

In some dairies it is the custom to use a "natural starter"; that is, a small quantity of cream that has developed the required qualities is added to the fresh cream. This procedure is simply seeding the new cream with the organisms known to be capable of producing the desired results.

A step further has been taken by the more progressive dairymen. The fresh cream is pasteurized in order to eliminate the action of any bacteria present and a pure culture of an organism already proved to have the requisite qualities is introduced. In this way butter of a uniform quality, at least as regards flavor, can always be depended on.

Unfortunately all cream is not pasteurized before it is churned, and pathogenic organisms are by no means rare in market butter. In an examination of 21 samples offered for sale in Boston two or 9.5 per cent were found to contain tubercle bacilli.[1] Other investigations in another locality report 15.2 per cent. Many experiments have been made to determine the length of time typhoid bacilli will continue to live in butter. It is generally assumed that they die after a few days.

Cheese. — The bacteriology of cheese making is somewhat more indefinite than that of butter making. It is proven that cheeses are ripened by the action of bacteria and molds and that the different flavors are due to the growth of different species during the process. As yet, however, the organisms concerned and the rôle they play is largely a matter of conjecture.

[1] Rosenau, "Preventive Medicine," p. 581.

PART II

CHAPTER IX

ABILITY OF BACTERIA TO PRODUCE DISEASE

Infection. — In the early days of bacteriology the presence of bacteria in or on the skin or mucous membranes was regarded as an evidence of a diseased condition. It is well known now, however, that organisms such as streptococci, staphylococci, and pneumococci are frequently present in the nose or mouth or on the skin of normal healthy persons. The intestines contain many thousands of different species, but only under unusual conditions do they produce disease. The mere contact, therefore, of microorganisms with bodies of animals or man does not necessarily mean a diseased condition. When, however, they pass the protective skin and membranes, invade the deeper tissues, and multiply there they may produce poisons which give rise to the various symptoms met with in disease. This invasion, multiplication, and resulting disease is spoken of as an *infection*.

In the production of an infection the main factors are (1) the defensive forces the body can command to resist the invaders and (2) the power of the invading organism to withstand all the opposing forces the body can produce against it, to multiply and to elaborate poisonous substances.

Certain bacteria may live and multiply in the body apparently without either causing or receiving injury. Such organisms may be harmless and incapable of producing poisons or there may be established between them and the body cells an equilibrium in that the amount of poison produced is neutralized and rendered inert by a corresponding amount of cell secretions. In the latter

case if the invaders retain their pathogenic powers the person thus harboring them and probably disseminating them is termed a germ "carrier." Thus pneumococci and influenza bacilli are present in the nose or throat of many individuals. Persons who have recovered from an attack of diphtheria or who have been in contact with those suffering from the disease frequently become carriers. The typhoid bacillus may remain located in the gall bladder and be discharged in the feces long after all symptoms of the disease have disappeared. It has been estimated that after convalescence from typhoid fever one to three per cent may remain carriers for months or years.

When the balance of such a relationship is disturbed by diminished resistance on the part of the body infection is likely to occur. Such an infection is spoken of as *autogenous*. Appendicitis resulting from infection by the colon bacillus following congestion due to fecal impaction may be taken as an example. Usually, however, infections result from contact with contaminated material outside of the body. Probably those conveyed by water or food are of the most frequent occurrence; for example, typhoid bacilli or cholera spirilla by water, tubercle bacilli by milk. Or infection may result from a scratch with a rusty nail on which are the spores of tetanus, or hydrophobia from the bite of a rabid dog.

Infections resulting from the introduction of bacteria from sources apart from the individual infected are spoken of as *exogenous*.

Contagious and Infectious Diseases. — Organisms that are strictly parasitic and therefore cannot grow apart from the human body must, in order to produce disease in a second person, be transferred from one person to another by direct contact; the leprosy bacillus may be taken as an example. Diseases produced by these organisms are said to be *contagious*. Other organisms not so strictly parasitic, which are able to adapt themselves to other conditions outside of the body, may gain access to a second individual by means of contaminated material. A disease thus produced is spoken of as *infectious*. No strict rule, however, can be adhered to in such a classification since bacteria commonly

transmitted by the latter method may under certain conditions be transmitted by the former. A simpler plan is the classification of all bacterial diseases as infectious, reserving the term contagious only for those which are contracted as a result of direct contact.

Defensive Forces of the Body. — The body possesses three natural defenses against bacterial invasion: (1) a covering more or less unsuitable for bacterial growth and penetration; (2) the ability to produce chemical substances which either kill the organisms or render their poisons inert; (3) the power of certain cells to engulf and destroy the invaders. Thus even though the first barrier be passed invasion does not necessarily mean infection; the other forces acting singly or together may speedily prevent injury.

Influence of Tissues on Bacterial Invasion. — Many species of bacteria find a temporary lodgment upon the skin, but it is a poor soil for growth and forms an effective barrier against entrance into the deeper tissues. A group of cocci, however, are habitually present, and injuries such as wounds, or burns, or sometimes a simple pin prick enable them to penetrate deeper. Certain varieties are able to produce direct action without the existence of previous injury. Thus staphylococci may reach the roots of hair follicles and sweat glands and cause suppurative conditions.

The warmth and moisture of the mucous membranes make them much better adapted for bacterial growth than the skin. The nasal cavity is somewhat cleansed by the nasal secretion. Nevertheless, the influenza bacillus, the diphtheria bacillus, and others find it possible to obtain a lodgment and by concentrating at one point to lower the vitality, destroy the epithelial tissue, and gain an entrance there. It is possible that the meningococcus and the virus of anterior poliomyelitis gain access to the body by this route.

Diminution in quantity or change in quality of the normal body secretions may favor the growth of bacteria and render invasion easier. For example, the saliva is ordinarily somewhat bactericidal, but during a fever the amount secreted is diminished and unless the mouth is carefully and frequently cleansed fetid

sores develop on the teeth and lips as a result of bacterial growth.

The gastric juice through the hydrochloric acid it contains has a marked germicidal effect. Nevertheless, many bacteria escape its action because they are protected in the food or because of its neutralization. Bacteria causing typhoid fever, cholera, tuberculosis, and other infections may thus pass unharmed into the intestines and there produce their respective lesions.

Most inhaled organisms which pass the larynx are gradually removed by the ciliated epithelium of the bronchi; the few which succeed in gaining entrance to the lungs are able to multiply and produce disease only when the lung tissues have lost some of their resistant powers.

Points of Entrance. — Infection occurs with certain bacteria only when they enter the body by an appropriate route and reach special tissues. Thus typhoid, cholera, and dysentery infection does not take place unless the organisms enter the gastro-intestinal tract; they never enter through the skin. Gonococci usually enter the body through the genital organs or occasionally the eye, but never by way of the respiratory or digestive tract. The avenue of invasion thus determines largely whether infection will or will not occur, and if it does its nature and severity. Diphtheria bacilli rubbed on an abrasion of the hand produces only a slight lesion, but rubbed on an abrasion of the throat they cause inflammation, necrosis of the tissue, and a general poisoning. Pneumococci lodging on the surface of the eye may cause a severe conjunctivitis, on the mucous membrane of the throat a pseudomembranous angina, and in the lungs pneumonia.

Several species of bacteria are normally present in the mouth, some of which may be the cause of caries of the teeth. The importance of this condition is becoming more and more recognized as having a direct bearing on the general health. A carious tooth may be the portal of entrance for microörganisms causing a general infection.

The mode of entrance of the tubercle bacilli is still a disputed point. The theory that they enter through the respiratory tract

accounts most readily for the far greater frequency with which tuberculosis affects the lungs than it does other parts of the body. Recent experiments, however, tend to show that they may be swallowed and pass through a practically intact intestinal wall and find their way into the mesenteric lymph glands. The cervical glands of children often become infected with tubercle bacilli without any visible trace being left in the mucous membrane of the larynx to indicate their passage. In such cases the crypts of the tonsils are strongly suspected of being their portal of entrance.

Bones are infected by organisms carried to them in the blood stream except in cases of direct injury; for this reason the periosteum and the bone marrow, being most abundantly supplied with blood, are first affected.

Influence of Numbers. — The number of pathogenic bacteria which succeed in invading the tissues is an important factor in determining whether or not an infection will take place. If only a few gain entrance all may be killed; if, however, a large number are introduced some are almost sure to survive, to multiply, and produce their specific disease unless the body is immune. When a chronic disease or other factors have reduced the body defenses fewer bacteria than would otherwise be required may give rise to infection.

Virulence. — The degree of ability which an organism possesses to overcome the defensive forces of the body and to give rise to disease is spoken of as its *virulence*. Bacteria whose virulence is great may produce disease when only few in number, whereas millions of a less virulent species might be required.

It is impossible, by any means, to make a known non-virulent organism virulent. It is not difficult, on the other hand, to decrease or increase the virulence of an organism already possessing pathogenic powers. The ability to produce poison may be lessened by repeated growth on artificial culture media; by exposure of a culture for a short period to a temperature just below the thermal death point, or to sunlight, or to small quantities of antiseptic or germicidal substances. These methods are frequently employed

to attenuate cultures in the preparation of vaccines to be used for purposes of active immunization.

Ordinarily, the passage of an organism through an animal increases its pathogenicity only for that particular species of animal. Thus the passage of certain bacteria through a guinea pig increases their virulence for guinea pigs and not for rabbits nor rats. A method of increasing the virulence of a given culture is to inclose it in a collodion capsule of suitable thickness and place the capsule within the abdominal cavity of the chosen animal. The body fluids are able to transfuse through the sac, destroying such bacteria as are unable to withstand their injurious influence. In this way only the strongest survive and a race of more virulent organisms results.

An exception occurs in the passage of the smallpox virus through the calf, where it loses forever its power of producing smallpox.

Certain bacteria increase their resistance against the body defensive forces, and consequently their virulence by the formation of a capsule. The capsule may be quickly lost when the organism is grown on artificial culture medium, and its virulence correspondingly lowered. Repeated passage through animals will again restore it and produce a race of capsulated virulent bacteria.

It is thought by some authorities that bacteria actively secrete substances that are able to paralyze the protective forces of the body, especially the leucocytes. Little is known of them or their action. Their existence, nevertheless, may explain the statement made by Metchnikoff, that a virulent microörganism is not so readily taken up by the leucocytes as a non-virulent one. Thus it would seem that in an infection a tremendous struggle is carried on between the bacteria and the body cells, each side provoked by the other to manufacture forces that will either attack the enemy or protect itself against counter attacks.

Mixed and Secondary Infections. — Several different microörganisms may invade the tissues at the same time and produce a *mixed infection*, or one may follow another or others and give rise to *secondary infection*. Associated organisms are often influenced by the activities of each other. Thus the presence of pus-produc-

ing cocci has an injurious effect on anthrax bacilli. On the other hand, aërobic bacilli make possible the growth of anaërobes by absorbing free oxygen. Tetanus bacilli and their spores would be less likely to develop in wounds were it not for the presence of aërobic bacteria introduced with them. Blood infections are usually due to one form of bacteria only. Even when several varieties are introduced only one as a rule survives and multiplies. It has been stated that the presence of one organism may increase the virulence of another; for example, the scarlet fever virus is said to favor the development of streptococci. It is rather more probable that the factor favoring the growth of the latter organisms is the reduced resistance of the tissues due to the poison produced by the former. On the other hand, the products of certain bacteria may rid the body of certain other forms. Pasteur was able with attenuated chicken cholera cultures to produce immunity against anthrax. The ingestion of soured milk with its enormous numbers of lactic acid bacteria is advocated in order that a harmless variety may crowd out in the intestines more dangerous organisms.

Pathogenic Effects Produced by Bacteria. — As already stated, bacteria may be roughly divided into two classes: *saprophytes* and *parasites*. No strict dividing line can be drawn since many species may enter one or the other class according to conditions. A similar statement applies to the terms *pathogenic* and *non-pathogenic*. No known organism will under all circumstances produce disease in all animals; conversely, ordinary saprophytes may develop both parasitic and pathogenic powers when the body resistance is sufficiently reduced by fatigue, exposure, or another infection. Again an organism that is highly pathogenic for one animal may be quite harmless for another. The terms then are relative. A microörganism is pathogenic only when the defenses of the body are not strong enough to resist it.

Bacteria may be termed pathogenic when they produce one or more of the following conditions: (1) mechanical injury to the tissues; (2) disintegration of tissues to furnish themselves with food, or (3) irritation and destruction of tissues by poisons.

Bacteria possessing great vitality may, as already stated, pass

into the deeper tissues and through the lymphatics gain access to the blood stream, thus giving rise to *bacteremia* or *septicemia*. When organisms are present there in great numbers or are bunched together or mixed with fibrin to cause thrombi and later emboli, they may by thus blocking the circulation cause serious mechanical injury. Emboli stationed in the capillaries of feebly resistant tissues usually result in local lesions; multiple abscesses may thus be formed, producing the condition known as *pyemia*. It will be noted that the term *septicemia* is applied to conditions in which bacteria circulate and multiply within the blood, giving rise to symptoms of general poisoning, without, however, the formation of abscesses. In *pyemia*, on the other hand, abscesses are produced in the internal organs and other parts of the body.

The tissue changes produced by bacteria are either of a degenerative or a recreative nature. In the former resistance is lacking and the tissue is finally disintegrated; in the latter case there is excessive activity on the part of the body cells, secretions are increased, phagocytosis is marked, until finally the invaders are either eliminated or gain the battle.

A local inflammatory reaction presents different characters in different conditions. It may be accompanied by an exudate serous, fibrinous, or purulent in character; it may be localized or show a tendency to spread; it may be followed by suppuration and lead to necroses. In many diseases the reaction is somewhat protracted and there is a tendency to the formation of new tissue. In leprosy, tuberculosis, syphilis, etc., such formation frequently occurs in separate foci so that nodules result.

Changes unassociated with the presence of bacteria may occur in certain organs, due to the action of bacterial poisons circulating in the blood; secreting cells and the walls of blood vessels may thus be permanently injured. Diphtheria poison produces marked degenerative changes both in the spinal cord and in the peripheral nerves. It is possible that some of the lesions of the nervous system occurring in syphilis are due to toxin.

Acute infections ordinarily pass through the following stages. First occurs the *period of incubation*, which begins at the time of

infection and continues until the first symptoms appear; the period is more or less constant for each species of microörganism. According to the theory of Vaughan the bacteria are at this time multiplying enormously and for their food are using the proteins of the body. Thus the soluble proteins of the blood and lymph are being converted into bacterial cells. The process is entirely one of construction; no bacterial poisons are being eliminated to disturb the body and consequently no symptoms appear. The period of incubation is nevertheless critical since a strongly virulent organism will develop with great rapidity and consequently consume a large amount of protein.

The second state is marked by the appearance of symptoms. The body cells have become aware of the presence of the invaders and are now pouring out ferments they have generated, which kill many of the bacteria and decompose their protoplasm into simpler substances, some of which are poisonous and produce the general symptoms of intoxication.

Then follows a period of high fever and the disease is at its height. Special symptoms appear according to the organs involved. The struggle between the invasive forces of the parasite and the defensive forces of the body nears a climax; supremacy seems to be first with one side, then with the other. Finally the battle ends, and if the body cells have proved victorious the symptoms begin to disappear and a period of convalescence commences, during which the body gradually overcomes the effects of the bacterial invasion and returns to a state of health. The latter period is decidedly critical; undue exertion or errors in diet may further weaken the already exhausted tissues, thus lowering their resistance and leading to a relapse.

Degrees of Infection. — When an infection occurs with a particularly virulent microörganism there may be so much poison liberated that the cells of the body are paralyzed and completely overcome. In such a case instead of a high fever being produced the temperature drops to subnormal, and rapid prostration and death of the patient quickly ensue. Such an infection is termed a *malignant infection.*

The type of infection already described, with its period of incubation, symptoms, high fever, decline, and convalescence, is spoken of as an *acute infection.*

A third type characterized by a slow development and mild symptoms and terminating after months or years either in death or recovery is spoken of as a *chronic infection.* In these infections it may be that the parasites develop and produce their toxins very slowly or that they are only partially absorbed by the tissues. In this way the body cells are only stimulated to produce sufficient protective substances for their immediate need. Gradually, however, the body cells may become exhausted and unless aroused by some such means as the administration of a vaccine to produce an oversupply of antibodies they may be slowly but surely overcome.

The Spread of Infection. — The amount of infectious material and the path by which it is discharged play perhaps the largest rôle in the dissemination of disease. In diphtheria, typhoid fever, cholera, influenza, gonorrhea, and pulmonary tuberculosis enormous numbers of virulent bacteria leave the body in the discharges from the mouth, nose, intestines, or genito-urinary tract. Conversely, in such diseases as streptococcic meningitis, gonorrheal rheumatism, and tuberculous peritonitis there is little danger of infecting others because few or no living bacteria are discharged from the body.

The species of animals that may be infected has a slight influence in the spread of disease. Anthrax, glanders, tuberculosis, hydrophobia, and some other diseases appear both in man and animals; certain other infections such as gonorrhea, syphilis, measles, typhoid fever, etc., never, so far as is known, occur in animals, and consequently are not transmitted by them.

Another important factor is the resistance of the specific organism to conditions outside of the body. Spore-bearing bacteria, such as anthrax and tetanus bacilli, will retain their pathogenic powers for years. Certain non-spore-forming organisms, such as those causing influenza, gonorrhea, and syphilis, are extremely sensitive; pneumococci and cholera spirilla are a little more resist-

ant, and a little hardier still are the typhoid, diphtheria, and tubercle bacilli and the staphylococci.

The " carrier " is perhaps one of the most potent factors in the spread of disease and one of the most difficult to control since he can only be detected by laboratory examination, and even when detected cannot always be isolated. Fortunately in many cases quarantine is not necessary. Specific treatment and cleanliness may speedily cure the condition or render him less dangerous.

Koch's Postulates. — According to Koch an organism can be considered the causal agent of a given disease only after it has fulfilled certain requirements: (1) it must always be associated with the disease; (2) be isolated in pure culture; (3) produce the disease when inoculated into a healthy animal, and (4) be obtained again in pure culture. For a long time these conditions were accepted as the only proof of such a causal relationship. Recent studies in immunology and the demonstration of specific serum reactions has, however, rendered such a procedure for the most part unnecessary.

CHAPTER X

BACTERIOLOGICAL EXAMINATIONS

THE general principles to be observed in obtaining material for bacterial examination are (1) extreme care that the material is not contaminated with organisms from other sources; (2) that the bacteria in the specimen are not injured by a disinfectant, heat, etc.; (3) that the material is examined with the least possible delay.

Whenever possible it is advisable to make films or inoculate media directly from the patient's body. If this cannot be done the material should be placed in a sterile container and sent as quickly as possible to the laboratory. On no account should a disinfectant be added. If a short delay is unavoidable the specimen should be kept in the refrigerator in order that development of the organisms may be arrested and the stronger species may not thrive at the expense of the weaker. The immediate examination of material containing a great number and variety of organisms cannot be over-emphasized. If, for example, a specimen of feces is to be examined for typhoid bacilli a delay of twenty-four hours may result in their entire disappearance if they were present in small numbers.

Material from abscesses, open wounds, and mucous membranes can best be obtained by means of a sterile swab; fluid exudates may be taken with a sterile syringe or capillary pipette. To make the latter, a piece of glass tubing about ten inches long is heated in the center in a Bunsen flame until it begins to soften, then withdrawn and quickly stretched until the center has the required diameter. The tube is cut with a file so as to obtain two tubes the needed length. A little plug of cotton is placed within the large ends and the pipettes are sterilized in a plugged test tube.

Examination of Pus. — Films should first be made and stained with methylene blue, with carbol fuchsin, and by Gram's method. Whenever the latter method is employed it is advisable to smear on the end of the same slide a small amount of a known Gram positive culture (staphylococcus) and of a known Gram negative one (B. coli) as a control. Occasionally this preliminary examination reveals all it is necessary to know; if not, a loopful of pus is inoculated into appropriate media and streaks are made on agar and blood or serum agar plates. In most cases it is well to make anaërobic cultures also. When colonies have developed on the plates, media is inoculated and a film made from each variety present; further methods of identification of species have already been described.

When the only information required is the knowledge of the presence or absence of a definite species, then only the special methods for the detection of those organisms need be employed.

Nose and Throat Cultures. — The exudate is obtained by means of a sterile swab, which is immediately replaced within its sterile container if the examination cannot be made at once. If the examination is to be made for the diphtheria bacillus the swab is lightly smeared over a sterile slide and then over the surface of Loeffler's serum media. The smear when stained with Loeffler's methylene blue may reveal the presence of B. diphtheriæ; if not, the serum culture should be incubated from twelve to eighteen hours. A film prepared from the resulting growth will reveal the organisms if they are present.

For septic sore throat the procedure is the same save, in addition, plates of blood-smeared agar should be streaked. Long chains of streptococci will, however, generally be seen in the original film.

For the detection of tubercle bacilli the procedure is the same as for sputum.

In the case of Vincent's angina the typical fusiform bacilli and spirochetes will be seen in the smear. A strong stain such as gentian violet gives the best picture.

Sputum. — Patients should be instructed to rinse the mouth well, so that particles of food may not be mixed with the sputum.

Since the early morning sputum coughed up from the lungs is most likely to contain the tubercle bacilli, that should be collected if possible in a wide-mouthed receptacle. In making the examination one of the yellowish white cheesy masses is taken on a sterile platinum loop and smeared on a glass slide. The film is fixed by heat in the usual manner and stained with Ziehl-Neelsen's carbol fuchsin.

If the bacilli in the sputum are not sufficiently numerous to be detected by the above method they may sometimes be found if a concentration be effected. Antiformin, a patented preparation consisting of sodium hydroxide and sodium hypochlorite, is mixed with the sputum in the proportion of about one sixth antiformin to five sixths sputum; the mixture is thinned with a little sterile water and centrifuged. The clear upper fluid is removed, more water added to the sediment, and recentrifuged. The upper fluid is again removed, and the sediment smeared on to slides and stained. The antiformin dissolves the sputum and kills most of the bacteria except the tubercle bacilli.

If negative results are obtained by both of the above methods the search for the tubercle bacilli may be continued by means of animal inoculation. About 2 c.c. of sputum thinned with a little salt solution is injected subcutaneously, preferably in the thigh, into a guinea pig. At the end of four to six weeks the animal will probably die; if not it is best to kill it by allowing it to inhale ether or chloroform. The animal is autopsied and the peritoneal nodes, the spleen, and the inguinal nodes at the site of inoculation removed. The tissue is cut in small pieces with a sterile knife and forceps. Films are made and portions of the tissue are gently rubbed over the surface of special media. In this way pure cultures may be obtained from material containing very few tubercle bacilli although other organisms may be abundant. Growth on artificial media is comparatively slow; it may not appear on the inoculated tubes before the end of seven or eight days.

Urine. — Specimens of urine for bacteriological examination should be taken by means of a sterile catheter and passed into a sterile container. The urine is centrifuged, the upper fluid poured

off, more urine added to the sediment, and recentrifuged. All the fluid is then removed and films and streak plates made from the sediment. If necessary an animal is inoculated.

Feces. — A great variety of bacteria are present in feces. Very few, however, are considered of pathological significance. The different forms may be isolated by inoculating a tube of broth with a small amount of feces and streaking pour plates with the resulting emulsion.

Bacterial examination of feces is generally made for the isolation of the typhoid bacillus, less often for the cholera spirillum. The methods employed will be considered in the special sections.

Examinations are occasionally made for the detection of tubercle bacilli in feces. It is considered of doubtful significance, however, since they may be present in the feces of persons suffering from pulmonary tuberculosis as a result of swallowing sputum.

Ear Cultures. — From the discharge a culture is made on Loeffler's serum and also two film preparations; one of the latter is stained by Gram's method, the other by a method appropriate for the detection of any special species thought to be present. The culture is examined after eighteen to twenty-four hours' incubation and if necessary the resulting growth is plated on agar to isolate the organisms in pure culture and prove their identity.

Eye Cultures. — The procedure is the same as above except that in addition to culturing on Loeffler's serum cultures should also be made on blood agar.

Blood Cultures. — In order to detect organisms occurring in the blood in relatively small numbers, such as the typhoid bacillus, it is often necessary to make blood cultures. The skin should be thoroughly cleansed as for a surgical operation and 10 to 15 c.c. of blood withdrawn from the median basilic vein of the arm by means of a sterile hypodermic needle and syringe. Whenever possible it is best to inoculate the culture media at the bedside while the blood is liquid. If this is not convenient coagulation may be prevented by drawing into the syringe before the needle is inserted into the vein an equal quantity of 2 per cent sodium citrate or by immediately transferring the blood to a test tube containing

the sodium citrate. Five c.c. of blood is added to a flask containing 100 c.c. of glucose meat infusion broth, 1 c.c. to each of two or three test tubes of broth, and 1 c.c. to two or three tubes of glucose agar, cooled to 40° C. The latter are thoroughly mixed by a rotary motion to avoid the formation of air bubbles, after which the necks of the tubes are flamed and the contents carefully poured into Petri dishes. This procedure not only makes possible the isolation of the organisms, it also enables a rough estimate to be made of the number present. From the broth tubes other tests may be made if the organism is obtained in pure culture.

Solid Organs. — When solid organs are to be examined about one inch of the surface is seared with a hot spatula or scalpel in order to kill all extraneous organisms. An incision is made in the seared area with a sterile scalpel and small quantities of the fluid within the organ removed with a platinum loop and transferred to suitable media.

Examination of Bacteria in Tissues. — In order to examine bacteria in body tissues the latter must be fixed, *hardened*, and *cut* in extremely thin section.

Fixation consists in treating the tissue in such a manner that it will preserve as far as possible its condition at the time of removal from the body; hardening renders it firm enough to be cut in very thin sections with a microtome.

The best results are obtained by embedding in solid paraffin. Impregnation with paraffin in the melted state gives, when solidified, support to the tissue elements and greatly facilitates the cutting process. After hardening, the tissue is dehydrated and then completely permeated by a solvent of paraffin which will rid the tissue of the dehydrating fluid and at the same time make possible the entrance of the paraffin. The solvents in general use are chloroform, cedar oil, xylol, and turpentine.

Several methods of more or less value have been devised. The following is simple and gives good results.

Fixation. — As soon as possible after removal from the body small pieces of tissue about $\frac{1}{4}$ inch by $\frac{1}{8}$ inch are cut and placed in the fixative prepared as follows: To a heated 0.75 per cent

solution of sodium chloride add sufficient bichloride of mercury to make a saturated solution. Small pieces of tissue are immersed in the solution for about ten hours; for larger pieces twenty-four hours is necessary. They are then tied in a piece of gauze and placed in a stream of running water for from twelve to twenty hours according to their size in order to wash out the excess of bichloride of mercury.

Hardening. — The pieces of tissue are placed for twenty-four hours successively in each of the following strengths of ethyl alcohol; 30 per cent, 60 per cent, 90 per cent, and finally absolute alcohol. They are then ready to be embedded in paraffin. If only a minute particle of tissue is to be examined for diagnosis all the stages may be compressed into twenty-four hours.

Embedding in Paraffin. — The tissue is transferred to (1) cedar oil or xylol until translucent; (2) equal parts of cedar oil and paraffin at 37° C. for two hours; (3) melted paraffin at 52° C. for four hours; (4) each piece of tissue is removed from the hot paraffin by means of forceps and placed in very small tin or paper box, after which it is surrounded with the melted paraffin. The paraffin used should be one that has a melting point about 52° C. When the block is cold the edges are pared and the preparation is ready for sectioning.

Cutting of Paraffin Sections. — A microtome is generally used for this purpose. Each section should be as thin as possible. When cut the sections are floated on the surface of a glass of warm water kept at about 40° C., where in a short time they become perfectly flat.

Fixation on Slides. — A clean slide is thrust obliquely into the water below the section, a corner of the section is held on to it with a needle, and the slide is withdrawn. The surplus water is wiped off with a cloth, the section carefully adjusted by means of a camel's hair brush, and the slide placed on a support with the section side downward in an incubator for from twelve to fifteen hours; it will then be sufficiently fixed for staining. Before staining, however, the paraffin must be removed by dropping on to it a little xylol. When the paraffin is dissolved the xylol is removed

by gently wiping with filter paper, after which a little absolute alcohol is dropped on to the section. If any crystals of bichloride of mercury are noticed they may be removed before staining by immersing the slides for a few minutes in equal parts of Gram's iodine solution and water and then washing out the iodine with alcohol.

CHAPTER XI

BACTERIAL TOXINS AND ANTITOXINS

THE ability of bacteria to produce toxin is their chief weapon of offense in the production of disease. Poisonous products such as acids, alkalies, hydrogen sulphid, etc., result from the growth of many species, and such substances may play a minor rôle in diseased conditions. They are, however, totally different to toxins. Fortunately only a comparatively few of the vast family of micro-organisms are able to produce the latter.

Bacterial toxins are divided into two classes: (1) those intimately connected with the bacterial protoplasm and liberated only after the death of the organism, and (2) those which result from living bacterial activity, are soluble, and pass out from the organism into the surrounding medium. The former are spoken of as *endotoxins*, the latter as *exotoxins*.

Endotoxins. — When the dead bodies of certain bacteria are injected into animals toxic effects are frequently observed. For example, if tubercle bacilli are killed by heat and injected into the tissues of a susceptible animal tubercular nodules will be found to have formed around the point of injection; the dead cells of typhoid bacilli and cholera spirilla likewise give rise to pathogenic effects. So far it has been impossible to obtain these toxins apart from the bacterial protoplasm. Since the death of bacteria must be constantly occurring both in culture medium and in the body during infection it is reasonable to suppose that these dead bacterial cells are disintegrated and the endotoxin thus set free. It is believed that the living members secrete a ferment that has a solvent action upon the dead organisms and that upon this process of *autolysis* the liberation of the poison depends. However this may be, it is definitely known that the body cells secrete a substance which has a strongly lytic action upon bacteria.

The action of the endotoxins on the body tissues differs from that of the exotoxins in that they do not give rise to symptoms of a specific character. There is no definite period before symptoms appear, *nor do they stimulate the body cells to the production of antitoxin.* Certain protective substances are elaborated against them, but they are of a totally different nature to antitoxin.

Aggressins. — In certain cases it is difficult to understand a result of bacterial growth which is due neither to endotoxins nor exotoxins but to some other substance which aids the organisms in combating the body defenses. If, for instance, tuberculous exudate is removed from an animal suffering from the disease, and after sterilization it is injected into a healthy guinea pig, it has practically no effect. If into a second guinea pig tubercle bacilli are injected the usual lesions appear in from four to six weeks. If, however, into a third guinea pig the sterile exudate and a comparatively small quantity of tubercle bacilli are injected death follows within twenty-four hours, indicating that the exudate had a paralyzing effect upon the defensive forces of the animal cells, thus greatly increasing the virulence of the bacilli. That the effect is not produced by endotoxin in the sterilized exudate is proved by the fact that the exudate alone produced no reaction. Similar results are obtained with other organisms such as typhoid and dysentery bacilli, cholera spirilla, etc. It has been assumed therefore that the exudate contains a substance which enables the bacilli to become more aggressive. Consequently this hypothetical substance has received the name *aggressin*. It is believed that it has a paralyzing effect upon the polynuclear leukocytes, which, as we shall see later, constitute one of the body's strongest defenses. In general the production of aggressins is most abundant when the body resistance is greatest. It is thought by certain authorities that aggressins are secreted by bacteria as a means of protection against the opposing forces of the animal cells, just as the body cells produce antitoxin to neutralize bacterial toxin. According to this theory toxins may be considered as the offensive agents of bacteria and aggressins as their defensive weapons.

Exotoxins. — There is evidence that the exotoxins or *true toxins* are of a protein nature, although nothing is known of their chemical structure; nor is it known whether they are secretory or excretory products. They are, however, undoubtedly produced by bacteria during their growth. They are poisonous in minute doses; reproduce characteristic symptoms and lesions of the disease after a period of incubation; are soluble in water; are destroyed by heat, and in the animal body stimulate the cells to produce defensive substances which neutralize them, — so-called *antitoxins*.

The three well-known exogenous toxins are those produced by B. diphtheriæ, B. tetani, and B. botulismus. They can be produced apart from the body by growing the organisms on culture medium; the toxin passes from the bacterial cell and is diffused throughout the substance on which the bacteria are growing. If broth is employed the toxin can be obtained germ free by passing the broth through a porcelain filter. All attempts to separate the toxin from the broth have failed. The only way its presence can be recognized is through its effects on animals. By this means it is possible not only to determine the presence of the poison but also its strength.

As already stated, true toxins are poisonous in exceedingly small amounts. Tetanus toxins have been prepared from cultures of the bacilli so strong that .0002 gram would be a fatal dose for a man weighing 140 pounds.

Another of the most distinguishing features of the true toxins is their ability to produce all the characteristic symptoms that arise when the disease is naturally contracted and the specific organisms are present. Thus after an injection of diphtheria toxin an animal will show depression, necrosis of the tissue at the site of inoculation, post-diphtheria paralysis, etc.

Small amounts of diphtheria or tetanus toxin prove fatal when circulating in the blood stream; larger quantities, however, when taken by mouth are not injurious because they are immediately destroyed by the digestive juices. On the other hand, the toxin of botulism is absorbed by the mucous membranes of the intestines and by this route gains entrance to the circulation. A strange

fact in connection with the latter toxin is that it is produced outside of the body and not within it. B. botulismus will grow and produce its poison on almost any form of protein. Sausages are probably the most frequent source of botulism. When the food enters the intestines, the poison which is intimately mixed with it is absorbed and the characteristic disease is produced. As in the case of diphtheria and tetanus a specific antitoxin is formed by the body cells seeking to protect themselves from this particular poison.

The difference between botulism and ptomain poisoning should be clearly understood; botulism results from eating food containing a true toxin elaborated by the Bacillus botulismus. Ptomain poisoning, on the other hand, results from the ingestion of food that has been decomposed perhaps by several species of bacteria into simple but poisonous compounds. In the former case the poison is produced by bacteria; in the latter the poison is the disintegrated meat, fish, cheese, or whatever the protein may be. Another important difference between the true toxins and ptomains is that the latter do not stimulate the body cells to the production of antitoxin.

Phytotoxins. — Substances resembling in action the bacterial toxins occur in the seeds of some of the higher plants; among them are ricin from the castor oil bean and abrin from the jequirty bean. These toxins of vegetable origin are, like the bacterial toxins, exceedingly poisonous in small amounts, act only after a period of incubation, are destroyed by heat, and produce specific antitoxin.

Zoötoxins. — Closely corresponding poisons are found in the blood and secretions of a number of animals. Snake venom, the poisons of scorpions and spiders, as well as poisonous substances present in eel blood, are well-known examples. They, too, like the true bacterial toxins cause the production of antitoxins when injected into the body of another animal.

Diphtheria Toxin. — Diphtheria bacilli usually find lodgment upon some portion of the upper respiratory tract, either the tonsils, the larynx, or not infrequently on the nasal mucous membrane.

Occasionally they find conditions suitable for growth on an abrasion of the skin, in the vulva, or on the conjunctiva. If they are able to develop they secrete during their growth the toxin which destroys the epithelial cells, and from this area of infection the poison is absorbed and carried to all parts of the body. Other microorganisms such as pneumococci and streptococci, which may be harmless on intact healthy membranes, readily grow in the necrotic tissue and may add to the severity of the local lesion and the general toxic condition.

Diphtheria bacilli rarely enter the blood stream; they usually remain localized, and only their toxin affects the susceptible body tissues. The outcome of the infection depends largely upon the amount and the strength of the toxin absorbed and the quantity of antitoxin present in the blood. The amount of tissue involved in the nose or throat is not always an indication of the severity of the disease; virulent bacilli in a small patch may produce more toxin than less virulent ones occupying a larger area. As a rule, the patient who has most antitoxin, whether naturally present or gained as a result of a previous attack of the disease or an injection of antitoxin, will be least affected even though the bacilli be extremely virulent.

Twenty years ago diphtheria was one of the most dreaded diseases; it had a mortality of at least 30 per cent. About 1888 Roux and Yersin discovered that it was produced by a soluble poison; two years later von Behring demonstrated that circulating in the blood of all animals recovering from an attack of the disease there was a certain substance, *antitoxin*, which rendered the poison harmless. It seemed evident that this substance must be elaborated by the body cells to save themselves from being attacked by the toxin. The idea was then conceived that if a serum rich in antitoxin could be injected into a person suffering from diphtheria it would afford protection by neutralizing the toxin until the cells of the patient could respond sufficiently to produce their own antitoxin in large enough amounts. At first the results were not as satisfactory as expected because the serum was not powerful enough. By 1896, however, it had been sufficiently perfected to

cause a marked decrease in the mortality from diphtheria in those communities in which it had been used. Since then thousands of lives have been saved by its means. Records show that when antitoxin is used on the first day of the disease practically no mortality occurs.

Production of Antitoxin for Therapeutic Purposes. — Toxin is first prepared by cultivating a virulent strain of the bacilli in a suitable broth medium for ten days or two weeks. The culture is then passed through a porcelain filter which retains the bacilli and allows the strong toxin solution to pass through. The potency of this toxin is estimated by injecting carefully measured doses into a series of guinea pigs. Usually about $\frac{1}{200}$ c.c. of a moderately strong toxin is fatal. The smallest amount of toxin that will kill in four days a guinea pig weighing 250 grams is spoken of as the toxin unit or minimum lethal dose. This preliminary titration serves to indicate how much toxin can with safety be injected into a horse of much heavier body weight.

Horses are chosen for the production of commercial antitoxin partly because large quantities of serum can be obtained from each animal and partly because the serum is normally of a bland nature. Strong healthy horses which have been proved to be free from tuberculosis and glanders are injected with gradually increasing doses of the toxin; the first doses are usually guarded by an injection of antitoxin given at the same time. As soon as the reaction passes and the temperature becomes normal a slightly larger dose of toxin is given, until by the end of about eight weeks the horse can tolerate many times the amount received on the first day. Injections are continued and weekly tests are made of the serum until it is found that the amount of antitoxin no longer increases. At the end of from four to six months the serum may contain from 700 to 1000 units of antitoxin per cubic centimeter. When no further increase appears the horse is bled aseptically from the jugular vein and the blood stored in the refrigerator for two days in order that the serum may separate from the clot. At the end of this period the serum is siphoned off, and after the addition of a small amount of an antiseptic as a preservative it is ready for use. A

method of concentrating such a serum has been devised which not only reduces its bulk but is said to lessen the probability of serum sickness.

After a short period of rest a horse that has been used for the production of antitoxin may be used in the same manner for a further supply. Given three months rest each year a horse is said to furnish a high-grade serum for three or four years.

Standardization of Antitoxin. — At first antitoxin serum was administered in so many cubic centimeter doses, but since some horses produce a much more powerful serum than others the results were altogether irregular. The adoption of a standard unit, therefore, was deemed necessary in order to obtain a certain degree of accuracy in dosage. As a result the standard unit of antitoxin was fixed as *the smallest amount that will just neutralize one hundred times the amount of toxin that will kill a guinea pig weighing 250 grams in four days.*

In order to standardize an antitoxin it is necessary to have a standard toxin against which to test it. Since toxin quickly deteriorates a standard antitoxin is supplied in small quantities by the Hygienic Laboratory at Washington to the various manufacturers for this purpose. A series of guinea pigs each weighing about 250 grams are inoculated with 1 c.c. of the standard antitoxin plus varying amounts of toxin. In this way the L_+ (limes death) dose, which is the amount of toxin plus 1 c.c. of antitoxin required to kill a guinea pig in four days, is obtained. Having determined the dose of toxin that just neutralizes the standard antitoxin this constant dose of toxin plus increasing amounts of the new antitoxin is injected into another series of 250 gram guinea pigs. At the end of four days it will be found that those receiving the smallest amounts of antitoxin have died and that the larger amounts have protected the animals. The strength of the antitoxin is estimated from the smallest protective amount. Thus if a guinea pig receiving .003 c.c. of serum had died and the next of the series receiving .004 c.c. had died also, but the third which had received .005 c.c. and likewise all the others receiving larger doses had been protected, then the serum would be said to contain 200 units of antitoxin

per c.c. One two-hundredth of a c.c. or .005 c.c. neutralized the standardized toxin; therefore each cubic centimeter of the serum contained 200 units of antitoxin.

Antitoxins are fairly stable bodies. When kept in a cool, dark place they may be preserved for a year or more with very little deterioration. It is customary for manufacturers to place a label on each package bearing a date beyond which the serum is not guaranteed to contain the original amount of antitoxin.

The most important point in the administration of antitoxin is that it be given as soon as possible after infection has occurred; 2000 units on the first day that symptoms appear is considered more efficacious than 5000 units on the second day. A total of 10,000 or even 20,000 units in severe cases is sometimes given.

Prophylactic Immunization against Diphtheria. — The subcutaneous injection of antitoxin will afford protection for a limited period to susceptible persons exposed to infection. The doses are relatively small and the injection does not usually produce any ill effects save a little soreness at the site of inoculation. Unfortunately antitoxin injected into the body is eliminated rather rapidly, so that protection thus gained lasts only from two to four weeks. As a prophylactic measure children under one year are usually given 500 units, older children and adults 1000 to 1500 units.

Seeing that antitoxin produced by the body cells in direct response to the presence of toxin remains for a much longer period in the body than that injected from another animal, a method has been advocated whereby the body cells may be stimulated to produce their own antitoxin in sufficient amounts to protect themselves in case of exposure to diphtheria. A mixture of toxin and antitoxin known as T-A is injected in graded doses subcutaneously. The method is based upon the principle that the union of toxin and antitoxin is not stable, and when a neutral mixture of the two is injected sufficient toxin may be dissociated to stimulate the body cells to produce their own antitoxin.

Schick Test. — In order to determine whether a prophylactic dose of antitoxin is necessary in case of exposure to diphtheria Schick has devised a simple skin test for detecting the presence

of natural antitoxin in the blood. A minute amount of toxin (about one fifth of the minimum lethal dose for a guinea pig) is injected intradermically. If the person receiving the toxin possess an amount of antitoxin equal to at least one thirtieth of a unit in each cubic centimeter of blood the injected toxin is neutralized and no reaction appears; if, on the other hand, he has no antitoxin, the toxin acts as an irritant to the skin and in from twenty to forty-eight hours produces a small inflamed area. This positive reaction indicates that the person has no natural antitoxin and therefore that he is susceptible to the disease; conversely, a negative reaction indicates that an individual has in all probability sufficient natural antitoxin to protect him, even in case of exposure, and a prophylactic dose is unnecessary.

Tetanus Toxin. — Tetanus, like diphtheria, is a local infection characterized by a general toxemia. The bacilli and spores never gain access to the blood; they remain at site of infection where they produce their toxin, which when absorbed is responsible for the disease. The blood, then, usually contains tetanus toxin, but is sterile so far as the organisms are concerned. Two different poisonous substances have been shown to exist in tetanus toxin: (1) tetanospasmin, which has a special affinity for nerve tissue and to the action of which the characteristic symptoms are due, and (2) tetanolysin, a substance of probably much less importance which attacks the red blood corpuscles, ruptures the envelope, and liberates the contents, giving rise to a more or less anemic condition.

One of the greatest dangers of tetanus lies in the fact that while the local lesion may show no sign of disturbance the central nervous system may suddenly develop symptoms of poisoning. The toxin is produced during or soon after the first twenty-four hours and from the point of infection it passes rapidly into the blood and lymph stream and according to certain authorities is absorbed by the end plates of the motor nerves and quickly travels along the axis cylinders to the spinal cord. The more general opinion, however, is that the toxin passes by way of the lymphatics of the nerves and not by way of the axis cylinders. So great is the affinity of tetanus toxin for nerve tissue that once

united with the nerve cells it is difficult or impossible to effect its neutralization; hence the greatest value of tetanus antitoxin lies in its administration as a prophylactic. As a prophylactic remedy it is even of more value than diphtheria antitoxin; therapeutically, it is less beneficial, since the tetanus toxin combines so rapidly and firmly with the nerve cells. Recently, however, a method has been employed which has met with considerable success. A moderate amount of spinal fluid is removed by a spinal puncture and from 3000 to 5000 units of tetanus antitoxin in a volume of from 3 to 10 c.c. of normal salt solution is injected slowly by gravity; at the same time 10,000 units are given intravenously or intramuscularly.[1] As a prophylactic measure from 1000 to 1500 units are injected intramuscularly.

It is generally agreed that since tetanus is almost invariably associated with a wound, proper treatment of the original lesion combined with the immediate administration of antitoxin will surely prevent its development.

Production of Tetanus Antitoxin. — As in the case of diphtheria a strong toxin is first prepared. Suitable broth is inoculated with tetanus bacilli and the organisms are grown anaërobically for two weeks at 37° C. At the end of this period the toxin broth is separated from the bacilli by filtration and its strength is determined by the same method as that employed for diphtheria toxin, except in this case heavier guinea pigs are used. Those weighing about 350 grams are chosen. The immunization of horses and procuring of serum are also conducted in much the same way as for the production of diphtheria antitoxin.

For the standardization of the new antitoxin a standard toxin with which to test it is obtained from the Hygiene Laboratory at Washington. The unit employed is not quite the same as that adopted for diphtheria. A unit of diphtheria antitoxin is defined as *the amount of antitoxin that will just neutralize 100 minimum fatal doses of toxin for a 250 gram guinea pig.* A unit of tetanus antitoxin is *defined as the amount of antitoxin that will just neutralize 1000 fatal doses of toxin for a 350 gram guinea pig.*

[1] Park and Nichol, Jour. of the A. M. A., Vol. 63, July, 1917, p. 325.

CHAPTER XII

IMMUNITY

For many years it has been observed that one attack of most of the infectious diseases will protect an individual against subsequent attack, or at least future attacks will be of a less severe nature. Crude attempts were frequently made by primitive people to obtain this protection. Thus South African tribes tried to defend themselves against snake bites by using a mixture of snake venom and gum; in the East in order to obtain protection against a severe attack of smallpox people deliberately placed themselves in contact with a mild case, hoping to contract the disease in a similar form and thus obtain protection against a disfiguring and probably fatal form.

The object of these procedures was to obtain *resistance or immunity*. The term *immunity* in its broadest sense may be applied to resistance in general. Ordinarily, however, it is applied to the power of resisting disease which certain forms of life possess. Health is a condition of immunity. So long as we are alive and our body cells can continue to manufacture their specific protective substances the bacteria on our skin and in our intestinal tracts can do no harm, but the moment we die they penetrate our tissues and disintegrate them.

Immunity is of course the contrary condition to susceptibility. It is not confined to the animal kingdom alone; it occurs also among plants. The theory of Welch attributes its possession even to bacteria; thus man is susceptible to the typhoid bacillus because the typhoid bacillus is immune to man; conversely man is immune to the hay bacillus because the hay bacillus is susceptible to man.

Pasteur tried to explain the production of immunity by his "exhaustion theory," a theory now entirely disproved. He be-

lieved that as bacteria developed in the tissues they used up some substances necessary to their existence, and that when this substance was exhausted they could no longer grow for lack of proper food. Chauveau took an exactly opposite view and proposed his "retention theory." He suggested that as in a test tube bacterial growth may cease because the organisms have produced substances harmful to themselves and not because the food supply is exhausted, so in the body these substances might be formed and retained there and prevent the future development of the organism. Both of these theories are now only of historic interest. It has been proved that the production of immunity is a much more complicated problem, resembling rather a battle between an invading army and defensive forces, during which both parties are extremely active.

When bacteria have succeeded in overcoming the normal defenses of the body and have invaded the tissues the body cells are by no means overcome; the battle is really only just about to begin. The presence of the invaders stimulates the body cells to defend themselves by actively producing substances termed antibodies, by means of which they endeavor to rid themselves of the invading force or neutralize their products.

Antigens and Antibodies. — Whatever offensive weapon the parasite may produce, the body cells manufacture a substance especially prepared to combat it. If the toxin of a microörganism is its chief means of inflicting injury, such for instance as the soluble toxin of the diphtheria bacillus, the body cells produce an antitoxin to neutralize it; if it is necessary to destroy the bacteria themselves then a substance, bacteriolysin, is poured out on the invaders, which dissolves them. In certain infections, and particularly those due to pyogenic cocci, the polynuclear leukocytes and certain other cells engulf the organisms and carry them off bodily. Another antibody, opsonin, appears to aid the leukocytes in their work.

The term *antigen* is generally applied to all substances that cause the formation and appearance of antibodies in the body fluids. This power is not confined to bacteria and their toxins,

but is shared, as we have seen, by certain substances in plants (phytotoxins) and poisonous secretions of certain animals (zoötoxins). Red blood corpuscles, serum of different animals, egg albumen, milk, etc., when inoculated into the tissues, are treated as foreign substances and are disposed of as quickly as possible by antibodies specially manufactured by the body cells for that purpose.

The term *antibody* is used to designate the entire group of specific substances produced by the body cells in reaction against the various antigens; certain antibodies act by neutralizing their antigen (antitoxin), others by agglutination or precipitation (agglutinins or precipitins); others act by completely dissolving their antigen (bacteriolysins, hemolysins); still others may so lower the resistance of their antigen as to render it an easy prey to the phagocytes (opsonins or bacteriotropins).

Mechanism of Immunity. — Of the many theories advanced regarding the mechanism of immunity two have claimed more attention than all the others; one, the "humoral theory," advanced by Ehrlich, attributes the protective forces of the body to the body fluids; the other, the "cellular theory," proposed by Metchnikoff, claims the honor for certain body cells. From recent studies, however, it is evident that in different infections the body employs different means of protecting itself. In certain diseases the production of immunity seems to depend upon the activity of the cells, in other diseases substances contained in the body fluids seem to possess the property.

Ehrlich's Side Chain Theory. — The "humoral theory" of immunity, which considers that the power of resistance to infection resides in the body fluids, is said to have originated when Foder discovered that the blood of a rabbit when placed in a test tube will kill anthrax bacilli without the apparent aid of the body cells. Later, in 1890, additional weight was given to the theory when von Behring and Kitasato demonstrated the presence of antitoxin in the blood. At first great importance was attached to the antitoxins, until it was discovered that they are produced only in a few diseases, notably diphtheria, tetanus, and botulism.

In 1894 Pfeiffer discovered that cholera spirilla introduced into the peritoneal cavity of a guinea pig highly immunized against them lost their motility almost immediately, gradually became swollen and granular, and finally passed into complete solution. These changes are generally spoken of as "Pfeiffer's phenomenon," or bacteriolysis.

As a result of these and many other observations Ehrlich offered an explanation based on a theory advanced some years before to account for the process of cell nutrition. He thought of the cell as possessing two functions: one physiological, such as that of a gland cell to secrete, and the second function one of nutrition concerned mainly in nourishing and repairing the cell. Moreover he attributed this double function to each molecule composing the complex cell. That portion of the molecule by means of which it secures nourishment is of greatest importance in relation to immunity. Ehrlich pictured this molecule of protoplasm as a functional center with a large number of side chains or receptions, each of these side chains possessing the ability to select the food atom it needs from the surrounding blood and lymph, to combine with it, and by a kind of absorptive process to incorporate it in the molecule. The food molecules are conceived as possessing a portion specially adapted for union with the side arm of the cell, so that when the two are brought in relation they fit together in much the same way that a key fits a lock. As food molecules vary in their chemical composition it is reasonable to assume that the cell receptors prepared to anchor them differ: they may be of a simple constitution and adapted only to the taking up of a very simple substance or they may be more complex and able to digest a larger food molecule.

It is easy, then, to conceive that when bacteria and other foreign substances are introduced into the body fluids certain receptors may anchor them as they pass in just the same manner as a similarly constituted food molecule. Combination with such substances, however, leads to a different result; instead of being nourished the cell may be poisoned and in all probability lose the receptor that had attached itself to the foreign substance. If,

for example, that substance is a toxin and it is sufficiently abundant and powerful, many receptors may be thus lost and many cells damaged. Symptoms of the specific disease may present themselves and death may ensue. On the other hand, if the injured cells possess sufficient vitality the loss of one or more receptors will stimulate them to an immediate attempt to repair the damage. Since nature is always lavish in her processes of repair it may happen that not only are the lost receptors replaced but a large number are added. These excess receptors having no place for attachment to the cell are thrown off into the blood stream. All possess the same structure as the original ones. Consequently when they meet in the blood stream with the same kind of substance which caused their production, their antigen, they are capable of combining with it and rendering it harmless before it is able to attack the body cells. In diphtheria and tetanus the antigen is the toxin of the bacteria, and the cast-off receptors produced as a result of its action constitute antitoxin.

It should be noted that an excess production of receptors or antibodies occurs only as a result of the chemical union of a receptor with a poisonous substance; the assimilation of food material is of benefit to the cell; consequently the receptors remain unharmed.

Three Orders of Antibodies. First Order (Antitoxins). — The simplest receptor of the cell molecule is conceived as possessing a single arm or haptophore for union with a correspondingly simple food molecule. A toxin molecule is imagined as possessing two portions, one the haptophore especially adapted to fit this simple receptor, and a second, the toxophore portion in which its toxic power resides. The cast-off receptors of this order constitute *antitoxin*. Fig. 21.

Second Order (Agglutinins, Precipitins). — All diseases are not produced by soluble toxins, and furthermore the production of antitoxin does not explain such phenomena as the agglutination of bacteria and the dissolving property of certain sera. Accordingly Ehrlich modified his theory and assumed that while simple molecules of food might be readily assimilated by the simple cell receptor, other more complex molecules might require some form

of preparation to render them assimilable. He conceived the possibility then of a second order of receptors furnished like the first with a haptophore portion for anchoring the food molecule, and also with an additional portion which he called the zymophore group, the special function of which was to prepare the food molecule for absorption.

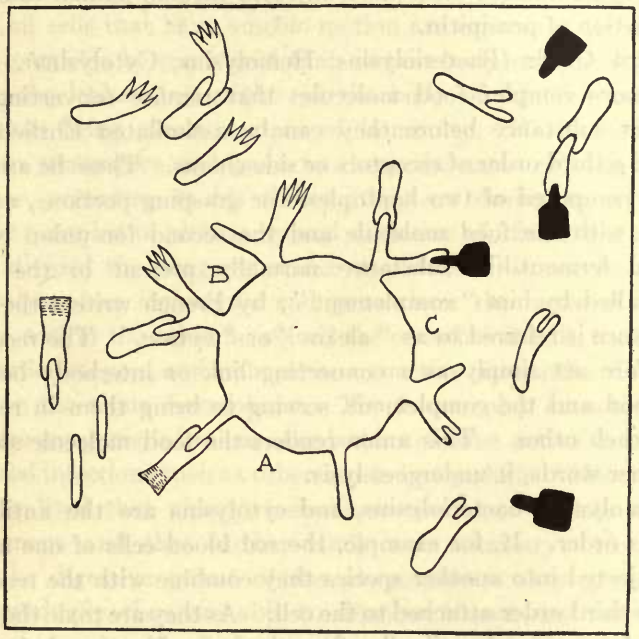


FIG. 21. — Diagram illustrating: *A*, Receptors of First Order; *B*, Receptors of Second Order; *C*, Receptors of Third Order.

Similarly, certain pathogenic substances which are more complex than the toxins he assumed would combine with receptors of this kind, the haptophore portion of the pathogenic molecule combining with the haptophore portion of the receptor and acted upon by its zymophore portion.

Two such antibodies are known; in one the zymophore portion of the antibody causes clumping or agglutination of its antigen and is consequently known as *agglutinin*. In typhoid fever the

bacilli or their products cause the production of an antibody of this nature, so that when the serum of a typhoid patient is mixed with its antigen, typhoid bacilli, the latter lose their motility and become clumped together in masses. The other antibody of this class appears to coagulate and precipitate soluble substances and accordingly is known as *precipitin*. Such protein substances as milk, egg albumen, etc., will if injected into an animal cause the production of precipitin.

Third Order (Bacteriolysins, Hemolysins, Cytolysins). — For still more complex food molecules that require converting into simpler substance before they can be assimilated Ehrlich conceived a third order of receptors or side chains. These he assumed to be composed of two haptophore or grasping portions, one for union with the food molecule and the second for union with a special ferment-like substance normally present in the blood and called by him " complement "; by French writers the same substance is referred to as " alexin " or " cytase." The receptors therefore act simply as a connecting link or interbody between the food and the complement, serving to bring them in relation with each other. This union renders the food molecule soluble. In other words, it undergoes lysis.

Hemolysins, bacteriolysins, and cytolysins are the antibodies of this order. If, for example, the red blood cells of one animal are injected into another species they combine with the receptors of the third order attached to the cell. As they are toxic the result is the formation of antibodies (hemolysins). If more of the antigen, which in this case is the foreign corpuscles, be injected, the hemolysins uniting them with the complement in the blood cause them to be completely dissolved. If certain bacteria are injected into an animal immunized against that particular species, consequently in whose blood bacteriolysins are circulating, they share the same fate as the corpuscles in the previous example. Thus the production of bacteriolysins offers a satisfactory explanation of " Pfeiffer's phenomenon." It should be remembered that although these antibodies prepare their antigens for lysis, or " sensitize " them, they are not in themselves lytic, final solution of the

antigen being accomplished by the ferment-like substance, the complement.

Ehrlich's conception of immunity, then, was that of a chemist, and his antibodies the result of a chemical union between the body cells and the foreign protein, bacterial or other.

"**The Cellular Theory.**" — Metchnikoff as a biologist naturally based his theory on his observations of biological phenomena. Since all cells that have amebic motion are capable of enveloping small particles he considered that certain body cells, and particularly the leukocytes, might rid the body of infective material and thus bring about a state of immunity. He likened these cells to scavengers and gave to them the name of phagocytes, because, according to his theory, they are during an infection mainly occupied in picking up and disposing of offensive material.

He divided the phagocytes into two classes; in one he placed the polynuclear leukocytes and gave them the name of *microphages*, and in the other he placed the endothelial cells, the mononuclear leukocytes, and embryonic connective tissue cells. To these he gave the name *macrophages*. The former play an active rôle in acute pyogenic infections, the latter are most active in chronic bacterial infections such as tuberculosis and syphilis. Metchnikoff soon realized that phagocytosis alone could not explain all the phenomena and that further study was necessary. He found that the digestive power of the phagocytes is very great, and that gradually they are able to dissolve almost any substance. He considered two of these substances, one derived from the microphages which he called microcytose, and the other obtained from the macrophages and to which he gave the name of macrocytose, to be identical with Ehrlich's interbody and complement.

The relative importance of the cellular and humoral theories rests largely on which of the body cells are most active in forming antibodies. Metchnikoff showed the important part played by phagocytes in any infection, and claimed that the antibodies in the circulating fluids are the products of their activity. Ehrlich, on the other hand, emphasized the presence of immune bodies in the body fluids and sought to explain the method by which they

are produced without attributing the action to any one group of cells.

Recent experiments tend to show that the leukocytes, the spleen, lymphatic tissue, and bone marrow are all actively concerned in manufacturing antibodies. In infections due to the various pathogenic cocci, phagocytes appear to be the most active agents; in other infections such as those due to typhoid and other bacilli it is probable that antibodies are chiefly operative in overcoming the infection and producing immunity. Thus there appears no warrant for holding one view to the absolute exclusion of the other. It would seem that all phases of immunity are cellular in origin and that this cellular activity is general rather than limited to one group.

Phagocytosis. — In order to understand phagocytosis it is necessary to consider its three phases; namely, the advance of the leukocytes, the engulfment of the particles, and their subsequent digestion. The question naturally arises as to how the leukocytes are made aware that their presence is needed at the point of infection; some attractive force must operate between this point and the leukocytes circulating in the blood. It is assumed that a chemical substance acts as an attractive force. Almost all motile unicellular organisms, whether animal or vegetable, will respond to chemical stimuli. Generally attraction is toward a given point, constituting what is known as *positive chemotaxis.*

Experiments show that such cell movement is due to a change in surface tension. If the chemical substances which cause this apparent stimulus decrease the surface tension the cell advances in the direction from which the substance comes, if it decreases the tension the cell recedes.

"This explanation may account for the behavior of cells in inflammation. At the point of injury or infection chemical substances are produced that tend to lower the surface tension; these are carried by the body fluids to the nearest capillaries where they enter through the vessel wall and come in contact with the leukocytes. Naturally the surface tension will be least on that side of the vessel wall through which the stimuli has passed

and the result will be the forming of pseudopodia on the part of the leukocytes and gradual motion in this direction. Once beyond the vessel wall the leukocyte will travel in the direction from which the chemotactic substance comes. If the leukocyte encounters a substance that further lowers the surface tension it will encircle and inclose it. If by chance it has engulfed bacteria their toxins may kill the cell or so equalize the tension that it ceases to move; otherwise the leukocyte moves forward until it is checked. It may be that movement continues always in the direction from which the chemical stimulus comes until it is blocked by a phalange of leukocytes which are being held by chemotaxis around the area of infection. This supposition would explain the wall formation which so often occurs in suppurative processes. If, on the other hand, recovery commences and the chemotactic substances cease to be formed, then it may be more abundant at some distance away from the center, and towards this point the leukocytes will move, following the attracting substance back to the lymph stream and blood vessels. This probably explains the dispersion of the living phagocytes at the end of an inflammatory process." (Kolmer.)

Nothing is known of the nature of these chemotactic substances. It is thought that they may be formed as a result of the death of tissue cells caused by bacterial poison or such irritants as coal or stove dust. In most infections chemotaxis is positive; in a few, however, and particularly those caused by virulent streptococci, the phagocytes do not appear to be influenced by any such substance. Whether stimuli are ever formed by bacteria that actually repel leukocytes is not as yet decided. If such substances do occur they must closely resemble the aggressins which neutralize opsonins and so retard phagocytosis.

The engulfment of bacteria is accomplished by the phagocyte protruding part of its protoplasm until it encircles the organism, which soon appears within the substance of the phagocyte.

After phagocytosis the fate of the inclosed bacteria depends largely on their nature. Generally they undergo a process of digestion similar to gastric digestion in higher animals; the

phagocytes are able thus to dispose of particles of all kinds, bacteria, cellular débris, the fragments of coal in anthracosis, catgut, and silk ligatures and other foreign substances. It may happen, however, that they can withstand the phagocytic ferments and even cause the death of the cell, in which case they become again liberated in the tissues. The inclosing of the parasites, then, is only a preliminary step. The process may be useless unless suitable digestive ferments are excreted and the bacteria dissolved.

CHAPTER XIII

OPSONINS, AGGLUTININS, PRECIPITINS, LYSIN

Opsonins. — The process of phagocytosis has been found to depend not so much on inherent properties of the phagocytes as upon a certain substance present in the body fluids. Apart from this substance the leukocytes do not become phagocytes. Metchnikoff suggested that the presence of this substance stimulated the leukocytes to become active. Later studies, however, have shown that the substance acts directly upon the bacteria and renders them more attractive to and easily digested by the leukocytes. These bacteriotropic substances have been named *opsonins* (from *opsono*, I prepare food for). Opsonins are present normally in the blood. They may, however, be greatly increased in immunization.

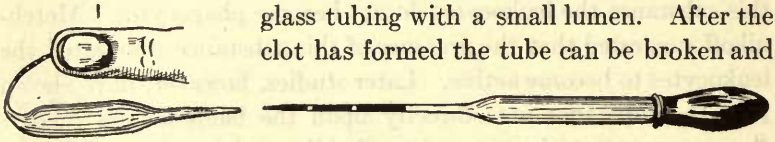
Bacteria differ in their susceptibility to opsonins. Their resistance may be due to capsule formation or, according to the theory of Welch, to actual self-immunization of the bacteria. Opsonins are much more active in some infections than in others; they are especially operative in pyogenic conditions, in which phagocytosis is recognized as the chief defensive force. The relation of normal and immune opsonins to the other antibodies is as yet unsettled; they may be of the same nature as amboceptor and complement or they may be separate antibodies. However that may be, they are an important factor in the production of immunity, since it is upon their action that phagocytosis depends.

The existence of opsonins in a given serum is easily demonstrated by mixing the serum with a suspension of bacteria and adding washed leukocytes; the leukocytes will in all probability take up large numbers of the bacteria. If, however, leukocytes washed

free from serum are added to a suspension of bacteria which have not previously been sensitized by opsonins practically no phagocytosis occurs.

A method has been devised whereby the relative amount of opsonins present in the blood of a sick person and a normal healthy person may be estimated. For the procedure it is necessary to have (1) blood serum from the patient and also from a healthy person, (2) washed leukocytes, and (3) a suspension of the organisms the opsonin for which is to be measured.

Serum is obtained from the patient by pricking the finger or ear lobe and allowing the blood to flow into a Wright's capillary tube (Fig. 22). The tube is easily made by bending in a flame glass tubing with a small lumen. After the clot has formed the tube can be broken and



A B

FIG. 22. — *A*, Wright's Capillary Tube for collecting Blood; *B*, Wright's Capillary Pipette used for Opsonic Index.

the serum withdrawn. Serum is obtained from a normal individual in the same manner.

An emulsion of leukocytes is prepared by dropping about twenty drops of blood from a pricked finger into 20 c.c. of normal saline. The mixture is centrifuged until the leukocytes appear as a layer of cream over the red corpuscles. The clear upper fluid is removed by means of a pipette and the upper layer of the sediment added to 10 c.c. of normal saline. This second mixture is centrifuged, after which the clear upper fluid is discarded and the remaining leukocytic emulsion used for the test. The purpose of this preparation is to wash the leukocytes free from influencing substances that might be present in the blood from which they were taken, and also to obtain a greater number of leukocytes in a small quantity of emulsion than would be found in the same amount of whole blood.

The bacterial suspension is prepared by gently rubbing a loopful of the growth of the organism taken from an agar culture into

normal salt solution. The suspension should only be moderately thick and when thoroughly mixed should be centrifuged to remove all clumps.

By means of a capillary pipette marked about one inch from the end, equal amounts of the three fluids, *i.e.* patient's serum, leukocytes, and bacterial suspension, are drawn up into the tube, an air space being left between each. All are then expelled upon a slide and thoroughly mixed by being drawn into the tube and again expelled several times. Finally the mixture is drawn into the tube, the end is sealed, and the tube is placed in an incubator at 37° C. Exactly the same procedure is repeated, using normal serum in place of the patient's serum. After fifteen minutes' incubation a large drop of the mixture from the tube containing the patient's serum is smeared on a slide by means of a second slide as for an ordinary blood film. A second slide is prepared in the same manner from the tube containing the normal serum, and both films are dried, fixed, and stained. The slides are then examined. The number of bacteria are counted in fifty or one hundred leukocytes and the average determined by dividing the total number of bacteria counted by the number of leukocytes in which they were found. This gives the phagocytic index. The opsonic index is then determined by dividing the phagocytic index of the patient by that of the healthy individual. Thus, if the average number of bacilli influenced by the opsonin in the patient's serum and taken up by the phagocytes is three, and the average number taken up by the leukocytes after being acted upon by normal blood is four, then the opsonic index will be $\frac{3}{4}$ of one or 0.75. For example:

$$\frac{3 = \text{patient's phagocytic index}}{4 = \text{normal phagocytic index}} = 0.75.$$

Normally the opsonic index may vary between 0.8 and 1.2. A higher index is regarded as indicative of increased resistance and an index below normal as diagnostic of infection with the specific organism tested. In the above case the patient's index is below normal, consequently an infection might be assumed.

The opsonic index as a guide for the administration of vaccine was at one time considered its most practical use. Observation showed that following an injection of vaccine the opsonins were decreased for a longer or shorter period, the so-called negative phase, and that later an increase occurred, the positive phase. The purpose of recording the opsonic index was so to determine the dose and the time of administering the vaccine that the negative phase should be as limited as possible. The opsonic index is much less used at present in vaccine therapy; it requires a considerable amount of time to determine and can only be relied upon when undertaken by skilled workers.

Agglutinins. — According to Ehrlich's side chain theory agglutinins are antibodies of the second order. They possess like antitoxins and lysins a portion for uniting with their antigens. They also possess zymophore portions to which their special reaction is due. Metchnikoff believed that the agglutinins are derived from the leukocytes and endothelial cells. It is generally thought, however, that the bone marrow and the spleen are most active in their formation.

If the serum of a patient suffering from typhoid fever is added to an emulsion of typhoid bacilli, and the mixture placed in the incubator for a short period, the bacteria which were formerly scattered throughout the fluid will be found to have clumped together in small masses at the sides of the test tube, and as they gradually fall to the bottom the fluid becomes clear. If a hanging drop preparation is made it will be observed that with the addition of serum the bacilli move closer together, gradually losing their motility, until finally they adhere in clumps.

In 1896 Widal applied this fact to the diagnosis of typhoid fever, and since agglutinins appear comparatively early in the disease it is a considerable aid both in typhoid fever and in other diseases in which agglutinins are formed. The test may be made macroscopically by sedimentation of the clumps in a test tube or microscopically in a hanging drop; the latter method is best adapted for diagnostic purposes when a quick report is necessary and only a small amount of serum is available.

Serum may be obtained by pricking the finger or ear lobe with a sharp-pointed instrument or needle and allowing the blood to pass into a Wright's capillary tube, as already described. Whole blood may be used, in which case one or two drops are placed on a slide and dried and later brought into solution again by the addition of salt solution. The use of serum from which the red blood cells have been removed is preferable.

When the serum has been obtained a 1 in 10 dilution is made by mixing one part of serum with nine parts of normal salt solution; from this dilution higher ones are made. For example, one part of the 1 in 10 dilution to one part of salt solution gives a 1 in 20 dilution; one part of 1 in 10 dilution to two parts of salt solution gives a 1 in 30 dilution; etc.

The culture with which the test is to be made should be either a twelve to eighteen hour growth in broth of the specific organisms or an emulsion of an eighteen hour agar slant culture in normal salt solution. The latter is prepared as already described for an opsonic index.

In making the microscopic test the procedure is much the same as for a hanging drop. A platinum loopful of the serum dilution and an equal quantity of the bacterial suspension are thoroughly mixed on a coverslip, and the coverslip is then inverted over a concave glass slide the rim of which has been greased with vaseline. Several dilutions are used and the slides are placed in the incubator for twenty to thirty minutes and then examined with the number 7 dry lens. In a series of dilutions all degrees of agglutination may be observed, from large clumps with clear interspaces to smaller clumps with a few motionless bacteria between, down to clusters of six or seven organisms partially agglutinated trying to free themselves. In a positive case of typhoid fever, within thirty minutes a 1 in 20 dilution will usually show complete agglutination, and a 1 in 40 dilution an almost complete reaction; partial agglutination may sometimes be observed in a dilution as high as 1 in 100. Properly killed bacteria respond to the test almost as well as living ones.

The macroscopic test is made as follows: a series of small tubes

are arranged in a rack and in them are placed equal amounts of serum dilution and bacterial suspension, each one of the tubes receiving a dilution of serum higher than the preceding one. It should be remembered that the addition of the bacterial suspension increases the serum dilution; thus a 1 in 10 dilution of serum when mixed with an equal volume of bacterial suspension becomes a 1 in 20 dilution.

The mixture of serum and bacteria is placed in the incubator for a few hours and then left at room temperature or placed in the ice chest for several hours. When agglutination takes place the clumps of bacteria fall to the bottom of the tube, leaving the fluid entirely or partially clear according to the amount of agglutinin present in the serum.

In typhoid fever a positive agglutination reaction may be given as early as the third day of the disease; ordinarily it does not appear before the seventh or eighth day. Occasionally the reaction may be absent or occur only during convalescence; as a rule it is strongest during convalescence, remains positive several weeks, and then disappears.

The agglutinins were regarded for a long time as absolutely specific. That is, that dysentery bacilli were agglutinated by serum of an individual or animal immune to dysentery and by no other. Later it was found that group agglutinins exist; that is, an immune serum will agglutinate closely related species though in a less degree. For instance, the serum of a typhoid patient may agglutinate typhoid bacilli in a 1 in 80 dilution; the same serum may agglutinate the closely related colon bacilli in a 1 in 10 dilution and may have no effect whatever on the unrelated diphtheria bacilli.

In addition to their diagnostic value the agglutinins may serve as an aid in the differentiation of bacterial species. Thus if serum obtained from an animal highly immunized against the typhoid bacillus agglutinate an unknown organism that has the cultural characteristics of the typhoid bacillus, the unknown organism is undoubtedly B. typhosus. Especially may this be regarded as a proof if the unknown strain is agglutinated by the same dilution of the serum as a known strain.

Precipitins. — Shortly after the discovery of the agglutinins it was shown that immune serum when mixed with the germ-free filtrate of a culture of the corresponding organism produced a cloudiness and afterwards a precipitate. Several authorities consider the precipitins and agglutinins are identical. It is reasonable to assume that in an old broth culture the bacteria undergo disintegration and pass into solution. Accordingly the substances which when in the bacterial body are agglutinated may, when in solution in the bacteria-free filtrate, be precipitated. The test is made in the same way as the macroscopic test described for agglutinins, except that the filtrate of a culture is used instead of a bacterial suspension.

It has been found that precipitins may be produced by injecting albuminous substances into suitable animals. Thus a rabbit immunized to human serum will produce a precipitate when mixed with human serum; similarly a rabbit immunized to horse serum will produce a precipitate when mixed with horse serum. This fact has found a practical application in forensic medicine; blood spots dissolved out in normal salt solution can be recognized as of human or animal origin even after months of drying. The test is also used in determining the nature of meat suspected to be horse flesh.

Lysins. — The bacteriolysins were discovered by Pfeiffer in his attempt to immunize animals against cholera by the injection of live cultures. It was soon discovered that the lytic action Pfeiffer had noticed, the so-called Pfeiffer's phenomenon, would take place not only in the peritoneal cavity of a guinea pig but also in a test tube when the immune serum of the animal was at once mixed with its antigen. According to Ehrlich this disintegration of bacteria occurs as a result of their union with an antibody and complement. To the antibody he gave the name of amboceptor because he considered it as an interbody linking together the antigen and complement. One of the extraordinary facts connected with the reaction is that the active part of the combination, the complement, is normally present in the blood, but only when united with a specific amboceptor can it affect the

antigen. Complement is a delicate substance, destroyed by a moderate temperature (55° C.), and disappears from serum that is kept for a few days. Like the ferments it is somewhat unstable, but it differs from them in being "fixed" or used up in definite quantities.

Bacteriolysins are produced only in the case of certain organisms, and of these the typhoid and cholera groups are the most notable.

Lysins may be produced by antigens other than bacteria. If an animal be injected with the body cells of another species it develops antibodies, **cytolysins**, which when combined with complement disintegrate the same type of cells that were employed for their production. Cytolysins have been obtained with leukocytes, kidney cells, and other organs and tissues. When the cytolysins were first discovered they aroused great enthusiasm in the hope that it might be possible to disintegrate and dissolve such foreign cells as cancer and other tumors. Unfortunately, the results have been disappointing; the cytolysins are comparatively weak and not very specific.

Hemolysins have perhaps been the most studied of all the lysins, owing to the ease with which the reaction may be observed. It has long been known that in some instances the blood serum of one animal has the power in a certain degree of dissolving the red blood cells of an animal of a different species. Bordet demonstrated that if an animal were given repeated injections of the red blood corpuscles of another species the serum of the former acquired the property of dissolving the red blood cells of the latter. He found also that this property disappeared when such serum was heated at 55° C., but as in the case of other lytic serum it was regained when fresh serum from a normal animal was added. It was evident that the immune serum contained a new substance, the amboceptor, which in the first instance had united the cells with complement and brought about their destruction, and in the second case it was amply demonstrated that not only is complement destroyed by a lower temperature than amboceptor, and that it is indispensable for the reaction, but also

that the complement is not specific but is present in normal blood.

Bordet and Gengou found that bacteria or red blood cells could be " sensitized " by placing them in immune serum that had been deprived of its complement; that is, the antigen and amboceptor could enter into a loose combination without the former being affected. If, now, measured amounts of fresh serum from a non-treated animal be added, all the complement contained in it is " fixed " or absorbed, with the result that these sensitized bacteria or red blood cells are dissolved. These facts, which emphasize the general law that when an antibody is demonstrated it may be assumed that the antigen is or has been present, form the basis of the Wassermann reaction for syphilis and the complement fixation tests for gonococcus infection, streptococcus infection, etc.

Thus when in suitable proportions a bacterial antigen (for example, a suspension of gonococci) is mixed with heated serum containing the specific amboceptor (serum of a patient with a gonococcic infection), and fresh serum containing complement (usually that of a guinea pig) is added, there is a union of the antigen and amboceptor, and all of the complement is absorbed. Since there is no visible sign that such a union has taken place, it is determined by adding in measured amounts an emulsion of red blood cells together with their specific amboceptor but no complement. The red blood cells will remain unchanged because there is no free complement left to unite with them.

If, on the other hand, the suspected patient had not a gonorrheal infection and consequently no specific amboceptors were present in his serum, then the complement could not be bound to the antigen, so that on the addition of the red blood cells plus their amboceptor the complement would be promptly absorbed by them and hemolysis occur. In this latter case a marked difference in the mixture would be noted; the disintegration of the envelope of the cells releasing the hemoglobin would give to the fluid a clear red appearance totally different to the opaque pinkish color of the positive case in which the red blood cells remained intact.

The reaction may be represented thus:

(1) Antigen + Specific amboceptor + Complement
Red blood cells + Hemolytic amboceptor
= Positive reaction; no hemolysis, the complement having combined with the antigen and specific amboceptor in patient's serum.

(2) Antigen + Serum containing no amboceptor + Complement
Red blood cells + Hemolytic amboceptor
= Negative reaction; hemolysis occurs. Since there is no amboceptor in the patient's serum the complement was free to unite with the red blood cells and their amboceptor.

The Wassermann test for syphilis has the same principle, save that the antigen is a different preparation.

The reaction may vary all the way from a complete absorption of complement to a non-absorption. Complete absorption of a given amount of complement is regarded as strongly positive and is frequently reported as four plus (++++). A one plus reaction is considered as only doubtful unless recovery is taking place as a result of medication.

The relation between the various antigens and their antibodies may be tabulated as follows:

Antigens	Antibodies
Soluble toxins (bacterial or other)	Antitoxins
Animal and vegetable protein	Precipitins
Bacteria	Opsonins
Bacteria and red blood cells	Agglutinins
Bacteria and red blood cells and animal cells	Bacteriolysins, hemolysins, cytolysins

CHAPTER XIV

TYPES OF IMMUNITY. PREPARATION OF VACCINE. ANAPHYLAXIS

RESISTANCE to bacterial infection may be inherent or *natural* or it may be *acquired* as a result of an attack of the disease or by artificial means. Natural immunity is an inborn quality of a species and of course is the converse of natural susceptibility; acquired immunity, on the other hand, is not inborn but gained during the person's lifetime. It is a state of natural susceptibility transformed into one of resistance.

Natural Immunity. — This type of immunity is an inherited character usually possessed by all individuals of a given species; thus man is immune to certain diseases of the lower animals, such as swine plague, fowl cholera, mouse septicemia, etc. On the other hand, animals are immune to many of the diseases common to man, such for example as measles, typhoid fever, chicken pox, etc.

Closely related species often show a marked difference in their degree of resistance to the same infection; white mice are practically immune to glanders whereas field mice are highly susceptible. Negroes are said to be more susceptible to tuberculosis and less susceptible to yellow fever than Caucasians. In man the difference in racial immunity is not so marked as was formerly supposed; opportunities for infection and diverse hygienic customs may in a large measure account for such differences. No race of mankind seems to possess absolute immunity to any disease to which the species is susceptible.

Individual differences are often noticed in the degree of resistance to infection in, for example, slight cuts and scratches. Indi-

vidual natural immunity is, however, a more or less relative term; in fact, in the same individual slight factors such as exposure to cold or fatigue may be sufficient to change the balance and convert a condition of resistance into one of susceptibility. In some cases resistance is so feeble that the equilibrium between health and disease is easily disturbed; in the case of tuberculosis in man the body possesses sufficient natural immunity to resist small amounts of infection, but this resistance is quickly broken down by any influence which undermines the general vitality. Hard work, mental and physical, which involves late hours and inadequate periods of rest and recreation; insufficient food and bad air — all tend to lower immunity and increase susceptibility to infection. Exposure to wet and extreme cold is well known as a factor in the etiology of colds and pneumonia. Experiments with animals give abundant proof of these facts. For instance, chickens ordinarily immune to anthrax may become susceptible if their feet are kept in cold water; white rats, also usually immune to anthrax, become susceptible after being compelled to turn a revolving wheel until exhausted before they are inoculated.

In epidemics of certain diseases many individuals escape, while in other persons infection appears in varying degree of severity; It is probable that the number of invading organisms, or their virulence, or the channel of infection may account for these apparent differences as well as varying degrees of immunity.

Acquired Immunity. — As its name implies, acquired immunity is the converse of natural immunity; it is acquired and not inherent. Acquired immunity occurs in two distinct forms, *active* and *passive*.

Active Acquired Immunity is resistance to infection due to the activity of the body cells as a result of an attack of the disease in question, or as a result of artificial inoculation with the specific organism in a modified form, or its products. Immunity of this kind is active in the sense that it occurs as the result of the active struggle of the body cells against the invading parasites, a struggle in which the foe is overcome and the body cells become more resistant than they were before. Active immunity may be gained by

(*a*) An attack of the disease;
(*b*) Introduction of vaccine consisting of the living causal agent in a modified form;
(*c*) Introduction of a vaccine consisting of dead organisms;
(*d*) Introduction of toxin.

An Attack of the Disease. — The degree and duration of immunity following an infection varies greatly according to the disease. Immunity following smallpox, yellow fever, measles, scarlet fever, typhoid fever, whooping cough, typhus fever, chicken pox, and mumps is generally lasting; in a few of the diseases, however, second attacks have been known to occur. Certain other diseases, such as pneumonia and erysipelas, seem to leave the individual more susceptible to a second attack; yet in these infections there must be a certain amount of immunity, even though it is of short duration, or the patient would not recover.

Introduction of the Modified Causal Agent. — Apart from an actual attack of the disease this method of imitating nature produces the highest and most lasting degree of immunity. Edward Jenner in 1798 established the fact when he succeeded in demonstrating that vaccination with material from cowpox protected an individual against smallpox. Eighty years later Pasteur applied Jenner's principle to other forms of disease. About 1888 the chickens in the neighborhood of Paris were being destroyed in great numbers by a virulent intestinal infection. Pasteur isolated an organism which he found to be the cause of the disease and which when injected into healthy chickens produced all the characteristic symptoms. Then he discovered that by prolonged cultivation on artificial medium the bacillus could be so attenuated that when injected into chickens no harm resulted, and, what was of much greater importance, these same chickens when inoculated with freshly isolated virulent organisms were found to be immune.

Pasteur then turned his attention to the study of anthrax. He found, however, that the methods applied to immunize chickens against cholera were not applicable in this case. Prolonged cultivation produced spore formation and not attenuated cultures.

Other investigators sought to obtain the desired result by heating the blood of animals suffering from the disease for a few minutes at 55° C. or by heating cultures of the organism at 80° C. and then using them for inoculation. Neither of the methods were very successful. After much experimentation Pasteur found that by cultivating the organisms at a high temperature, 42° to 43° C., they could be so attenuated that finally they were entirely robbed of their disease-producing power. In this manner they could be modified at will, and by inoculating animals first with a highly attenuated culture and then with a moderately attenuated one he was able to immunize them against anthrax.

Immunization against hydrophobia was the next study undertaken by Pasteur, and here as in the two previous cases his efforts were successful. Again he was confronted with a difficulty not met with before; in this case the causal agent was unknown. First, Pasteur established the fact that the virus of rabies finds lodgment in the brain and spinal cord since by injecting an emulsion of these tissues taken from an infected animal into rabbits he was able to reproduce the disease. Then he discovered that if the spinal cords were removed from these rabbits and subjected to a drying process the virulence of the virus contained in them could be attenuated to whatever degree wished, depending upon the length of the period of drying.

Pasteur taught, then, at least three methods of so modifying organisms that they may be used for the artificial production of active immunity: (1) prolonged cultivation on media, (2) growing at a high temperature, and (3) drying. He also demonstrated at the same time that the causal agents of each disease have their own characteristics and must be dealt with accordingly.

In certain diseases which are greatly influenced by the channel of entrance of the invading organisms still another method is frequently employed; the most resistant tissues are chosen as the site of inoculation. In the case of cholera, for example, there is much less danger in injecting living organisms into the subcutaneous tissues than in taking them by mouth.

Introduction of the Dead Causal Agent. — This method is of course safer than the preceding one, and the immunity produced is identical with that produced by the injection of living organisms save that it is of a lower degree and is not so lasting.

Vaccines usually produce a general reaction, such as malaise, headache, pains in the muscles, and slight fever, and a local reaction at the point of inoculation. The reactions appear as a rule within a few hours and last from one to two days.

A vaccine of dead organisms is prepared from a twenty-four-hour growth of a pure culture on an agar slant. The growth is washed off with salt solution, and after the number of organisms present in the suspension are determined they are killed by being exposed to a temperature of 56° C. for one hour. A higher temperature for a shorter period would be equally effective in killing the bacteria, but it would at the same time so alter the organisms chemically as to make them less effective.

Several methods are in use for determining the number of bacteria present in such a suspension; the following is one of the most frequently employed. Blood is taken from a pricked finger and a blood count made to ascertain the number of red blood corpuscles present in a cubic millimeter. Then with a capillary pipette one volume of blood is taken from the pricked finger and mixed with one volume of the bacterial suspension and two or three volumes of sterile salt solution. The mixture is then spread evenly on a slide as in making a blood smear, and after staining with one of the special blood stains it is examined with the oil immersion lens. The red blood cells and the bacteria are counted in a certain number of fields and the ratio between them determined. If, for example, in twenty fields the average shows two red blood cells to one microörganism and there are five million red blood cells in each cubic millimeter of blood, then there will be approximately half that number of bacteria, or two million five hundred thousand, in a cubic millimeter of the suspension and in a cubic centimeter one thousand times more. A vaccine containing any number of bacteria desired can thus be obtained by diluting the original suspension. A simpler method is that of

centrifuging the suspension in a special tube. A sediment of organisms up to a certain mark gives an approximate number when diluted with a given quantity of salt solution.

Sensitized Vaccines. — The bacteria living or dead are left in contact for some time with the serum of an animal immunized against that particular species in order that a combining of antigen and antibody may take place, after which the serum is removed by centrifuging. It is claimed that immunity produced by sensitized vaccines is more quickly developed and of longer duration; also the local and general reactions are lessened.

Polyvalent Vaccines. — Cultures of several different species of bacteria may be mixed in definite proportions and administered at the same time. A vaccine containing typhoid, paratyphoid, A and B bacilli, and cholera spirilla is reported to have been used with success.

Bacterial vaccines are always given in subcutaneous injections. Three or four doses are usually given at intervals of about five to ten days.

In addition to being used as a prophylactic measure, vaccines are often employed therapeutically in local infections such as acne, pustule, or a boil. It is assumed that while the local resistance has been lowered it is probable that the general antibody producing tissues have not commenced to react; the vaccine may thus stimulate the latter and cause the infected area to be flooded with antibodies.

Frequently an autogenous vaccine is prepared for such cases; that is, the infecting organism is isolated from the discharge, grown in pure culture, and prepared as a vaccine. In most instances immunity produced by the introduction of a bacterial vaccine lasts from two to five years and may, of course, be renewed.

It is not definitely known how long a vaccine may be effectively used after the date of its preparation; usually after a period of from four to six months it is supposed to lose its potency.

Immunization with Toxin. — Soluble toxin such as produced by the diphtheria bacillus may be obtained free from bacteria

by filtration of a broth culture. The process of immunization is started by giving exceedingly small doses usually in conjunction with antitoxin; afterwards the doses are gradually increased. The method has been employed in the case of snake venom and a high degree of immunity thus produced.

Passive Acquired Immunity. — As the name indicates, this form of immunity is passively acquired by virtue of receiving antibodies formed by the body cells of an animal that has had to resist the infecting agent in order to produce them. Thus in order that a child may become passively immune to diphtheria an animal must first combat the disease; horses are injected with successive doses of toxin and are required to overcome its effect and acquire an active immunity of a high grade due to the production of antitoxin. The horse then is actively immune because it has manufactured its own antibodies. When its antitoxin-laden serum is injected into the child, the child becomes passively immune; protection being due not to the activity of its own body cells but to those of the horse.

Passive immunity is specific; that is, the serum of an animal immunized against one microörganism will protect an individual or another animal against that and against no other. Immunity of this type is gained just as soon as the immune serum has become mixed with the blood of the person or animal injected. It is of much shorter duration than active immunity and the degree is seldom equal to that of the latter. It is, however, especially of value as a prophylactic measure against an acute infection that has a relatively short incubation period.

Ordinarily the injection of immune serum as a therapeutic measure causes very little disturbance to a patient and this little is more than counterbalanced by the release of the body cells from combat with the toxic substances which are overcome by the antibodies injected.

Passive immunity may be antitoxic or antibacterial, according to the antigen employed for the production of the specific immune serum.

ANAPHYLAXIS

Ordinarily when an animal is given repeated injections of an antigenic substance, the antibodies produced by the first injection are increased and eventually a high degree of immunity is established. Under certain circumstances, however, the reverse seems to be the case. A second injection will produce severe and even fatal symptoms, so that it would seem instead of immunity a state of hypersusceptibility or hypersensitiveness has been produced.

To this state of hypersusceptibility Richet gave the name anaphylaxis, meaning "without protection," because to him it represented the reverse of prophylaxis. Recent researches, however, tend to show that the two conditions may not be opposed, but may even be closely related. The term "*allergy*" or "altered energy" has been suggested as a more appropriate one.

According to the theory of Vaughan the phenomenon of anaphylaxis may be explained by supposing that when a protein such as horse serum or egg albumin or bacteria is injected it is broken up by an enzyme present in small amounts in the body into a toxic and a non-toxic portion. At the first injection the disintegration takes place slowly and the body is slightly or not at all affected. By the second time the injection is given, however, considerably larger quantities of the splitting enzyme have been elaborated, so that a large amount of the toxic portion is immediately liberated and symptoms quickly appear. The second injection causes no symptoms unless, as in all immunity reactions, sufficient time is allowed to elapse for the cells to combine with the proteins and for the specific ferments to be produced. The exact nature of the reaction is as yet unknown. Recent research tends rather to support the view that anaphylaxis occurs as a result of the union between antigen and antibody, taking place in the body cells and not in the blood stream. Further work may reveal that both factors are concerned.

Experimental anaphylaxis in animals shows that the first injection of a foreign protein which in itself is not poisonous so

sensitizes an animal that a second injection after an interval of about twelve days may cause the reaction known as anaphylactic shock. The guinea pig is apparently the most susceptible of all animals to horse serum, yet a first large dose gives rise to no symptoms. A second injection of a minute amount may cause, within five or ten minutes, a condition of restlessness and spasmodic respirations and probably partial or complete paralysis. Recovery may take place at this stage or convulsions may develop and the guinea pig may die within twenty or thirty minutes.

The symptoms of anaphylactic shock are not the same in all animals, and this has been explained on the ground of slight differences in anatomical structure. It has been demonstrated that smooth muscle cells are the most hypersensitive. In the case of the guinea pig the mucosa of the bronchi is relatively thick compared with the lumen and the muscular contraction throws it into folds, with the result that the guinea pig is asphyxiated. The bronchi of dogs have relatively less smooth muscular tissue. This probably accounts for the few cases of death from asphyxia in anaphylactic dogs. In the latter contraction of the smooth muscle of the intestines starts a vigorous peristalsis; the muscles of the heart and arteries are also affected.

Fortunately the severe and fatal forms of anaphylaxis are extremely rare in man; most cases have occurred in persons known to be susceptible to horse protein. This undue hypersusceptibility is revealed by the asthmatic attacks which such a person exhibits when entering a stable or nearing a horse. Serum anaphylaxis or "serum sickness" in man sometimes occurs following a dose of antitoxic serum; the characteristic symptoms are a skin eruption, swelling of the lymph glands, joint pains, and albuminuria. These symptoms are altogether independent of the antitoxin contained in the serum and are purely dependent on the serum as such. For this reason a concentrated antitoxin is less likely to produce serum sickness. In the majority of cases symptoms do not appear for from eight to ten days. Presumably an amount of the antibody or protein splitting substance has been generated by that time and all of the horse serum that remains in the circulation

is attacked, with the result that there is an excess of liberated poison which gives rise to the anaphylactic reaction. If the dose of serum has been small or if a second injection follows the first in less than six to eight days there is seldom any reaction. If, however, a second injection is made a few months after the first an immediate reaction often follows. If a year or more elapse between the injections there is usually no danger of a reaction. By that time the antibody has disappeared from the circulation. The rare fatal cases so far reported have followed first injections, presumably because the individuals were already hypersensitive.

Anaphylactic or allergic skin reactions are frequently employed as an aid in diagnosis. For instance, when tubercle protein (tuberculein), syphilis protein (leutin), glanders protein (mallein), etc., is applied or injected into the skin of an individual sensitized to that particular protein a local reaction occurs characterized by congestion and edema. If, for example, tuberculein be rubbed into the skin of a normal individual he will not react because he has not been sensitized, whereas a tuberculous patient, except one in the last stage, reacts promptly. The difference between the normal individual and the one in the final stage of tuberculosis is that the former has not been sensitized while the latter has had his anaphylactic powers exhausted; consequently he presents little or no resistance against the advance of the infection.

The so-called food idiosyncrasies are also instances of anaphylaxis. The articles of diet usually responsible are fish, tomatoes, strawberries, pork, eggs, etc.; the symptoms produced are skin eruptions, gastro-intestinal disorders, and vaso-motor disturbances. When there is a difficulty in determining which food is responsible the skin test may be employed by rubbing a drop of the food itself or a watery extract into a scratch upon the skin. The reaction comes on within thirty minutes and is demonstrated by a pink red edematous area.

The Shick skin test should be distinguished from the above. It is not an allergic skin reaction, it depends upon an entirely different principle.

PART III

CHAPTER XV

THE PYOGENIC COCCI

THE microörganisms most frequently found in suppurative processes, such as boils, abscesses, and purulent inflammations, belong to a group of bacteria known as *pyogenic cocci*. Of these the two most important because of their virulence and frequent occurrence are *Staphylococcus* (*pyogenes*) *aureus* and *Streptococcus pyogenes*.

Early investigators noticed the frequent presence of small round bodies in the pus discharged from abscesses and sinuses and gave to them a variety of names. In 1880 staphylococci were first obtained from pus by Pasteur. In 1881 Ogston studied the question and found that the staphylococci were most common in circumscribed acute abscesses and the streptococci in spreading suppurative conditions. Rosenbach in 1884 differentiated by means of cultures several different varieties of pyogenic micrococci to which he gave the special names *staphylococcus pyogenes aureus*, *staphylococcus pyogenes albus*, *streptococcus pyogenes*, etc. Other organisms are met with less frequently in suppuration; such for example as *staphylococcus pyogenes citreus*, *micrococcus tetragenus*, *bacillus pyocyaneus*, etc. The pyogenic cocci are constant inhabitants upon the skin and mucous membranes. Consequently not only may they cause a pathogenic condition themselves but may readily enter into an infection started by another organism and further increase the injury by giving rise to a "mixed infection."

STAPHYLOCOCCUS AUREUS

Morphology and Staining. — The organism is a small coccus about 0.8 μ. in diameter, sometimes appearing in pairs or isolated groups of three or four but most commonly in irregular clusters resembling bunches of grapes. It stains with the usual basic anilin dyes. It is Gram positive; that is, when stained by the method of Gram it retains the gentian violet dye; it does not form spores; it does not possess flagella and is consequently non-motile, although marked Brownian movement may sometimes be noticed in a hanging drop preparation. (Fig. 23.)

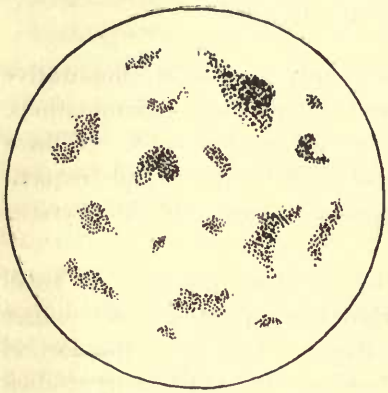


FIG. 23. — Staphylococci.

Cultivation. The organisms grow readily on ordinary artificial culture medium made of meat extract. The optimum temperature for growth is about 30° C. although they possess a range from 10° C. to 43° C. in which multiplication will occur. In stab cultures in peptone gelatin a line of growth may be observed the day after inoculation, and on the second and third day liquefaction commences at the top of the medium. As liquefaction progresses the growth falls to the bottom as a flaky deposit of a golden yellow color, while a yellowish film may remain on the surface. In gelatin plates colonies appear as small yellowish disks around which liquefaction soon commences, giving a cuplike appearance with the small colony mass at the bottom. A stroke culture on agar gives an abundant orange-yellow growth with a smooth shiny surface. Single colonies on agar appear as small disks of the same color. On potato staphylococci grow luxuriantly with an abundant production of pigment; in broth the growth appears as a turbidity which later settles to the bottom as a sediment. Growth is rapid; it is estimated that a broth culture may contain

in twenty-four hours about 500,000,000 organisms per cubic centimeter. They are able to ferment dextrose, lactose, and saccharose, and from them form various acids. Fermentation, however, does not result in the formation of gas. Milk is coagulated and indol produced in peptone solution as a result of their growth.

If a little blood is added to nutrient agar and staphylococci are smeared over it, a clear zone surrounding each colony appears after twenty-four to thirty-six hours' growth; this effect is produced by a hemolytic substance in the organism which dissolves the envelope of the red blood cells and sets free the hemoglobin.

Resistance. — Among the non-spore-bearing bacteria staphylococci are perhaps the most resistant; cultures on gelatin or agar will remain alive for a year or more. Suspended in water the thermal death point varies with different cultures, averaging about two hours at 50° C., one half hour at 60° C., and ten minutes at 70° C. They are killed by mercuric chloride 1 in 1000 in from fifteen to thirty minutes, and by carbolic acid 1 in 100 in from twenty to thirty minutes.

They are very resistant to sunlight, drying, and low temperatures.

Pathogenesis. — Animals appear to be considerably less susceptible than man to staphylococci infections. Large amounts of a pure culture injected into a rabbit may cause the formation of abscesses which generally heal without treatment; or if the culture is sufficiently virulent and a large enough amount be given the animal may die in from two to eight days. On autopsy, abscesses are found in the various internal organs, particularly the liver, kidneys, and in the walls of the heart. These appear as small yellowish masses about the size of a pea surrounded by a zone of intense congestion. Many of the capillaries and smaller arteries are blocked with thrombi consisting of staphylococci.

Investigators have produced carbuncles in man by rubbing a pure culture of staphylococci upon the unbroken skin. The organisms supposedly gain entrance into the deeper tissues through the base of the hair follicles or sweat ducts. Lowered vitality of the tissues in almost any locality may permit a local invasion

resulting in a boil or a carbuncle. Endocarditis, septicemia, or pyemia may result from a local abscess through the introduction of the organisms into the lymph or blood stream. Bone tissue seems to be particularly susceptible. The majority of all the attacks of osteomyelitis and periostitis are due to staphylococcic infection.

Two substances have been isolated from cultures of staphylococci which explain in part their ability to produce disease. One, staphylolysin, acts on the envelope of the red blood cells in such a manner as to dissolve out the hemoglobin, and is consequently responsible in part for the anemia present in such infections. The other substance, leukocidin, has an injurious effect upon leukocytes. Both of these substances resemble the true toxins in that they stimulate the body cells to produce neutralizing antibodies. It is more than probable that these organisms generate other toxic bodies, but as yet nothing definite is known about them. Dead culture of staphylococci when injected subcutaneously may produce local abscesses.

Immunity. — Phagocytosis in this case is, without question, the chief factor in immunization; the amount of opsonin is increased, positive chemotaxis occurs, and the phagocytes actively engage in carrying off the invaders. An immune animal serum has been prepared containing antibodies that neutralize staphylolysin and leukocidin. Its effect, however, is relatively weak and it is seldom used except to confer passive immunity in cases in which the general vitality is so low as to contraindicate the attempt to produce active immunity by the introduction of vaccine. As a therapeutic measure vaccine treatment has given most satisfactory results, doses commencing with 2 to 20 million organisms gradually increasing to 100 to 1000 million are given.

Staphylococcus pyogenes albus. This coccus is identical with staphylococcus aureus except that it does not produce a yellow pigment and its pathogenic powers are somewhat feebler. Surface cultures have a milk-white appearance. It has been suggested that it may be a degenerate descendant of aureus, but no one has succeeded as yet in transforming one form into the other.

Staphylococcus epidermidis albus. It is probable that this organism is identical with staphylococcus albus. It is slightly virulent and is frequently found in the upper layers of the epidermis; it is the common cause of " stitch abscesses " following a surgical operation.

Staphylococcus pyogenes citreus. This organism also differs from aureus only in the color of its pigment, which is usually of a bright lemon yellow. It is less often met with in wounds, however, than either of the preceding cocci. A number of other staphylococci exist, few of which are in any degree pathogenic so far as is known. They differ in minor details, such as ability to liquefy gelatin or to form pigment.

MICROCOCCUS TETRAGENUS

In 1887 Gaffky isolated the organism from the pus of a tuberculous patient. It has been observed associated with other organisms in pulmonary tuberculosis, in acute abscesses, and also in the pus of empyema following pneumonia. It is also frequently found in the saliva of healthy persons, and it is generally assumed that while it rarely incites disease its presence helps to contribute to the progressive destruction of tissue in diseased conditions.

Morphology and Staining. — The cocci are somewhat larger than staphylococci; their diameter averages about 1 micron. They are arranged regularly in groups of four or tetrads, and often when first removed from pus appear to be surrounded by a capsule. They are readily stained by the basic anilin dye and are Gram positive.

Cultivation. — The optimum temperature is from 35° C. to 38° C. Growth is slow, but will occur both in the presence and absence of oxygen. On agar the colonies appear as small, round points, at first transparent and later becoming a grayish white. Gelatin is not liquefied; acid and coagulation is produced in milk.

Pathogenesis. White mice are especially susceptible to infection by the micrococcus tetragenus; other animals are much less so. In man it is usually non-pathogenic except in the condi-

tions already referred to; a few rare cases are on record in which it has been cited as the sole producer of a pyogenic condition.

STREPTOCOCCI

The pathogenic streptococci were first discovered by Koch in stained sections of diseased tissue, and by Ogston in 1881 in the pus of acute abscesses. Later in 1883 Fehleisen obtained pure cultures from a case of erysipelas. Because of the variety of pathologic conditions in which streptococci were found it was at first thought that each was produced by a different species; now it is generally assumed that the slight differences between the streptococci of erysipelas, of acute abscesses, of septicemia, of puerperal fever, etc., are only acquired variations of organisms of the same species.

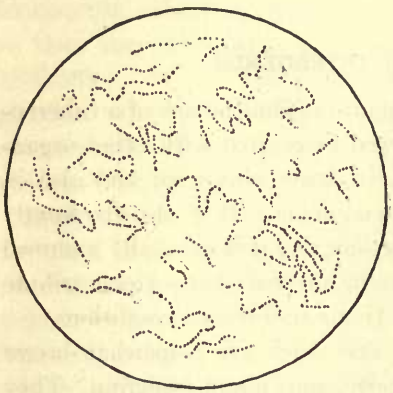


FIG. 24. — Streptococci.

All spherical bacteria which divide in one plane only and remain attached in longer or shorter chains, resembling somewhat a string of beads, are classified together under the name of streptococci (Fig. 42). The classification is simply a morphological one and includes both saprophytic and parasitic varieties. The relationship between the streptococci from different sources is by no means clear. Those of greatest importance, however, because of the power which they possess of inciting disease in man and because such diseases are frequently of a suppurative character, are roughly grouped together as *streptococcus pyogenes*.

Streptococcus pyogenes. Morphology and Staining. — The cocci are relatively small, measuring from 0.5 micron to 1 micron in diameter; they have no flagella nor do they produce spores. As a rule the pathogenic streptococci show a tendency to remain united

in long chains. A differentiation, however, cannot be based on this feature, since cultivation on media relatively unsuitable causes it to disappear. All streptococci are stained by the usual anilin dyes; the pyogenic group are for the most part Gram positive.

Cultivation. — The most favorable temperature for their growth is from 30° C. to 37° C.; below 15° C. and above 43° C. growth rarely takes place. They are faultative anaërobes growing both in the presence and absence of oxygen. Upon ordinary nutrient agar their growth is rather scanty. Much more satisfactory results are obtained by cultivating them on media having meat infusion as a basis or on media to which blood, serum, or ascitic fluid has been added. One to two per cent of glucose added to the medium favors development also. In a gelatin stab growth is seen about the second day as a thin line, later it appears to be formed of a row of minute rounded whitish colonies; the growth does not spread on the surface and no liquefaction occurs. On agar or gelatin surface colonies appear as fine grayish, opalescent points, smooth and round, or, as seen under the low-power lens, with a lacelike edge composed of chains of streptococci arranged in loops. Acid is produced in milk and usually coagulation of the casein. In slightly alkaline broth after twenty-four to forty-eight hours the growth frequently appears as a deposit of tiny flakes. The addition of blood or serum to the broth seems to have a marked effect on their chain formation; the same strain will often remain attached in long chains in the latter case, whereas in ordinary broth they may separate in twos or threes. On Loeffler's blood serum medium growth is rapid and luxuriant.

The pyogenic streptococci have been divided into two groups on the basis of their ability to cause hemolysis. If blood agar plates are prepared by adding 1 c.c. of fresh or defibrinated blood to 6 c.c. of agar at 43° C. and then inoculated with hemolytic streptococci and incubated for twenty-four hours, each of the colonies will appear to be surrounded with a clear zone due to the destruction of the red blood cells. Related streptococci, on the other hand, produce a zone of a greenish color. The latter are not so virulent and cause rather a chronic form of inflammation.

The inulin serum medium of Hiss is frequently used to differentiate the streptococcus pyogenes from pneumococci. The latter produce acid and coagulation of the serum, while the former are unable to ferment inulin.

Resistance. — On culture media, unless transplanted, streptococci do not live more than two to fourteen days; in body discharge they may live for several weeks. They are killed by exposure to a temperature of 54° C. for twenty minutes; low temperatures have less effect upon them. Exposure to sunlight kills them in a few hours. Mercuric chloride 1 to 1000 destroys them in from five to ten minutes, carbolic acid 1 to 100 in from five to forty-five minutes.

Pathogenesis. — Attempts have been made to classify the streptococci according to their pathogenicity or their growth on artificial culture media; such attempts have not been very successful because the differences observed are not constant. What may have been thought to be a definite characteristic may become totally changed under other conditions. The animals ordinarily used for experimentation are not so susceptible to streptococci infection as man, and different animals show different degrees of susceptibility to different cultures. A virulent strain when injected into a mouse will cause septicemia; those of a little less virulence will produce the same result if the quantity is increased; others still less virulent will produce septicemia if injected into a vein, but if introduced into subcutaneous tissue will produce an abscess or erysipelas. Others even less virulent when injected in large amounts will only produce a slight inflammation or no reaction at all.

Experiments have shown that streptococci originally virulent may become non-virulent after long cultivation on artificial culture media, but that after passage through an animal they regain their lost power.

In man streptococci are responsible for a greater variety of lesions than any other microbes, and in addition to the number of diseases they themselves cause they are present in " secondary " or " mixed " infection more often than any other organisms.

Erysipelas, a spreading inflammatory condition of the skin, is almost invariably due to streptococci. The organisms are found in large numbers in the underlying tissues and lymphatics; they may extend to serous and synovial cavities and give rise to peritonitis, meningitis, and synovitis. As a rule in erysipelas the cocci are not present in the central portion of the inflamed area, but may be isolated from the swollen edge by excising a small piece of the skin.

The fact that puerperal fever might be caused by infection from an erysipelas case was noticed long before it was discovered that the same organism could produce both conditions.

Observers have noted that patients suffering from malignant tumor seem to improve, and in some cases the tumor has diminished after an attack of erysipelas. Fehleisen, accordingly, inoculated hospital patients suffering from inoperable growths with cultures of streptococci and produced in them typical erysipelas and often a favorable influence on the growth. Later Coley modified the treatment by using a mixture of dead streptococci and bacillus prodigiosus or their products. The sarcomatous tumors are most favorably affected by " Coley's mixture "; carcinomatous growths slightly or not at all. Many observers, however, have failed to note any favorable results following its use.

Suppurative conditions in different organs of the body may result from streptococcus invasion. Ulcerative endocarditis, bronchopneumonia, pleurisy, empyema, otitis media, enteritis, are included in the list of diseases of which they may be the primary cause.

In throat affections of all kinds they play an active rôle; their constant presence on the mucous membranes and tonsils make possible a speedy invasion whenever there is a lowering of the local vitality.

In smallpox and scarlet fever streptococci can be isolated from the internal organs in a large number of the fatal cases. Certain authorities regard the streptococcus as the causal agent of scarlet fever; the view, however, is not generally accepted.

Isolated from the blood of rheumatic fever cases they have pro-

duced when inoculated into rabbits characteristic arthritic lesions. The question as to the specificity of the streptococcus found in rheumatism is as yet unsettled. In certain cases of chronic arthritis hemolytic streptococci have been isolated from the tonsils which when injected into certain animals have invariably produced arthritis. Removal of the tonsils in such cases generally results in marked improvement or recovery.

Several epidemics of sore throat due to streptococci have arisen from time to time, many of which have been traced directly to the milk supply. It is thought that such streptococci come originally from a septic human throat, find their way into the milk ducts, probably from the hands of a milker, and multiply there without causing any perceptible inflammatory condition in the cow. The bovine type of streptococci which produce inflammations of the udder of the cow has different cultural characteristics and is apparently not identical with those found in human septic sore throat.

As already stated, a satisfactory classification of streptococci from different sources is extremely difficult. Association with a specific pathologic condition is not conclusive that the organism is the causal agent of that and of no other condition. A strain isolated from suppurative processes may produce erysipelas, and conversely abscess formation may be produced by one isolated from erysipelatous lesions. Classification according to agglutinating reactions or the ability to ferment different sugars have likewise given insufficient aid in determining whether the streptococci are one or several species.

Immunity. — Streptococcus inflammations apparently do not stimulate the human body cells to produce immunizing antibodies. It is true that some protective substances must be produced or recovery would not take place. Evidently, however, they soon disappear, leaving the individual as susceptible as before.

An interesting experiment was tried by Koch and Petruschky on a man suffering from a malignant growth. They inoculated him subcutaneously with streptococci obtained from a case of erysipelas and produced in him a moderately severe attack of

the disease. The symptoms disappeared after about ten days and they then reinoculated him over the same area and again obtained the same result. Ten successive attacks were produced in the same manner, which proved, at least, that immunizing substances were not present in sufficient amounts to afford protection. It is a well-established fact that opsonin is increased and phagocytosis consequently active in all infections with the pyogenic cocci.

A degree of active immunity may be produced in rabbits and horses by inoculating them with gradually increasing doses of streptococcus cultures. Experiments with animals have shown that the serum from such immune animals will protect to a certain extent against the organism used for its production, but not against other strains.

Accordingly, in order that the serum may contain antibodies to combat the different streptococcic infections, a number of different strains isolated from different forms of disease are used for inoculating the horses. The " polyvalent " serum obtained from animals so treated is not so efficient as one prepared from the organisms infecting the treated case, but it is moderately effective for all cases. The use of such serum seems to have been of benefit in certain cases, but on the whole the results have been disappointing. Large doses must be given in order to obtain a sufficient amount of antibodies to produce an appreciable effect.

Vaccines are administered in subacute conditions. The initial dose is 5 to 10 million organisms; increasing amounts may be given to 500 million as a maximum.

CHAPTER XVI

PNEUMOCOCCUS, MENINGOCOCCUS, GONOCOCCUS

THE term pneumonia is used to designate a variety of pathogenic conditions of the lung or the parts which compose it. Of these the two forms most generally met with are: lobar pneumonia (acute croupous), an inflammatory process accompanied by abundant fibrinous exudate rapidly involving the entire tissue of a lobe or a large portion of it, and lobular pneumonia (broncho-pneumonia), a catarrhal inflammatory type spreading from the capillary bronchi to the air vesicles and often resulting in consolidation of patches of the lung tissue.

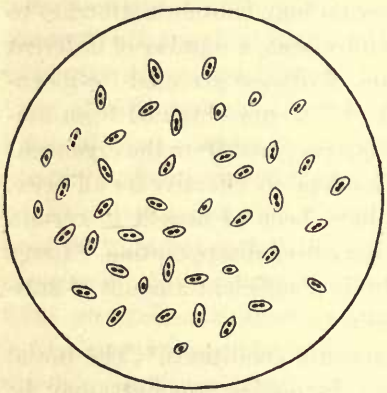


FIG. 25. — Pneumococci.

A number of microörganisms may give rise to pneumonia; such for example as B. mucosus capsulatus, B. diphtheriæ, B. pestis, B. typhosus, streptococci, and staphylococci. For the most part, however, these organisms produce the lobular type.

In lobar pneumonia about 95 per cent of all the cases are caused by a lancet-shaped micrococcus upon which various names have been bestowed, such as *pneumococcus*, *diplococcus pneumoniæ*, *micrococcus lanceolatus*, or after its discoverer, *Frankel's pneumococcus*. (Fig. 25.)

The pneumococcus was observed almost simultaneously by Pasteur and Sternberg in 1880 in the blood of rabbits inoculated

with human saliva. They did not, however, associate the organism they saw with lobar pneumonia. Later, in 1886, Frankel and Weichselbaum demonstrated beyond question that the large majority of the cases of lobar pneumonia are caused by the pneumococcus.

Morphology and Staining. — Ordinarily the organism appears as a small, slightly oval coccus, one side of which is somewhat pointed. The pneumococci may occur singly or in chains, and for this reason they are classed by certain authorities with the streptococci; usually they appear in pairs with the broad ends of the oval in juxtaposition and the pointed ends turned outward. When observed in fresh sputum or blood smears they are surrounded by a well-defined capsule. Cultivated on artificial media the capsule is rarely seen unless serum or blood has been incorporated in the medium. The organisms are non-motile, possess no flagella, and do not form spores. They stain readily with the ordinary dyes and are Gram positive. The capsule may be easily demonstrated in blood or sputum preparations by the method already described.

Cultivation. — The temperature range of the pneumococcus is rather limited, growth taking place as a rule only between 22° C. and 42° C. It grows equally with or without oxygen. When freshly isolated, growth is very feeble unless blood or serum is added to the medium; on agar or gelatin the colonies appear similar to those of the pyogenic cocci except that they are more delicate in appearance. The same may be said of stab cultures in gelatin. Along the line of inoculation a row of minute points appear which remain of a small size; there is no liquefaction of the medium. In broth a slight turbidity is produced which settles to the bottom of the tube as a fine deposit. Milk is quickly acidified and often but not always coagulated; growth on potato is seldom visible.

The pneumococcus is non-hemolytic; its colonies on blood agar are of a greenish color. Glucose, saccharose, lactose, and inulin are fermented; the ability to ferment the latter sugar and the fact that the organisms are dissolved in bile are two important

characteristics which aid in differentiating them from the streptococci.

The pneumococci may be isolated from mixed cultures or sputum by smearing the material over blood agar plates. After twenty-four hours the fine colonies are easily distinguished from all others except the streptococci; from the latter they differ only in that they are transparent, a little more delicate in appearance, and have a smoother edge.

By another method a small mass of sputum is washed free of extraneous organisms by gently rinsing it in salt solution and then injecting it into the peritoneal cavity of a white mouse. If virulent pneumococci are present, death will occur in from twenty-four to forty-eight hours and the organisms will be found in pure culture in the heart's blood and in the peritoneal exudate.

Resistance. — The pneumococcus is decidedly frail. Apart from the body or body discharges growth soon ceases. On artificial culture media it must be transplanted every three or four days in order to keep it alive, and even then occasional passage through a mouse or rabbit is sometimes necessary to maintain its virulence. In dried sputum it may live for several months and retain its pathogenic power. Low temperatures slightly above zero are quite favorable to the preservation of its vitality. In direct sunlight it dies within an hour. It is quickly killed by a moderate degree of heat; ten minutes' exposure to 52° C. is sufficient. Mercuric chloride 1 to 1000 will destroy it in five minutes and carbolic acid 1 to 100 in from five to ten minutes.

Pathogenesis. — The pneumococcus is frequently found in the saliva of healthy individuals. The New York Commission reported its presence in 45 per cent of a number of persons examined. Thus many apparently normal individuals are pneumococcus " carriers." It has been shown, however, that the majority of such cases are only " temporary carriers." The organism leaves the body mainly in the discharges from the mouth and nose and enters the system through the same channels.

Most strains are pathogenic for a number of animals. A small amount of sputum containing virulent pneumococci will cause

the death of a mouse or rabbit within twenty-four to forty-eight hours from septicemia. After death the blood will be found to contain enormous numbers of organisms.

Little is known of the toxic substances produced by the pneumococcus; it is thought that they are of the nature of endotoxins and are closely bound to the cell substance.

In characteristic pneumonia in man the organisms are found in the bronchioles and alveoli of the infected lung and in the lymphatic channels and blood capillaries; from the capillaries they find their way into the general blood current. So abundant are they in a certain percentage of cases that they may be found in cultures made from 5 to 10 c.c. of blood.

In all cases of lobar pneumonia and in many cases of bronchopneumonia pleurisy occurs, caused by the same organism that gave rise to the pneumonia; recovery from pleurisy due to pneumococci is generally more speedy than that caused by streptococci or staphylococci.

Other infections frequently complicating pneumonia are those of the pericardium, endocardium, meninges, and middle ear. They are probably explained by the fact that the infecting organisms are conveyed by means of the blood and lymph to all parts of the body.

Immunity. — Following an attack of pneumonia immunity lasts only for a short time. Two or three attacks of pneumonia are not unusual for the same individual. A serum of some protective and curative value has been produced by successive injections of gradually increasing doses of virulent pneumococci into horses. In addition, such serum possesses specific agglutinins which not only are an aid in diagnosis but by means of which investigators have been able to divide the pneumococci into four groups according to their specific reaction. Group I is the cause of the greatest number of infections. An immune serum has already been produced which has met with considerable success in conferring passive immunity in infections with this type. Fewer cases are caused by Group II and Group III, but the mortality is much higher than with Group I. A serum has been prepared

against Group II, but it has not had the same success as that prepared against Group I. Group IV is not really a group but an assembling together of all the remaining isolated strains.

Vaccines have been employed for the production of active immunity as a prophylactic measure; their curative value is doubtful in a disease so acute and relatively brief.

Relation of Pneumococci and Streptococci. — A group of cocci have frequently been found in various diseased conditions such as pneumonia and meningitis, which besides possessing a voluminous capsule are surrounded by a viscous substance which gives a slimy consistency to cultures and to exudates. So closely do they appear to be related both to the pneumococci and to the streptococci that it is difficult to determine with which they should be placed. It has been suggested that they be divided into two groups: (1) *pneumococcus mucosus*, which resembles the true pneumococcus in that it is non-hemolytic on blood agar, is soluble in bile, gives rise to acid and coagulation in serum inulin medium, and is very pathogenic to white mice; on the other hand, it forms much larger colonies than the pneumococcus, and the individual cocci tend to be less pointed. Recent investigators regard it as Group III in the pneumococcus classification. (2) *Streptococcus mucosus* is usually non-hemolytic, is not soluble in bile, and does not ferment inulin; the colonies are less transparent, the individual organisms are round and occur in chains. Thus while the pneumococcus mucosus is practically a true pneumococcus the streptococcus mucosus appears to form a connecting link between it and the streptococci.

MENINGOCOCCUS

Inflammation of the membranes surrounding the brain and spinal cord may be caused by several different organisms; it may occur as a primary or secondary infection. As a secondary infection it not infrequently occurs during pneumonia as a result of the pneumococcus being carried to the meninges by the blood stream; sometimes the tubercle bacillus is the invader. In-

flammation of the middle ear or frontal sinuses may by extension produce meningitis. In such cases the infecting organisms are usually staphylococci or streptococci. Sometimes meningitis is part of a septicemic or pyemic condition; occasionally it is due to a mixed infection, and not infrequently the pneumococcus has been found associated with the tubercle bacillus and also with meningococcus.

It has been estimated that about 70 per cent of all acute cases of meningitis appear in the form designated *epidemic cerebrospinal meningitis*, due to the organism usually termed the *meningococcus*. In 1884 Weichselbaum found the organism in six cases of meningitis, two of which were not complicated with pneumonia. He studied it in pure culture and showed that it possessed characteristics which clearly distinguished it from the pneumococcus. Because of its frequent presence in the interior of pus cells he gave to it its name of *diplococcus intracellularis meningitidis*.

Morphology and Staining. — The organisms appear as small cocci usually arranged in pairs, the adjacent sides being somewhat flattened against each other. Occasionally they are seen in groups of four or in small masses. They are non-motile, non-spore-bearing, and form no visible capsule. They stain with all the ordinary dyes and are Gram negative.

Cultivation. — The optimum temperature for the meningococcus is about 37.5° C.; growth will occur between 25° C. and 40° C. They can rarely be isolated on plain nutrient agar; the addition of a body fluid is usually necessary. On glucose ascitic agar the colonies appear as small, grayish white, finely granular disks. In broth development is slow and takes place near the surface. Different strains vary in their power to ferment carbohydrates and in their ability to grow on artificial culture media. Cultures may remain alive for several weeks. Certain strains, however, tend to die within three or four days and consequently require transplanting to a fresh medium at very short intervals.

Resistance. — The organism is readily destroyed by sunlight or drying or by exposure to a moderate degree of heat or cold. It is killed in from one to five minutes by 1 to 1000 solution of

mercuric chloride or 1 to 100 solution of carbolic acid. Its low resistance to influences outside of the body, together with the fact that it has not been demonstrated in the air or dust, is an evidence of its parasitic nature.

Pathogenesis. — The organisms vary in their pathogenicity for animals. Certain strains injected into the peritoneal cavity of a guinea pig will produce a septicemia. Killed cultures may also have a fatal effect, in which case death is probably due to a bacterial poison of the nature of endotoxin, liberated by the disintegration of the organism.

In human beings the course of the disease is very rapid; the lesion is of a suppurative nature involving the meninges, the base of the brain, and the surface of the spinal cord. During life the surest method of diagnosis is the detection of the specific microörganism in the spinal fluid withdrawn by means of a lumbar puncture. In cases of meningitis due to the meningococcus, the fluid appears somewhat cloudy, contains a high percentage of polynuclear leukocytes, and the characteristic Gram negative diplococci free or engulfed within the leukocytes. The mortality without serum treatment is about 70 per cent.

The organisms in all probability enter the body by way of the nasopharynx, passing out of the nose and its adjoining cavities along the path of the lymphatics toward the base of the skull. They are present in great numbers in the nasal cavity during the first twelve days of the disease, after which they disappear.

Epidemics usually occur in the winter and spring months and commence in localities where overcrowding is most likely. Because of the low vitality of the organism outside of the body "carriers" may be largely responsible for the majority of outbreaks, since the disease is undoubtedly transmitted from person to person. During an epidemic not all the persons who harbor the organism develop the disease; the carriers have been shown to outnumber the actual cases by ten to one. Apart from epidemics the meningococcus is rarely found on the membranes of healthy persons, but evidently there are those who carry it always and thus perpetuate the disease.

Agglutinins. — An agglutination reaction towards the specific strain of meningococci causing the disease is often given if death does not occur within the first few days. A positive reaction may appear by the fourth day in a dilution of 1 to 50; at a later stage it may even occur in as great a dilution as 1 to 400. A certain number of strains, however, do not give the reaction, and for this reason the test is unreliable and practically never used for diagnosis.

An antiserum has been prepared by injecting horses with a mixture of several strains of meningococci. A serum prepared by Flexner and Jobling has been extensively used both in America and Europe and has given very satisfactory results; the use of such an antimeningococcic serum has reduced the mortality from 70 to 30 per cent and has greatly diminished the tendency to chronic lesions in those who survive.

To administer the antiserum a lumbar puncture is made in about the third or fourth lumbar space, the cerebrospinal fluid is allowed to flow until only about three or four drops come per minute, and then the serum, which has been previously warmed to body temperature, is allowed to flow in by gravity. The average dose for a child is from 2 to 20 c.c. and for an adult from 20 to 40 c.c. More depends on the amount of fluid withdrawn than the age of the patient. In severe cases the serum is injected every twelve hours until there is an improvement; in milder cases it is repeated each day for the first four days. Usually from four to six injections are necessary although as many as fifteen have been employed.

Vaccines. — As a prophylactic measure three injections at weekly intervals of 250 millions, 500 millions, and 1 billion respectively have been advocated. In cases where lumbar puncture and serum treatment has had little effect an autogenous vaccine is sometimes employed therapeutically.

THE GONOCOCCUS

Gonorrhea is one of the most widely disseminated of all the infectious diseases. When it was first recognized is not

known; mention is made of it, however, in the earliest medical records.

Neisser in 1879 described a coccus constantly present in the pus of gonorrheal infections, to which he gave the name of gonococcus.

In 1885 Bumm succeeded in isolating it and cultivating it upon coagulated human blood serum. Later he proved its specificity beyond doubt by inoculating it into men and producing the characteristic disease.

Morphology and Staining. — The organism usually occurs in the form of a diplococcus closely resembling the meningococcus. The adjacent sides of the two cocci appear to be slightly concave, so that in stained preparations they have somewhat the appearance of two coffee beans placed side by side. In the early stages of the disease the organisms appear free or lying on the surface of desquamated epithelial cells. When the secretion becomes purulent they may be seen within the protoplasm of the leukocytes in such numbers that the latter may appear to be filled with them. As the disease becomes more chronic the phagocytes appear to become less active and fewer organisms are found engulfed within them. (Fig. 26.)

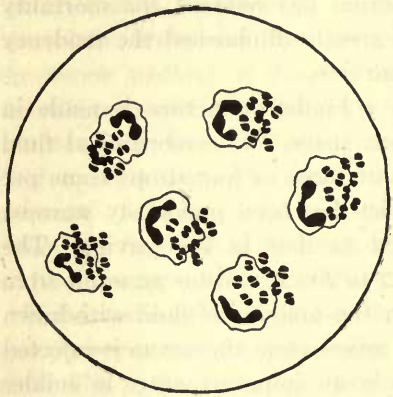


FIG. 26. — Gonococci within and near to Leukocytes.

The gonococcus is not motile, does not produce spores, is easily stained with any of the basic dyes, and is Gram **negative.**

Cultivation. — Growth takes place best at body temperature; below 25° C. and above 40° C. no development occurs. It is advisable to inoculate media as soon as possible after obtaining material from the body and to place the tubes in the incubator at once.

It is seldom a strain of the gonococcus will grow on ordinary nutrient agar; as a rule it requires the addition of blood serum or other body fluid. Colonies appear within twenty-four to forty-eight hours as delicate, finely granular disks with scalloped margins; in color they are grayish white with a tinge of yellow. When freshly isolated the organisms die in from two to three days unless transplanted; older cultures may live three weeks and if kept in the ice box even longer.

Comparison with Meningococcus. — Morphologically and culturally the two organisms resemble each other very closely. The following points are of importance in differentiating them. The meningococcus grows on culture media more readily than the gonococcus. After the first subculture it will frequently grow on nutrient agar, whereas the gonococcus will rarely grow on ordinary agar. The colonies of the latter are less opaque and have a more irregular margin than those of the meningococcus. The meningococcus grows well in broth with a neutral reaction, producing a general turbidity; whereas the gonococcus does not grow, and even if serum is added growth is very scanty and falls to the bottom as a deposit, leaving the medium clear. Of the sugars usually employed the gonococcus ferments glucose only, the meningococcus ferments maltose also. As a rule the part of the body from which the organisms are obtained gives sufficient information.

Resistance. — The gonococcus is very feebly resistant to harmful influences. It is quickly destroyed by drying when in thin layers of pus; in comparatively thick layers smeared on linen it has been found alive after several days. The organism has never been found apart from the human body or material obtained from it.

Pathogenesis. — All attempts to reproduce the disease in lower animals has so far failed. Intraperitoneal injections of living cultures into white mice produce peritonitis, but the organisms appear to be unable to multiply and soon disappear. Injected into the joints of rabbits and dogs, they cause an acute inflammation which soon subsides and the gonococci can no longer be

found. A similar result is obtained when dead cultures are used. Thus it is evident that while large numbers of the organisms can produce a certain amount of inflammation they are unable to multiply and spread in animal tissues.

In human beings infection is usually a result of sexual intercourse. Gonorrhea in men frequently results in prostatitis and epididymitis; in women vaginitis and endocervicitis, or spreading by direct continuity of tissue, the Fallopian tubes, the ovaries, or even the peritoneum may become involved. Sterility may result as a consequence of a salpingitis which obstructs the Fallopian tubes, or in man epididymitis may have a similar result.

The organisms penetrate the mucous membranes and passing between the epithelial cells cause an inflammatory reaction in the tissues below. Secretion increases and numbers of leukocytes gather around the infected area. The infection may remain more or less localized or the organisms may be carried through the blood and lymph to more distant parts of the body. One of the most serious and disabling complications is gonorrheal arthritis; neuralgic affections, muscle atrophies, and neuritis often accompany or follow a gonorrheal infection.

In gonorrheal conjunctivitis microscopic examination of the secretion generally reveals the identity of the invading organism. As the condition becomes chronic the gonococci are less numerous and a greater proportion of other organisms appear. Of all cases of ophthalmia neonatorum about two thirds are caused by the gonococcus.

Immunity. — After recovery from an infection immunity seems to be very slight if indeed it exists. Gonococci may persist in the genito-urinary secretions for years after apparent recovery has taken place and may at any time cause an acute gonorrhea in another person or even in the individual carrying them. There is no limit to the time an individual may remain infected or may infect others.

A slightly bactericidal serum has been produced by animal inoculations. A moderate degree of passive immunity is said to

result from its use in cases of arthritis; in acute gonorrhea it has no effect whatever.

Vaccines. — Vaccines have been employed with good results in joint inflammations and chronic lesions of the urethra and bladder. They are more effective when prepared with the organisms infecting the patient to be treated. Experiments have shown that at least ten different strains of gonococci exist. Hence a polyvalent vaccine in which each type is represented is advisable if an autogenous vaccine is not employed. The dose is from 25 to 500 millions, increasing to 1 billion every three to seven days.

Other Pathogenic Micrococci Resembling the Meningococcus and Gonococcus. *Micrococcus catarrhalis.* The organisms usually occur in pairs, resemble the meningococcus in form, and are negative to Gram's stain. On nutrient agar they will develop between 20° and 40° C. and appear as small yellowish white colonies; on serum agar their growth is much more luxuriant. They are often found on the mucous membrane of the respiratory tract and not infrequently excite a catarrhal inflammation.

Micrococcus Melitensis. — In 1887 Bruce discovered the organism in a case of Malta fever. It was formerly thought that the disease was confined to the shores of the Mediterranean and its islands. Cases have occurred, however, in India, China, South Africa, and some parts of North and South America. The organism is a slightly oval coccus occurring singly or in pairs, non-motile and negative to Gram's stain. Its growth is slow. Colonies on agar are not visible until the third day, when they appear as small round spots, somewhat transparent with bluish white margin and yellowish tinted center. The organism is destroyed by a temperature of 60° C. in twenty minutes, and by carbolic acid 1 to 100 in fifteen minutes. It shows rather a marked resistance to drying.

The milk of goats is considered the chief source of infection, although the disease may be conveyed by other means. A case is reported as contracted by the use of a clinical thermometer; a fatal case occurred as a result of laboratory infection.

In man the disease appears as an intermittent fever, often accompanied by pains of a rheumatic or neuralgic character. The fever usually lasts from one to three weeks and may recur from time to time during a period of several months. The organisms appear in the blood at the height of the fever and are present in various organs and in the urine from the second day to the end of the disease. Autopsies reveal degeneration both of the liver and spleen.

As an aid to diagnosis, blood cultures are usually made during the period the fever is highest. A typical characteristic of Malta fever is the appearance of agglutinins in the serum, which give a marked reaction in high dilutions. The serum of a patient may agglutinate the micrococcus melitensis in a dilution as high as 1 to 1000. Animals injected with the organism will produce a serum which will react in dilutions as high as 1 to 100,000. By this means suspected cultures can be readily identified.

CHAPTER XVII

THE DIPHTHERIA BACILLUS

DIPHTHERIA under various names has been described almost since the earliest days of history. In 1821 Bretonneau of Tours published a very comprehensive essay on the subject and gave to the disease its present name. Little further information was gained until about 1840. Observers began to notice the presence of microörganisms in the pseudomembranes and suggested they might be the causal agents. In 1883 Klebs described a bacillus of rather peculiar appearance which could be almost invariably demonstrated in the false membrane of the throats of those dying of true diphtheria. A year later the organism was isolated and cultivated in pure culture by Loeffler, who described its character and its pathogenic effects on animals. Loeffler was able by inoculation with the bacillus to produce the false membrane on damaged mucous surfaces. But he hesitated to conclude definitely that the organism was the direct cause of the disease, because he was not able to find it in every case thought to be diphtheria, and also he had found it in the throat of a normal, healthy child. Such conditions are more clearly understood now. Similar clinical symptoms are not necessarily produced by the same agent, and the knowledge recently gained that healthy persons may become carriers explains the occasional appearance of the organism in normal throats. Additional confirmatory evidence was given when Roux and Yersin in 1889–1890 showed that the most important features of the disease could be produced by means of the toxins separated from the organisms. By clinical and bacterial observations the relationship of the Klebs-Loeffler bacillus to the disease is now so definitely established that it has become a necessity to find the

organism in the lesion before a diagnosis of diphtheria can with certainty be made.

Morphology and Staining. — The diphtheria bacillus is a slender, straight or slightly curved rod ranging from 1 μ to 6 μ in length. The different strains vary considerably in form and even the same strain may assume a somewhat different shape under changed conditions. Freshly isolated organisms often possess granules which give them a beaded appearance. Others that have been grown on culture media may develop swollen ends that give to them the appearance of an Indian club; others again are thicker in the center and taper at one or both ends. When thickened at one end only they appear somewhat like a wedge. Stained with Loeffler's methylene blue the bacilli may appear uniformly colored or they may present a barred or striated appearance. The round bodies in the granulated forms (metachromatic granules) stain much more intensely than the rest of the organism. This peculiarity of form and staining appears to have a certain relation to the period of growth. A twelve-hour culture is most likely to show granular forms. A twenty-four-hour growth will show more club forms than at twelve hours. Older cultures still stain very faintly. Thus one may often see in a stained preparation the different forms side by side. The round or oval bodies which are so intensely colored with methylene blue appear even more distinct when Neisser's stain is used. Colored by the latter method the granules are almost black, while the remainder of the bacillary substance is of a yellowish brown. Serum cultures of about twelve hours' growth should be employed for this method. It was originally thought that the presence of granules in the bacilli indicated

Fig. 27. — Diphtheria Bacilli.

a degree of virulence, and that by the use of Neisser's stain the virulent forms of the diphtheria bacilli could thus be distinguished from the non-virulent without the delay of inoculating animals. Experiments have shown, however, that the variation in form and staining properties has no relation to pathogenicity and that by no means at present known can the virulent strains be distinguished from the non-virulent except by animal inoculations. The bacilli are non-motile and do not form spores. (Fig. 27.)

Cultivation. — Growth takes place best at body temperature. Development will occur at a temperature as low as 20° C.; below that, however, it usually ceases. The organism is aërobic and facultative anaërobic. When freshly isolated it grows much more readily on media containing serum. The mixture of Loeffler has been found to be one of the best for making cultures direct from suspected throats. At the end of about twelve hours colonies of the diphtheria bacilli appear as pearl-gray or occasionally yellowish gray, slightly raised points a little larger than the colonies of streptococci and a little smaller than those of the staphylococci.

On gelatin at 22° C. a stab culture shows a beaded appearance along the line of inoculation, while at the surface growth forms a small disk. No liquefaction occurs. Milk is an excellent medium. Growth is rapid and luxuriant; the lactose is not fermented nor is the casein coagulated. In broth a cloudiness is first produced which soon settles to the bottom and along the sides of the tube as a fine, powdery deposit. If the broth is inoculated on the surface and the tube is allowed to remain undisturbed growth is apt to occur as a fine but distinct scum upon slightly alkaline nutrient agar to which has been added 1 per cent dextrose. Good growth will result after one or two generations have been cultivated on serum media. The appearance of the colonies on agar is peculiarly characteristic and for this reason it is of value for the isolation of the organism. Surface colonies appear to have a dark, coarsely granular, piled-up center with a thin irregular border which sometimes appears jagged or torn.

Isolation. — Petri plates are first prepared by pouring into them nutrient glucose agar and allowing it to solidify. If the mixed

culture has been grown on ascitic broth a small portion of the pellicle is removed on a platinum loop and lightly streaked over several of the prepared plates; if instead of broth the mixed organisms are removed from serum medium the portion showing colonies which most resemble those of the diphtheria is chosen. The plates are incubated for about sixteen hours at 37° C., after which the most characteristic colonies are "fished" and transferred either to Loeffler's serum tubes or to the ascitic broth.

Resistance. — In cultures the bacilli will live for a long time at room temperature. They may survive for two months or more without transplanting. They are particularly resistant to cold at temperatures just below freezing; they will remain alive for weeks. In a moist condition, whether in cultures or a membrane, their resistance to heat is comparatively low. Ten minutes' exposure to a temperature of 60° C. is sufficient to destroy them. On the other hand, in a dry condition they possess a much greater power of endurance. In a membrane which is perfectly dry they can resist a temperature of 98° C. for one hour. Vigorous toxic diphtheria bacilli have been found on dried membrane four months after its removal from a throat. On toys, pencils, paper money, etc., they may live for several weeks. Their resistance to disinfectants is much the same as that of other non-spore-bearing bacteria. They are killed in a solution of mercuric chloride 1 to 1000 in from one to five minutes and in carbolic 1 to 100 in from five to ten minutes.

Pathogenesis. — With the exception of rats and mice most of the lower animals are susceptible to the toxin of the diphtheria bacillus, yet it is extremely rare that the disease appears in them. In fact the cat is the only animal known to have contracted diphtheria from contact with the disease. False membranes similar to those produced by the diphtheria bacillus in human beings may occur in animals, but only when the membrane has been first abraded and then virulent organisms either rubbed on to it or injected into it.

Very small quantities of a virulent broth culture injected subcutaneously will produce symptoms of toxemia in a guinea pig

within six to eight hours. If the animal does not succumb to a rapid intoxication signs of paralysis appear in the lower extremities, gradually extending to the entire body, and causing death by paralysis of the heart or respiratory muscles. Upon autopsy the site of inoculation is found to be congested and the neighboring lymph nodes swollen; the adrenals are congested; an excess of fluid appears in the serous cavities and in the heart; voluntary muscle fibers and nervous tissue show signs of degeneration.

In human infection the disease is characterized by a pseudomembrane on a mucous membrane, or occasionally upon the surface of a wound and a general toxemia. The site of the pseudomembrane is usually the throat, larynx, or nose; diphtheritic infection of the middle ear is not uncommon; infection of the conjunctiva sometimes occurs as a result of a patient's coughing or sneezing into the eye of another person. The local lesion is the result of bacterial invasion, and consequent degeneration of the epithelial cells gradually extends to the underlying tissues. A profuse fibrinous exudate is poured out, and soon spreading over the surface a false membrane appears composed of fibrin, leukocytes, dead tissue cells, and bacteria. The pseudomembrane may be so thick and firmly adherent as to leave a torn and bleeding surface when displaced.

The most serious injuries caused by the diphtheria bacillus are the systematic lesions due to the absorption of its poisons. Diphtheria is primarily a toxemia. As a result fatty degeneration takes place in the muscle fibers of the heart, in the myelin sheath of the peripheral nerves, and in the white matter of the brain and spinal cord and in the kidneys. These changes in muscle and nerve explain the paralysis and cardiac weakness so often following an attack of diphtheria. When death occurs as a result of the infection it is usually due to toxemia, laryngeal obstruction, or broncho-pneumonia.

Diphtheria Toxin. — Diphtheria bacilli when growing in nutrient broth produce a soluble toxin which diffuses from their bodies into the surrounding medium. Loeffler assumed the presence of such a poison but Roux and Yersin were the first to obtain it apart

from the living bacilli by filtration through a porcelain filter. Little is known regarding its chemical nature, but it has many of the properties of protein substances. It is completely destroyed by boiling for five minutes and loses a great deal of its strength when heated to 75° C. Its toxicity is lost when exposed for a few hours to direct sunlight. On the other hand, kept in cool and dark storage it deteriorates very slowly.

The symptoms produced in animals are practically the same whether cultures of living bacilli or the germ-free toxin be injected, except that when toxin only is introduced no false membrane is formed. The lesions which occur in the heart and other organs are identical; consequently there is sufficient proof that the chief injury to the body is caused by the powerful poison secreted by the living bacterial cells grouped together in enormous numbers in the false membrane of the throat. The organisms pour out their poison, which readily passes into the underlying tissues and diffuses through the body, injuring particularly those cells for which it has a special affinity.

There is a wide variation in the ability of diphtheria bacilli to produce toxin. The great majority of organisms isolated from throat exudates or pseudomembranes which possess the characteristics of the diphtheria bacillus are found to be strongly toxic. There are, however, grades of toxicity until finally we reach a small group sometimes found in slightly inflamed or normal throats which are morphologically and culturally identical with the Klebs-Loeffler bacillus yet do not produce in culture media or test animals the diphtheria toxin. From a public health standpoint such organisms are harmless, since it has not been proven that a non-toxin producer ever develops the power. It may be that the ancestors of these organisms were true diphtheria bacilli and that succeeding generations have by attenuation lost the power of producing toxin. That, however, is only a supposition. Certain investigators claim that a true diphtheria bacillus never completely loses its ability to produce toxin, however attenuated, and that related bacilli which do not possess the power never gain it. The passage of diphtheria bacilli through the body of a susceptible animal has little effect on their toxin production.

The severity of the disease produced by the diphtheria bacillus cannot be regarded as an index of the virulence of that particular strain. Association with other organisms and the presence of varying amounts of antitoxin in the blood of the patient may mask the real power of the invader. Descendants of the same organism may give rise to mild symptoms in one person and to a fatal infection in another.

Persistence of Diphtheria Bacilli in the Throat. — The length of time the bacilli continue to live in the throat after apparent recovery varies greatly. "Diphtheria bacilli disappear in about 50 per cent of cases by the time the local membrane has disappeared. They persist in about 5 per cent of persons at the end of two months, about 2 per cent at the end of three months, and approximately 1 per cent continue as chronic bacillus carriers." (Rosenau.)

Immunity. — The fact that fully toxic bacilli have been frequently found in the throats of healthy persons who have been brought in contact with diphtheria patients, yet who have not contracted the disease, demonstrates that diphtheria, like other infectious diseases, requires not only the presence of the specific organism but also a susceptibility on the part of the individual. It is estimated that about 70 per cent of all persons are protected from infection because of an antitoxin present in their blood. Conditions therefore which impair vitality and diminish the production of specific antibodies increase susceptibility.

Immunity following an attack of diphtheria usually lasts for several months or even years. Occasionally, however, it is of much shorter duration. Infants and adults possess relatively more immunity than young children between the ages of two and ten years. It is known that young animals born of immunized mothers inherit a certain degree of resistance. This may explain the relative insusceptibility of children during the first months of life. Passive immunity is only of short duration; the antitoxin injected usually disappears from the blood in less than three weeks. The nature of antitoxin and its prophylactic and therapeutic use is discussed in Chapter II.

Mixed Infections. — The diphtheria bacillus is not the only organism usually found in the false membrane. Associated with it are frequently found the pyogenic cocci. The streptococcus is an especially useful ally in disintegrating the surface cells of the mucous membrane, and making possible the penetration of the diphtheria bacillus into the deeper tissues, thus facilitating the absorption of its toxin. Certain suppurative conditions of the throat are unquestionably due to the pyogenic cocci. In most cases of fatal broncho-pneumonia following diphtheria streptococci or pneumococci or both are usually the inciting organisms and as diphtheria antitoxin has absolutely no effect on them they frequently are the cause of death. The presence of certain other bacteria may often be detected by a difference in the exudate; for example: B. fusiformis gives rise to an offensive odor, B. pyocyaneus to a bluish green color.

Bacteriological Diagnosis. — A pseudomembrane in the nose or throat is usually but not always the result of an infection by the Loeffler bacillus. Many other organisms frequently present in the throat secretions, such for example as streptococci and pneumococci, can under certain conditions produce a local lesion very similar to that of a mild case of diphtheria. Vincent's angina and the pseudomembrane in scarlet fever somewhat resemble that produced by the diphtheria bacillus. Generally, however, the deposit in the first-named diseases appears rather as an exudate than a membrane.

Nearly all membranous affections of the nose are diphtheritic, as are also thick, grayish membranes spreading over a large portion of the tonsils and the soft palate. Seen on the tonsils alone the presence of the diphtheria bacillus is less sure.

In uncertain cases bacterial examination is of the greatest value since the disease has such a rapid onset and the early administration of antitoxin is necessary both for the suspected case and those who may come in contact with it.

The examination of cultures made from suspected throats is usually a routine procedure in municipal laboratories. Outfits are supplied to physicians on request. These consist as a rule

of a tube of freshly prepared Loeffler's serum, a tube containing a sterile swab of absorbent cotton firmly wound on a strong iron wire, and printed directions and record form. The whole outfit is inclosed in a metal or wooden box.

In order to obtain a satisfactory culture the patient is placed in a good light; the tongue is depressed and the swab, removed from its tube, is gently but firmly rubbed against any visible membrane without being allowed to touch any other part of the mouth or throat. The swab is immediately inserted in the serum tube and the portion which has touched the exudate rubbed on the surface of the media; it is then returned to its tube, the plug inserted, and both tubes with the record blanks filled out are returned to the laboratory.

When there is no visible membrane it is advisable to make two cultures, one from the nose and another from the throat. Needless to say, cultures should not be made shortly after the application of a disinfectant.

On reaching the laboratory the inoculated tubes are placed in an incubator at 37° C. for twelve hours; at the end of that time and often before the serum will be found to be dotted with small colonies. A microscopic preparation is made by first placing a platinum loopful of sterile water upon a glass slide, and then by means of a platinum needle a number of typical colonies are removed from the culture tube and smeared in the droplet of water over the slide. The preparation is fixed in the usual way and stained with Loeffler's methylene blue for about five minutes. Examined with the oil immersion lens the film may show enormous numbers of diphtheria bacilli with few cocci, or the reverse, or an equal number of both forms.

An immediate diagnosis can often be made without the use of cultures by smearing a little of the exudate from the swab directly over the slide. The result is a little less satisfactory; the bacilli appear less typical and are mixed with fibrin and epithelial cells.

Animal Inoculations as a Test of Toxicity. — No means of determining with certainty the virulence of diphtheria and diphtheria-like organisms found in the throats of patients not showing signs

of diphtheria or in the throats of healthy individuals suspected of being carriers is known save that of animal inoculation. For this purpose an alkaline forty-eight-hour broth culture is employed and two guinea pigs are inoculated subcutaneously, one with two c.c. of the culture, the other with the same amount of culture plus a protective amount of antitoxin. If within four days the guinea pig receiving the toxin only dies, and the one receiving the toxin plus antitoxin lives, the organisms injected were undoubtedly diphtheria bacilli.

Another and more economical method is as follows: the hair is removed from the abdominal surface of two 250 gram guinea pigs, by shaving or plucking. The twenty-four-hours growth on Loeffler's serum of the organism to be tested is emulsified with 20 c.c. of salt solution, and 0.15 c.c. of this suspension is injected intracutaneously into the prepared abdominal surface of each of the two guinea pigs. One of the animals is injected intracardially at the same time with 250 units of antitoxin or an intraperitoneal injection of the antitoxin is made twenty-four hours before. In this way six cultures may be tested on the same animals. Virulent diphtheria bacilli produce an infiltration and superficial necrosis at the site of inoculation in from two to three days, while in the guinea pig protected by the antitoxin the skin remains normal.

Bacteria Resembling Bacillus Diphtheria. — *Bacillus Hoffmanni* organisms often spoken of as **pseudo** diphtheria bacilli are frequently found in normal throats and in some instances in those of diphtheritic individuals. At first they were regarded as attenuated diphtheria bacilli. Later investigators, however, consider them as a different species. They appear as short, thick rods, stain solidly with methylene blue, do not show granules when stained with Neisser's stain, are not motile, and do not form spores. Their colony growth on Loeffler's serum media closely resembles that of the diphtheria bacillus. They differ from the latter organism, however, in that they are unable to ferment any of the sugars; they do not produce toxin and are not pathogenic for guinea pigs.

Bacillus Xerosis. — Diphtheria-like bacilli were found by Hutschert and Neisser in 1904 in a chronic form of conjunctivitis known as xerosis, which they believed to be the causal agent. Since then the organism has so frequently been isolated from normal eyes that it is no longer considered as the cause of the disease. Morphologically it is almost identical with the diphtheria bacillus. It differs from B. diphtheriæ and B. Hoffmanni in its ability to ferment sugars, but it resembles the latter in that it produces no toxin and is non-pathogenic for animals.

Still other organisms exist which closely resemble the diphtheria bacillus structurally. They are apparently numerous and have been found both in normal and diseased conditions, although it is considered somewhat doubtful whether they ever incite disease. As yet no classification of these organisms has been made and they are grouped together under the term *Diphtheroids*.

CHAPTER XVIII

THE TUBERCLE BACILLUS AND OTHER ACID-FAST ORGANISMS

It is estimated that in the United States 160,000 persons die each year of tuberculosis. For centuries the disease has been recognized, but only within comparatively recent times has its infectiousness been scientifically established. The fact that tuberculosis might be induced by inoculation with tuberculous material was demonstrated by Villemin in 1865. Baumgarten early in 1882 described the bacilli in tissue sections, but it remained for Robert Koch to isolate the organism, to grow it in pure culture, and with the pure culture to reproduce the characteristic lesions in animals. Koch announced his discovery in 1882 and in 1884 he submitted his full report.

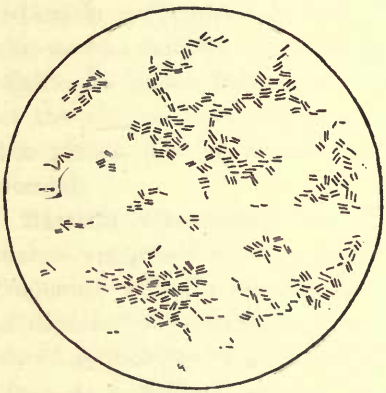


Fig. 28. — Tubercle Bacilli.

Morphology and Staining. — Tubercle bacilli appear as slender, non-motile rods about 2 μ to 4 μ in length and 0.3 to 0.5 μ in width. In colored preparations they may appear straight or slightly curved, single or lying together in heaps. Ordinarily they stain uniformly (Fig. 28). Frequently however, deeply stained thickenings are seen which give to the organism a somewhat beaded appearance. At first these granules were thought to be spores. Soon, however, it was shown that the bacilli containing them were no more re-

sistant to heat and drying than others in which they were not found and that consequently they could not be regarded as spores.

Occasionally long thread-like branching forms, sometimes with swollen ends, are seen. They are considered by some observers as involution forms; others regard them as normal and on this basis class the tubercle bacilli either with the higher bacteria (trichomycetes) or with the true molds; still others place them and closely related forms in a separate group intermediate between the ordinary bacteria and the higher forms. Their classification is still an unsettled question.

Another peculiarity of the tubercle bacillus is the possession of a waxy envelope, which not only confers upon it an additional degree of resistance, but which also prevents it from readily taking up the ordinary anilin dyes. Once stained, however, it is with difficulty that the color can be removed even by the use of strong acids. The property is so characteristic that the tubercle bacillus and certain other closely related organisms are termed "acid-fast." This staining peculiarity makes it possible to recognize the organism immediately in film preparations from pus, sputum, etc. Details of the special stains and methods employed have already been described.

Cultivation. — On account of their slow growth tubercle bacilli are difficult to cultivate and even more difficult to isolate. Koch succeeded in obtaining a pure culture by carefully rubbing tuberculous tissue over the surface of coagulated beef serum, and then after about two weeks' incubation there appeared minute points of irregular whitish growth which he compared to small, dry scales. Once isolated the organism will grow readily on egg medium or on agar containing 3 to 5 per cent glycerin; in from ten to fourteen days growth appears as a dull, whitish, wrinkled film. In thin layers of glycerin broth, if a small amount of growth is carefully placed on the surface it spreads as a wrinkled pellicle from one side of the container to the other; this method of cultivation is usually employed for the production of tuberculin.

It frequently happens that in tuberculous tissue tubercle bacilli are found free from contaminating organisms and a practically

pure culture is obtained on media inoculated with such tissue. Sputum, on the contrary, usually contains many other varieties which grow with much greater facility than the tubercle bacillus and so completely inhibit the growth of the latter. Isolation from such material is best accomplished by injecting it into guinea pigs; the animal will die in from four to six weeks and the bacilli may be obtained in pure culture from the lymph nodes near the point of injection and frequently from tubercles in the various organs. The optimum temperature for growth is 37° to 38° C.; below 30° and above 42° C. development rarely occurs.

Resistance. — Tubercle bacilli show a greater degree of resistance to external influences than most non-spore-bearing organisms. When completely dried they can withstand a temperature of 100° C. for forty-five minutes; separated in fluids such as milk they are destroyed by exposures to 60° C. in twenty minutes. Cold has little effect upon them. In sputum exposed to direct sunlight the organisms are killed in a few hours; in diffuse daylight in a few days. Dried in rooms that have little light, they have been found alive after ten months. It is not probable that the tubercle bacilli ever multiply outside of the body save in freshly expectorated sputum and on artificial culture media. In sputum they are destroyed in six hours by the addition of an equal quantity of 5 per cent carbolic acid. Bichloride of mercury is unsatisfactory as a disinfectant because it combines with the mucus present and has little effect upon the bacteria.

Pathogenesis. — Tubercle bacilli do not produce true toxins, but their bodies contain poisonous substances, probably of the nature of endotoxins. In the animal body the local lesion produced is usually in the form of a tubercle or nodule which varies somewhat in the different tissues. If the bacilli gain entrance to the connective tissue their first action appears to be on the connective tissue cells which soon begin to show that some irritant is acting upon them. The cells become swollen and mitotic division occurs, the resulting so-called epitheloid cells being much larger than the parent cell and possessing paler nuclei. Very soon small foci of epitheloid cells are formed about the bacilli and at

the same time numbers of leukocytes begin to appear in the neighborhood. When living bacilli are present and sufficiently virulent to multiply the lesion increases, the central cells degenerate into a cheese-like mass, and later a cavity results.

The most characteristic lesions caused by the tubercle bacilli are the so-called miliary tubercles which, before they undergo degeneration, appear as hard, gray, translucent nodules rather smaller than a millet seed in size. Instead of the miliary tubercles, however, a diffuse growth of tissue may occur similar in structure to the former and which also tends to undergo cheesy degeneration.

The general symptoms of tuberculosis, — fever, perspiration, and emaciation — are due to the absorption and distribution throughout the body of the bacterial poison.

Modes of Infection. — Occasionally the organisms attack the abraded skin or mucous membranes and lupus develops or a nodular growth. Their main entrance to the body, however, is through the respiratory tract or the digestive tract.

The organisms leave the body chiefly in the sputum of open cases of pulmonary tuberculosis and in other cases in any discharges from tuberculous lesions opening into the skin. In pulmonary cases the sputum is often swallowed, with the result that tubercle bacilli may be excreted in the feces. Thus all of the discharges from the body may be infective.

Because pulmonary tuberculosis is of far more frequent occurrence than any other form, tuberculosis was for a long period considered as an air-borne infection. The opinion was strongly expressed by Koch in 1884 and was for many years practically universally accepted. An interesting point in support of this theory is that it requires very few organisms by inhalation to give rise to the disease, whereas thousands are necessary by mouth to produce infection of the alimentary canal. On the other hand, the lungs are greatly protected from external infection both by their location and by the moist ciliated epithelium lining the nasal and pharyngeal passage. Also the fact that the lesion in the lungs is usually at the apex and not in the direct line that floating

particles in the air would be mechanically carried seems to indicate that tubercle bacilli may find their way to the lung tissue by other means than direct inhalation. Further, it has been demonstrated that sputum exposed to direct sunlight will, especially in summer, be disinfected by the time it is in a condition to be carried into the air as dust. Tuberculous sputum when expectorated in shady parts of the street, or in houses, or in dark places, however, constitutes a real menace. It has been estimated that as many as five billion tubercle bacilli may be expectorated by a single individual in twenty-four hours. Consequently the neighborhood of tuberculous individuals who expectorate without taking any precautions to prevent the spread of infection is exceedingly dangerous. In rooms occupied by such persons dried sputum containing virulent bacilli may be constantly in the air blown about by sweeping, walking, the closing of doors, etc. As long as sputum remains moist there is no danger of infection by inhalation; the only danger then lies in direct contact.

During ordinary breathing the expirations of a patient suffering from pulmonary tuberculosis are normally free from bacteria. In forced efforts, however, such as coughing, sneezing, and loud speaking, fine particles of throat secretion are thrown out as a light spray, which may be laden with organisms that may have been present in the mouth. Tubercle bacilli thus sprayed may fall directly on the mucous membrane of a healthy individual or may be conveyed indirectly by food or other objects.

As early as 1868, and many years before the discovery of the tubercle bacillus, Chauveau suggested that the causal agent might gain entrance by way of the intestinal canal. Later investigators have proved beyond question that such often is the case. Tubercle bacilli ingested in food and drink may pass through the mucous membrane of the digestive tube without leaving any trace of their passage, gain access to the blood, and so be carried to distant parts of the body. The fact that the disease usually localizes itself in the lungs may be because this organ presents the least resistance. It is even claimed by some authorities that no matter how the tubercle bacillus reaches an individual, whether by dust

or droplets, fingers or food, it passes either through the tonsils or the mucous membranes of the upper respiratory tract or is carried to the intestines and passed through the tissues there.

Infection by drinking milk from tuberculous cows has been clearly demonstrated. It has also been shown that such infection does not necessarily come from cows with tuberculous lesions of the udder, but may be conveyed in milk from cows showing no lesions of the udder whatever. Perhaps in all such cases dried feces falling into the milk from the skin of the cow is responsible for the presence of the tubercle bacillus. Human infection by this means must necessarily pass by way of the tonsils or the alimentary tract. The majority of cases of cervical adenitis and abdominal tuberculous in young children are undoubtedly contracted in this manner.

It may be stated, then, that the two chief modes of infection are by inhalation and by ingestion of the tubercle bacillus. In the former the organisms are for the most part derived from human beings; in the latter, milk and milk products from tuberculous cows or food contaminated from human cases are responsible.

Heredity.— In the strict sense of the word tuberculosis is not considered hereditary. It is extremely unlikely that spermatozoa or ova infected by tubercle bacilli would undergo normal development. It is generally conceded that a hereditary tendency or disposition to the disease may be transmitted from the parent to the offspring, although what the tendency is has not been clearly defined; it may be a feeble constitution, or a structural peculiarity or possibly an inability on the part of the body cells to generate defensive antibodies when infection occurs. Congenital infection, though rare, does occasionally occur, in which case tubercle bacilli pass from the mother to the fetus by way of the placenta. Extrauterine infection is much more likely to be the cause of tuberculosis in infants. Animal experiments have shown that the young of infected mothers are usually infected only when suckled by the tuberculous parent; when nourished by a healthy foster mother they remain normal. The fact that tuberculosis seems to persist in certain families may be due solely to the intimate associations

of home life and the lack of precautions in preventing the sick from infecting the well.

Immunity. — Although recovery from tuberculosis is of frequent occurrence the processes involved are extremely obscure. Many attempts have been made to produce artificial immunity in tuberculosis as in other infectious diseases, but so far all have failed. Tuberculous infection appears to differ in many respects from other infectious processes. Tubercle bacilli may invade the tissues and foci develop without sufficient bodily disturbance to attract attention; also an infection, so far as clinical symptoms are concerned, may be completely cured, yet the focus may remain and though completely walled off and non-progressive may contain virulent tubercle bacilli during the whole of the individual's life.

Koch discovered that infected animals reacted differently to an injection of living bacilli than did normal animals. When healthy animals are inoculated with virulent organisms tubercles develop near the point of inoculation; the infection is usually carried to the various organs and the animal dies of generalized tuberculosis. A tuberculous animal on the contrary shows an immediate and violent reaction. A marked inflammatory area around the point of injection occurs, followed sometimes by necrosis and sloughing, but with no advance of the infection beyond the point of injection. The reaction well illustrates the phenomenon of hypersusceptibility. Following a first injection of the tubercle bacilli or infection by other means the tissue cells offer no immediate resistance and the disease progresses. The presence of the organisms, however, so sensitizes the cells that a second invasion is resisted immediately and vigorously, and protecting substances and phagocytic cells are concentrated upon the point where they are most needed, as is evidenced by the prompt inflammatory reaction.

Koch, as a result of these observations, concluded that the resistance of tuberculous individuals might be further increased by the injection of disintegrated bacteria and the products of their growth, and with this in view he prepared tuberculin. Un-

fortunately the great hopes at first entertained have not been realized. A certain number of cases of increased resistance and clinical cures have, however, been reported from its use as a therapeutic agent.

The tuberculin reaction is local, focal, and general. The local reaction appears as an inflammatory condition at the point of inoculation. The focal reaction consists of an increased blood supply around the infected area and a consequent softening of the focus and a liberation of toxic products which give rise to the general reaction.

If the dose of tuberculin is not too large the focal reaction soon subsides, with the result that increased cellular activity has caused a further proliferation of connective tissue and fortified the wall surrounding the tuberculous process. In chronic lesions of the bones or inactive skin or ear cases, in which the body cells are only feebly active in self-defense, small doses of tuberculin are reported to have a stimulating beneficial effect. Should the dose of tuberculin be too large or the body cells incapable of reacting, the focal reaction may be a softening and breaking down of the lesion with liberation of the bacilli and spread of the tuberculous area. Thus tuberculin as a therapeutic agent is a somewhat dangerous weapon. Its success appears to rest upon administering just the right amount to call forth sufficient response without overtaxing the already sensitized body cells.

Tuberculin as a Diagnostic Agent. — The allergic or hypersensitive state of the tissue cells in tuberculous individuals makes possible the use of tuberculin as a diagnostic agent, although the phenomenon is as yet little understood.

A number of tuberculin preparations have been employed. The following is the method originally employed by Koch and usually designated "O.T." A six-weeks-old culture of tubercle bacilli in 5 per cent glycerin broth is killed by heat, filtered, and evaporated down to one tenth of its original volume. The resulting fluid thus contains the products of disintegrated bacilli, substances formed from the medium during their growth and the medium itself.

The Intracutaneous Test of Mantoux. — The test is made as follows: the skin of the forearm is cleansed with alcohol and then with ether and a series of injections of different dilutions of tuberculin are made intracutaneously. Four dilutions are used: 1 to 10,000,000, 1 to 1,000,000, 1 to 100,000, and 1 to 10,000. The amount injected of each is generally 0.1 c.c. A control injection of 0.1 c.c. of sterile normal salt solution is made at the same time. A positive reaction appears in six or eight hours and usually subsides in six to ten days.

The Percutaneous Test of Moro. — Equal parts of lanolin and tuberculin are made into an ointment and a small amount is rubbed into the skin on the chest. A positive reaction appears within one to four days as an eruption of slightly elevated papules.

The Cutaneous Test of Von Pirquet. — The test is carried out as follows: the forearm is cleansed with alcohol and ether and two small scratches are made about three inches apart, care being taken not to cause bleeding. On one scratch a drop of tuberculin is placed, the other is left as a control. A positive reaction may appear in from three to ten hours as a slightly raised reddening of the skin, usually circular and about 10 mm. in diameter.

Ophthalmic Test of Calmette. — For this test either a 2 per cent solution of Koch's old tuberculin or a purified form is used. One drop of the solution is placed in the conjunctival sac and the fluid is allowed to spread over the surface. In a positive reaction the conjunctiva is inflamed. The lids become congested and their inner surface of a bright red color, and varying amounts of a fibrinous exudate appears. The reaction reaches its maximum in from six to ten hours and disappears usually in from two to three days. The ophthalmic test is easily applied, but is little employed, owing to serious dangers that may result.

So far as is known there is no positive skin reaction without infection. A positive reaction, however, tells nothing of the location or extent of the lesion nor if it is a progressive or an encapsulated focus. Very advanced cases frequently show little response to tuberculin tests, the tissue cells being evidently incapable of further effort.

Varieties of Tubercle Bacilli. — At least four types of tubercle bacilli are recognized: human, bovine, avian, and fish. The human and bovine varieties closely resemble each other. The former appear somewhat longer and more slender than the latter, show a greater tendency to irregularities in staining, and grow more luxuriantly on culture media. The important difference lies in the fact that the human type is very pathogenic for man, but is considerably less so for cattle and other animals. On the other hand, the bovine type is very pathogenic for almost all mammalians except man; it is pathogenic for man, but much less so than the human type. The critical laboratory test for differentiating the two varieties is made on rabbits; a one hundredth of a gram of a young bovine culture injected intravenously into a rabbit will cause generalized tuberculosis in about six weeks, whereas fifty to one hundred times the amount of the human variety produces at most a slight tuberculous lesion.

About 10 per cent of all cases of tuberculosis in young children under five years of age is due to bovine tubercle bacilli. The fact that such infections are usually localized in the cervical or abdominal lymph nodes strongly suggest that the portal of entry is the tonsils or small intestines and that cows' milk is the source of origin.

Avian tubercle bacilli correspond in morphology and staining reactions with the above types. They differ in that they grow luxuriantly on culture media at 45° C. and can even multiply at a temperature as high as 50° C. On glycerin agar or blood serum an abundant growth appears within ten days, white, moist, and fat-like, and totally different to the dried and wrinkled appearance of the human type. Chickens, pigeons, and pheasants are very susceptible; geese and ducks appear to be immune.

The tubercle bacillus of fish was first isolated from lesions in a carp. Microscopically it resembles the other forms. On culture media growth is thick and moist like that of the avian type. Its temperature requirements, however, are very different to the varieties found in warm-blooded animals; growth occurs between

12° and 36° C., the optimum temperature being 25° C. Neither the avian nor the fish tubercle bacilli are pathogenic for man.

OTHER ACID-FAST BACILLI

Bacillus of Leprosy. — Hansen in 1874 reported the presence of a bacillus in the tubercles of leprous individuals which somewhat resembled the tubercle bacillus. Later many other observers confirmed Hansen's report. In tissue sections the bacilli appear as thin rods generally within the cells of the granulation tissue and are often so numerous that the cell structure is hidden; usually they are arranged parallel to one another and present the appearance of small bundles. They take up the basic anilin dyes more readily than the tubercle bacilli, but like them they resist decolorization with the mineral acids and alcohols. The organisms differ from the tubercle bacilli in that they grow with difficulty on artificial culture media and are much less if at all pathogenic for the lower animals.

In 1908 certain workers succeeded in growing an acid-fast organism on plain agar in symbiosis with ameba and other bacteria, and then by killing the other organisms by means of heat they obtained a pure culture of "acid-fast" bacilli. There is no satisfactory evidence, however, that such organisms will reproduce the disease in experimental animals.

The negative results following attempts to produce the disease by inoculations of leprous tissue have led to the assumption that the bacilli in such tissue must be for the most part dead. An apparently successful attempt was made upon a criminal in the Sandwich Islands, who obtained pardon on condition that he allow himself to be inoculated with leprosy. He consented and the disease did develop two or three years later. The experiment is open to objection, however, because the man had, before inoculation, been frequently in contact with lepers and had thus been exposed to the infection in a natural way.

It has been supposed by some observers that leprosy is a form of tuberculosis. There is little ground for the supposition, although

it has been found that a considerable portion of lepers react to tuberculin like tuberculous patients.

Rat Leprosy. — A disease occurs in rats which closely resembles leprosy. The relation between the disease and that occurring in human beings has not as yet been established.

Smegma Bacillus. — The organism is present in the secretions of the external genitals. It has much the same appearance and staining reaction as the tubercle bacillus. It is non-pathogenic.

Bacillus of Johne's Disease or Paratubercular Dysentery of Cattle. — The disease is characterized by chronic diarrhea and emaciation. The organisms isolated from the lesions closely resemble the tubercle bacillus.

Timothy Grass Bacillus. — Other organisms showing various degrees of acid-fastness have been isolated from grass, cow manure, milk, butter, etc. They have little interest apart from the fact that they can only be distinguished from the tubercle bacillus by animal inoculation.

CHAPTER XIX

INTESTINAL BACTERIA. THE COLON–TYPHOID GROUP

DURING life a great variety of organisms find suitable conditions for development in the intestinal tract. At birth the meconium of healthy infants is sterile. Very soon, however, bacteria gain entrance to the alimentary canal through the rectum or by way of the mouth from swallowing saliva or food. As the reaction and amount of oxygen at different levels vary, each species tends to remain in that portion of the tract in which it finds its optimum conditions. Aërobic organisms are most abundant in the mouth, although hidden in the crypts of the tonsils and in folds of mucous membrane anaërobes may flourish. Those organisms which are able to withstand the acidity of the gastric juice and succeed in reaching the duodenum find the alkaline reaction there much more favorable to development; also the diminished amount of oxygen renders conditions particularly suitable for anaërobic or facultative anaërobic growth. The available food supply is probably the most important factor in determining the type of organisms likely to develop. In breast-fed infants, for example, lactose being more abundant than any other ingredient in the milk, fermentative organisms predominate. Later as protein is added to the diet proteolytic bacteria become more numerous and the fermentative type relatively decrease. Many investigators have tried to determine whether the presence of bacteria in the intestinal tract is of physiological benefit to the individual. Successful experiments have shown that at least they are not a necessity. An infection with pathogenic organisms, such as dysentery or cholera, totally changes for a time intestinal condi-

tions. The invaders multiply, produce local lesions, and more or less crowd out the normal inhabitants.

Apart from infections abnormal conditions may result from the unbalanced activities of the organisms ordinarily present in the intestines. So far as at present known two distinct processes may be concerned: (1) excessive bacterial proteolysis, by means of which toxic substances are produced from the protein ingested as food which when absorbed by the body cells give rise to the condition known as " auto-intoxication," or (2) excessive carbohydrate fermentation which may result in an overproduction of acids or other irritating substances and cause a chronic diarrheal condition.

Of the many varieties of bacteria occupying the intestines of man and animals a certain group, ordinarily non-pathogenic, are classed together as " colon bacilli " because they live in the colon and have similar characteristics. Closely related morphologically and biologically to the colon bacilli are a number of other organisms which when they gain access to the intestines give rise to distinctly morbid conditions. The entire group is termed the *colon-typhoid group*. Such members as the typhoid and paratyphoid bacilli, including the types responsible for meat poisoning, are specifically pathogenic; the colon bacilli and their near relatives are pathogenic only under certain circumstances.

The group is usually arranged in four subdivisions:

1. Colon Group. Normally present in the intestines, rarely pathogenic.
2. Para-typhoid Group. Possessing varying degrees of pathogenicity.
3. Bacillus typhosus. Pathogenic.
4. Dysentery Group. Pathogenic.

All members of the entire group possess certain common characteristics. They are rather short, non-spore-bearing bacilli; stained by Gram's method they are decolorized; none of them liquefies gelatin.

Apart from these general features there appears to be a variety

of cross relationships, and in order to determine the subdivisions and to differentiate the members of each subdivision careful observation of their fermentative action on the various carbohydrate media, and their agglutinative reaction in immune sera is necessary.

From the above it will be understood that around each particular type a number of variants are grouped. Thus, for example, in different epidemics of dysentery different strains of bacilli have been isolated, varying from each other only in minor details, but corresponding in certain points which mark them as close relatives and warrant their classification as one group.

The Colon Group. — The first description of a member of this group was given by Emmerich in 1885, who isolated the organism from the dejecta of a patient suffering from Asiatic cholera. In 1886 Escherich obtained a similar bacillus from the feces of healthy infants, to which he gave the name Bacillus coli communis. Later it was shown that closely allied types are normal inhabitants of man and animals.

The organisms are widely distributed in nature. Transferred through the feces as manure or sewage, they are found on cultivated land and in surface waters. They are most abundant, however, in the intestines of man and animals and particularly in that portion from which they derive their name. Apart from the fact that under certain conditions the organisms may excite disease, they have special hygienic interest in that their presence in water or milk is an indication of fecal pollution. The presence of colon bacilli does not necessarily mean the presence of typhoid or dysentery bacilli, but it indicates the possibility when the contamination is of human origin.

B. Coli Communis. — The colon group contains many varieties; of these B. coli communis has probably been the most studied and may be considered as the most representative.

Morphology and Staining. — The typical forms appear as short rods with rounded ends ranging from 1 to 3 μ in length and 0.4 to 0.7 μ in diameter. They possess seven or eight peritrichic flagella. Motility, however, varies in the different

strains; sometimes in young cultures it is quite active; in others it may be so sluggish as to be hardly distinguishable from Brownian movement. B. coli communis stains readily with the ordinary aniline dyes, is Gram negative, and does not form spores.

Cultivation. — The organism is an aërobic and facultative anaërobe. It grows best at 37° C., but multiplication will occur as low as 10° C. It develops on the simplest culture media; in broth it grows rapidly causing a general clouding of the medium. In gelatin stabs growth occurs along the line of inoculation and spreads along the surface of the medium almost to the sides of the tube. Surface colonies on agar are of a grayish color, round and glistening, and often showing a peculiar structure somewhat resembling a grape leaf. Colonies growing deep in the medium may be oval or the shape of a whetstone. On potato growth is abundant, changing from a grayish white in young cultures to a yellowish brown in older ones. In milk coagulation occurs from one to four days, principally due to the production of lactic and acetic acid from the lactose present. In lactose-litmus-agar the medium becomes red and gas bubbles frequently appear by the side or under the colonies. The organism is able to ferment a number of carbohydrates; it produces acid and gas in media containing dextrose, levulose, galactose, lactose, maltose, and mannite. It develops especially well on media containing urine or bile.

B. coli communis does not peptonize albumins; it does, however, break down some of the higher nitrogenous compounds into smaller molecules. Indol is one of the most important products of its activity, although little appears to be formed in the intestinal canal in health. Nitrates are reduced to nitrites and from them ammonia and free nitrogen are produced.

Resistance. — The organisms are able to resist a higher degree of acidity or alkalinity than most non-spore-bearing forms. They are killed in from five to ten minutes by a temperature of 60° C. Frozen in ice, a certain percentage will live for six months. In carbolic acid 1 to 100 they are destroyed in five to fifteen minutes.

Pathogenesis. — Intraperitoneal injections of 1 c.c. or more of a broth culture into a guinea pig or rabbit may cause death within

twenty-four to forty-eight hours. There is a rapid decline in temperature, and finally the development of a fibrino-purulent peritonitis, due undoubtedly to the endotoxins liberated from the disintegrating bacteria.

In man they are considered as the cause of the majority of cases of cystitis and should such an infection spread they may give rise to pyelitis or suppurative nephritis. They have been isolated from abscesses of the liver and gall-bladder. Numerous epidemics of diarrhea in young children, cases of broncho-pneumonia, pleurisy, meningitis, and endocarditis, have also been attributed to them. In ulcerative conditions of the intestines they may readily pass through the injured intestinal walls and with associated organisms give rise to peritonitis. Ordinarily in such cases streptococci and staphylococci are also present, and it is probable that the latter are more actively concerned in producing the lesions. Shortly before death the colon bacilli frequently pass through the intact intestinal mucosa into the circulation.

It is somewhat surprising that an organism constantly present in such large numbers in the intestines should at times give rise to disease; it might naturally be expected that the body cells had developed a complete state of immunity towards it. A number of explanatory suggestions have been offered. It may be that none of the toxic products of the bacilli are absorbed through the intact mucous membranes, in which case no process of immunization would be likely to occur; or, on the other hand, it may be that temporary lowered resistance may permit the organisms to overcome the forces by which they have previously been held in check.

Immunity. — Bacteriolytic and agglutinating antibodies are produced in animals following injections of gradually increasing doses of living or dead organisms. The normal serum of animals and man will frequently agglutinate B. coli in dilutions as high as 1 in 10 or 1 in 20. The formation of such agglutinins may probably be the result of their habitual presence in the intestinal tract. The serum of patients recovering from typhoid fever or dysentery will agglutinate B. coli in even higher dilutions. The fact may be

explained either on the ground of their group relationship or the absorption of the toxic substances of the organism through the diseased intestinal mucous membranes.

Vaccines. — Vaccines have been found beneficial in cases of cystitis and appendicitis due to the colon bacillus. The dose ranges from 25 to 500 million organisms.

B. Coli Communior. — The organism is probably as abundant in the intestinal tract as B. coli communis itself. Morphologically and culturally they are identical, save that the latter does not ferment saccharose, whereas B. coli communior is able to form both acid and gas from it.

Capsulated Bacilli. — Closely related to the colon bacilli, and by some authorities included with them, are a number of organisms which differ from the latter in that they are non-motile and that they are usually heavily capsulated. Of these *B. lactis aërogenes* is perhaps the most frequently met with. It is normally present in the intestines, in sewage, and in water. It is almost always present in milk and cream and is one of the principal agents causing them to become sour. Another of the capsulated bacilli, *B. pneumoniæ* or *Friedlander's pneumobacillus*, isolated by Friedlander in 1882, was regarded for a time as the cause of lobar pneumonia. More recent discoveries have proved, however, that the pneumococcus is responsible for the vast majority of the cases of the disease. The type of pneumonia caused by Friedlander's bacillus is relatively infrequent and has a very high mortality. Still other members of the capsulated group are *B. ozenæ* the causal agent of fetid rhinitis, and B. of rhinoscleroma, which receives its name from the disease it gives rise to.

PARATYPHOID GROUP

Between the colon and typhoid bacilli are a large number of organisms which possess the common group characteristics, *i.e.* they do not liquefy gelatin, are Gram negative, and do not form spores, yet they show wide variations in their reaction on sugars and are not agglutinated by either typhoid or coli immune serum. Near the typhoid end of the scale organisms exist which

differ culturally from the typhoid bacillus in that they produce acid and gas from glucose while the former produce acid only. At the other end of the scale are organisms just as closely related to the colon bacilli. Possibly the members of the group are links in a chain connecting the two groups.

Attention has centered around these organisms mainly because of their connection with "food poisoning." Such poisoning is usually spoken of as "ptomain poisoning," and is the result of poisonous products formed from the food itself. The food at fault may not appear in any way unusual, a bacteriological examination being necessary to determine the presence of the bacteria. Preserved foods or sausages are the most frequent cause of poisoning; cases have been reported from milk and milk products. It is important to note that ptomains are relatively resistant to heat and that the organisms themselves, while not especially resistant, may escape unharmed by the usual cooking processes when embedded in sausage or joints of meat.

In 1888 in a village in Saxony a cow which had been sick for two days with profuse diarrhea was slaughtered and the meat sold for food. Fifty-seven persons who ate the meat became ill and one case ended fatally; a young man who had eaten the meat raw died in about thirty-six hours. From this fatal case and also from the flesh of the diseased cow Gärtner isolated an organism to which he gave the name *B. enteritidis*. As its name implies, the lesions produced by the organism are intestinal. Notably an inflammation of the mucous membrane and occasionally hemorrhages occur. When infection is caused by the bacillus itself symptoms do not usually appear until about twenty-four hours or more after the food has been eaten. When they appear at once they are undoubtedly due to the poisonous ptomains already formed from the food.

Paratyphoid Bacilli. — In 1896 Acharde and Bensaude obtained from the urine of a patient suffering from an infection similar to typhoid fever an organism which they named the *paratyphoid bacillus*. In 1900–1901 Schottmüller isolated from the blood of patients showing much the same symptoms two bacilli closely

resembling the former, which he termed *B. paratyphoid A* and *B. paratyphoid B*. The symptoms produced are of the enteric form, and very soon the organisms appear in large numbers in the stools and in the blood.

Infection with Type A lasts from nine to fourteen days and is characterized by headache, pains in the neck and back, fever, and occasionally diarrhea. Infection with Type B is manifested by a more sudden onset and symptoms very similar to those produced by B. enteritidis, such as vomiting, chills, and diarrhea. The two organisms are readily distinguished by their production of specific agglutinins.

As a rule paratyphoid fever is much milder and has a lower mortality than typhoid fever. It is not known what degree of immunity is conferred by one attack, but it is known that an attack does not protect against typhoid nor does typhoid protect against paratyphoid. When exposure to both infections is anticipated a mixed vaccine is usually administered in doses commencing with 500 millions of typhoid bacilli and 250 millions each of paratyphoid A and B. Second and third doses are usually double the amount.

Members of the Group Found in Animal Diseases. B. suipestifer. — The organism was isolated from cases of hog cholera. It is usually considered as a secondary invader; the primary cause of the disease is a filtrable virus. The organism closely resembles B. paratyphoid B, and can only be distinguished from it by serological tests.

B. psittacosis. — Parrots imported from the tropics often die of an enteritis and general septicemia caused by this organism. Rabbits, pigeons, fowls, and mice are also susceptible. From birds or animals the disease is readily communicable to man by the infected dejecta.

Rat Virus. — Dangez isolated an organism belonging to this group from an epizoötic in field mice, which he introduced commercially for the purpose of killing rats. Other similar preparations are obtainable in the market under the names of Ratin, Liverpool virus, etc.

CHAPTER XX

THE COLON TYPHOID GROUP

(CONTINUED)

B. Typhosus. Dysentery Group

B. Typhosus. — In 1880 Eberth found the organisms now known as B. typhosus in the spleen and diseased portions of the intestines of persons who had died of typhoid fever. In 1884 Gaffky obtained the organism in pure culture and was able to study its growth characteristics. Its causal relationship to typhoid fever, however, was particularly difficult to prove because, although the organism was pathogenic for many animals when inoculated subcutaneously or intravenously it was impossible to produce infection by feeding and the characteristic symptoms of the disease as they appear in man. Experiments with anthropoid apes, increased knowledge concerning specific antibodies produced in immune serum, the presence of the bacillus in the blood and feces of typhoid patients and not in healthy persons other than " carriers " have been sufficient to establish the fact that B. typhosus is the causal agent of typhoid fever. Recent work tends to show that there are several strains slightly different culturally which have hitherto been classified as B. typhosus, and that in the future these organisms will be considered as the typhoid group rather than the typhoid bacillus.

Morphology and Staining. — Typhoid bacilli are short rods with rounded ends, varying from 1 μ to 3 μ in length and 0.5 to 0.8 μ in width. In hanging drop preparations they are seen as single individuals or they remain attached and appear as threads. Morphologically they are identical with B. coli save that they are

often more slender (Fig. 29). When growing under favorable conditions they are actively motile; in hanging drop preparations made from young cultures the short forms move rapidly with a darting movement, while the attached, thread-like forms have a slower and more undulating motion. They possess twelve or more peritrichic flagella, which are longer and more wavy than those of the colon bacillus (Fig. 30). With the ordinary anilin dyes they stain rather slowly, they are Gram negative, and do not form spores.

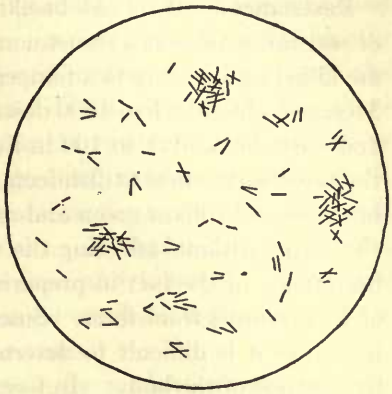


FIG. 29.— Typhoid Bacilli.

Cultivation. — The typhoid bacillus is aërobic and facultative anaërobic. Its optimum temperature is about 37° C. On culture media growth does not take place below 9° C. or above 42° C. In a gelatin stab there is a fine white growth along the line of inoculation. The main growth, however, is on the surface, which spreads outwards toward the sides of the tube as a thin leaf-like film. On agar colonies appear in twenty-four hours as thin disks, white or bluish gray in color, and with slightly scalloped margins. Broth is uniformly clouded. Occasionally a thin film forms on the surface after eighteen to twenty-four hours' growth.

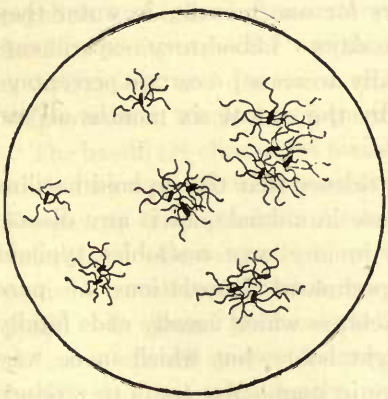


FIG. 30.— Typhoid Bacilli, showing Flagella.

The tests with sugars furnish a means of differentiating the typhoid bacillus from other members of the colon-typhoid group. The typhoid bacillus produces acid without gas in maltose, glu-

cose, and mannite, but has no effect upon lactose or saccharose. The identification of the organism is readily established by means of the agglutination reaction.

Resistance. — Typhoid bacilli exhibit about the same degree of resistance to heat as most non-spore-bearing organisms. They are killed by exposure to a temperature of 56° C. in fifteen minutes. Mercuric chloride 1 to 1000 destroys them in one to five minutes and carbolic acid 1 to 100 in five to fifteen minutes. As a rule they are less resistant to disinfectants than B. coli. Such substances, however, as brilliant green and crystal violet inhibit the growth of the latter without affecting the typhoid bacilli. Application has been made of the fact in preparing special media for the isolation of B. typhosus from feces. Since the organisms are rarely found in nature it is difficult to determine the length of time they will live outside of the body. In feces in privy vaults or on the ground they tend to die out rapidly; the majority may be dead within twenty-four hours. Some, however, may persist for a much longer period. According to certain authorities they may remain alive in feces during the winter for five months. They have been reported to remain alive in oysters for one month; in water they seldom live longer than seven days. Laboratory experiments show that in ice the numbers rapidly decrease; a certain percentage may live for four months, but by the end of six months all are killed.

Pathogenesis. — There is no evidence that the typhoid bacillus is ever associated with any disease in animals nor is any disease known amongst animals which in any way resembles typhoid fever. Subcutaneous or intraperitoneal inoculations of pure cultures produce a short, acute sickness which usually ends fatally in from twenty-four to forty-eight hours, but which in no way resembles the disease as it occurs in man. Attempts to produce the disease by feeding animals on typhoid dejecta mixed with their food have been equally unsuccessful except in the case of anthropoid apes.

In human infection inflammation and ulceration in the Peyer's patches and solitary glands of the intestine are the characteristic

lesions. In the early stage of the disease there is acute inflammation; leukocytes gather in great numbers in the invaded area and suppuration and necrosis result. In the severest cases the lesions may involve the muscular and peritoneal coats of the intestinal wall and perforation may occur; peritonitis and death usually follow. Passing through the injured mucous membrane into the blood stream the bacilli are carried to all parts of the body and become localized in groups or foci in the various organs. Of these the spleen usually contains the greatest number of bacteria; it becomes enlarged and congested and in tissue sections the bacilli appear as clumps between the cells. A similar but less marked invasion may take place in the liver and kidneys. In the gall bladder they may occur in enormous numbers, and even after recovery they may persist there for years.

In addition to the local changes in the various organs toxic poisoning is manifested in typhoid fever as in other infectious diseases by disturbances in the circulatory, respiratory, and heat regulating centers.

Occasionally complications occur, such as pneumonia, osteomyelitis, and other inflammatory conditions, in which the typhoid bacillus seems to be the exciting cause. Usually, however, such complications are due to a secondary or mixed infection with staphylococci, streptococci, pneumococci, or the colon bacilli.

The bacilli are eliminated mainly in the feces. About the second week they are apt to appear also in large numbers in the urine. Frequently they persist until several weeks or months after convalescence. Occasionally they are found in the secretions; organisms have been found in the roseolar spots which occur in typhoid. It cannot be concluded, however, that their presence is the cause of such spots. In cases of pneumonia due to the typhoid bacilli they are abundantly present in the sputum. There is strong evidence that the bacilli may persist in the gall bladder for many years and from thence find their way into the intestines. It is probable that the catarrhal inflammation they produce there causes a deposit of the bile in a solid form, resulting in gallstones. In operations on the gall bladder years after recovery from the

disease typhoid bacilli have been found. They have even been found within the calculi.

Typhoid Carriers. — In the majority of cases of typhoid fever the bacilli disappear from the feces during the first three or four weeks of convalescence, but in a certain number of cases, about 1 to 5 per cent, they persist for many months and even years after an attack of the disease. Such carriers have been classified as "temporary" when they cease excreting the bacilli within a year of convalescence and as chronic when this period is exceeded. The distinction is unimportant since both types are a menace to the community in which they reside. A danger lies in the fact that carriers generally appear to be in good health or only suffer occasionally from slight pain in the region of the gall bladder. The majority of traced carriers are women. Since in such cases the chief danger lies in their conveying the bacilli to foodstuffs, a carrier occupied as a cook or waitress or on a dairy farm is a special menace. The following remarkable case of typhoid carrier is cited by Dr. Park of New York:

"A visitor in the family of which this woman was cook developed typhoid fever some ten days after entering the household. This was in 1901. The cook had been with the family ten years and it is difficult to say which infected the other. The cook went to another family. One month later the laundress in this family was taken ill.

"In 1902 the cook obtained a new place. Two weeks after her arrival the laundress was taken ill with typhoid fever; in a week a second case developed, and soon seven members of the household were sick.

"In 1904 the cook went to a home in Long Island. There were four in the family as well as seven servants; within three weeks after arrival four servants were attacked.

"In 1906 the cook went to another family. Between August 27 and September 3 six of its eleven inmates were attacked with typhoid. At this time the cook was first suspected. She entered another family on September 21st. On October 5th the laundress developed typhoid fever.

"In 1907 she entered a family in New York City and two months after her arrival two cases developed, one of which proved fatal.

"The cook was removed to the hospital March 19, 1907. Cultures taken every few days showed bacilli off and on for three years. Sometimes the stools contained enormous numbers of bacilli and again for days none would be found. She was released on parole in 1910, promising to report to the Health Department and not to engage in cooking. She broke her parole and disappeared. In 1915 in an epidemic of typhoid at a maternity hospital a total of twenty-five cases developed. Investigation showed that the food was the cause and the cook was identified as 'Typhoid Mary.' During the period of disappearance she infected a friend and was the cause of several cases in a small private sanitorium. She is known to have been the cause of at least fifty cases of typhoid fever."

The tracing of typhoid carriers is an important and at the same time difficult problem. A Widal reaction cannot be depended upon since the agglutinins in the serum vary in amount from time to time. The actual proof that a person is a carrier lies in the isolation of the typhoid bacillus from the feces or urine, and as the organism may not always be present, several examinations must be made if negative results are at first obtained in suspected cases.

Modes of Communication. — The typhoid bacillus probably always enters the body by way of the mouth, fingers or food being responsible for its conveyance. Water-borne epidemics still occur, though with much less frequency. Fortunately typhoid bacilli do not multiply in water; they usually die within seven days except in winter, when a covering of ice or snow affords them some protection. Water-borne epidemics of typhoid almost always occur in the spring, fall, or winter, since most fecal material eventually finds its way to water, and as watercourses draining inhabited regions are likely to be contaminated with human feces, there is always the possibility of surface water containing typhoid bacilli. The first big epidemic in America definitely traced to the water supply occurred in 1885 at Plymouth, a small mining town near Philadelphia. Of the 8000 inhabitants 1000 contracted

the disease. Plymouth received its water from a mountain stream which drained an almost uninhabited watershed. The infection was traced to a man who had spent his Christmas holidays in Philadelphia, had contracted the disease there, and had returned home in January. During his sickness the excreta were not disinfected but were thrown on the banks or into the frozen stream. In March a thaw came and the entire mass was washed into the brook and on into the water main. Three weeks later the disease began to appear in the town with such rapidity that some days as many as 100 new cases were reported. In all there were 114 deaths. The epidemic proved at least that freezing alone for a short period is not sufficient to destroy the organism.

Milk-borne epidemics, like those due to water, have a sudden onset and then subside rather sharply. Up to 1907 statistics showed 317 epidemics caused by infected milk. Most milk outbreaks are reported from England or America. The custom of boiling the milk in many other countries undoubtedly affords them a certain amount of protection against typhoid infection. Milk-borne epidemics usually have certain definite characteristics. As a rule contamination comes from a case or a carrier on the farm and the outbreak is localized to the area receiving milk from that farm. Usually people of the better class and those who drink milk raw are affected, and several cases may occur simultaneously in one house. Milk products have been responsible for a certain number of outbreaks; oysters and other shellfish have also contributed their quota. Vegetables such as celery, lettuce, and water cress grown on land fertilized with fresh night soil may account for a few cases.

Flies have been justly condemned as spreaders of the disease. They breed in fecal and decomposing masses of all kinds and deposit the organisms they accumulate on the food they walk over. In a recent experiment typhoid bacilli were isolated from five out of eighteen flies captured in the privy and on a fence near the sick room of a typhoid patient.

A number of cases occur due to lack of knowledge of caring for the sick. The danger of fomites containing living bacilli

is very real, and the utmost care in disinfecting all articles used by a patient as well as all excreta cannot be overemphasized.

Immunity. — One attack of typhoid fever usually confers immunity which lasts for several years; in about 2 per cent of persons having had one attack a second attack occurs which is usually very mild. Immune serum is highly bactericidal and possesses abundant agglutinins, precipitins, and opsonins. Gradually increasing doses of living or dead bacilli injected into animals produces a similar serum, but attempts to use it therapeutically have not met with much success.

Serum Diagnosis. — The fact that the serum of typhoid patients will in high dilutions agglutinate typhoid bacilli, while the serum of normal individuals or those not suffering from the disease has no effect upon them, has been of enormous aid in the diagnosis of typhoid fever. The first application of the test was reported by Widal in 1896. Details of the method have been given in a previous chapter. Usually the reaction is given about the seventh day and gradually increases until convalescence. In about 95 per cent of all cases it is said to appear at some period of the disease.

Bacterial Diagnosis. — A blood culture is generally positive during the first week of the disease in all cases; during the second week in about 50 per cent of cases, and as the disease progresses the organisms tend to disappear from the blood stream.

Bacilli seem to be most numerous in the feces during the second, third, and fourth weeks of the disease. The short life of the organisms outside of the body makes it imperative that specimens be examined as soon after passage as possible.

A small portion of the feces is emulsified if solid in a tube of broth, if fluid it can be plated without further preparation. Poured plates are made of special media such as that of Conradi-Drigalsky, and a loopful of the fecal material is streaked over the solidified media. The usual method is to use three plates for each specimen, streaking the second and third plates without recharging the loop. After twenty-four hours' incubation a blue typhoid-like colony is fished into broth and at the end of from eight to ten hours sufficient growth will have developed for the

next procedure. Hanging drop preparations are made of a mixture of the broth culture and a dilution of immune horse serum, the strength of which is already known. With appropriate dilutions agglutination will take place almost immediately if the organism tested is the typhoid bacillus. The result may be confirmed by inoculating media containing lactose and dextrose. Acid production will take place in the latter and no change in the former.

Vaccines. — Wonderfully good results have been obtained from the injection of killed bacilli as a prophylactic measure against typhoid fever both in military and civil life. Statistics show a steady decline of typhoid in the U. S. Army since the introduction of compulsory vaccination in 1910. Only one case occurred in 1913 among over 80,000 men. In the British Army the reduction of morbidity is estimated at 50 per cent. An excessive dose of infectious material may break down the protection resulting from the action of the vaccine, yet in such cases the severity of the disease will be considerably modified.

In the army 500 million, 1 billion, and 10 billion bacteria are given usually on three successive Saturdays by means of a subcutaneous injection near the insertion of the deltoid muscle. Occasionally a slight local inflammation and a general feeling of malaise develops which disappears within twenty-four to forty-eight hours. Consequently it is customary to give the vaccine in the afternoon so that any reaction which may develop will occur while the individual is in bed. The degree of immunity decreases after two and a half years. It is advisable, however, in cases of constant strain and exposure to revaccinate each year.

Attempts have been made to use small doses of vaccine as a therapeutic measure in typhoid fever. Excellent results are reported in a certain number of cases; in others, however, they have been unsatisfactory.

THE DYSENTERY GROUP

The term *dysentery* is usually applied to diseases which show such symptoms as intestinal pain and diarrhea with mucus and

blood in the stools. Within recent times two distinct forms have been distinguished: one variety, *amebic dysentery*, is caused by a protozoön; the other form, *bacillary dysentery*, is caused by bacilli of the colon-typhoid group.

In 1898 Shiga, a Japanese bacteriologist, isolated an organism from the stools of dysentery patients which he found would agglutinate with the serum of those patients and not with that of normal individuals. Moreover, he was not able to find the organism in the feces of patients suffering from other diseases nor in those of normal individuals. Shiga's bacillus is now considered the causal agent of the majority of acute dysentery epidemics which occur in temperate climates.

In 1899 Flexner, while investigating dysentery in Manila, isolated a bacillus from dysenteric stools which at that time he considered identical with that isolated by Shiga, but later found that it differed in agglutinative reactions. In the same year Kruse in Germany isolated similar bacilli from cases of dysentery. In 1902 Park and Dunham obtained an organism from a severe case of dysentery at Seal Harbor, Mt. Desert, Maine, which proved by its different agglutinating characteristics to be still another strain. Since that time several others have been described. One writer has even described fifteen different forms which have fermentative characteristics distinguishing them one from the other. The following classification of Hiss, in which all members fall into one of four groups, is the generally accepted one:

Type 1. Shiga. Ferments dextrose.
Type 2. Park-Hiss. Ferments dextrose and mannite.
Type 3. Flexner-Strong. Ferments dextrose, mannite, and saccharose.
Type 4. Harris-Wollstein. Ferments dextrose, mannite, saccharose, and maltose.

Of the four types it is generally agreed that Type 1 appears most frequently in the severest forms of the disease; types 3 and 4 are found more frequently than the others in the dysentery or summer diarrhea of young children.

Morphology and Staining. — The organisms closely resemble the typhoid bacilli save that they are somewhat thicker, and

filamentous forms rarely are seen. Their staining reactions are the same as those of other members of the colon-typhoid group.

Cultivation. — In gelatin stab cultures a thin line of growth develops, very little appearing on the surface. Colonies on agar and gelatin plates are much the same as those of the typhoid bacilli and are smaller and more transparent than those of B. coli. In broth a uniform cloudiness is produced with sometimes a pellicle or a slight deposit. As already stated the different strains behave differently towards the different sugars; they all ferment dextrose and none of them are able to ferment lactose.

Resistance. — Dysentery bacilli show much the same degree of resistance to heat and disinfectants as the typhoid bacilli. In feces they usually die in one or two days.

Pathogenesis. — With the exception of monkeys the characteristic disease cannot be produced in animals by feeding them with cultures of the bacilli. Many animals, however, are sensitive to subcutaneous or intravenous inoculations, and the surprising result of such inoculation is that the animals show all the symptoms of the disease, and on autopsy the mucous membrane of the cecum and colon are found to be excessively inflamed. It is evident that the cells of the intestinal mucous membrane have a strong affinity for the bacterial toxin.

In man the organism does not enter the blood stream and the lesions are especially confined to the intestinal mucous membrane. In mild cases the disease takes the form of a catarrhal inflammation only; in severer cases necrosis of the epithelium may occur and the intestines may be lined with a pseudomembrane consisting of fibrin, dead cells, and bacteria.

Since the bacilli are found only in the intestines the spread of the disease is due to fecal contamination direct or indirect. Food, soiled linen, carriers: all may play a part. Water may become contaminated as in the case of typhoid, although comparatively few water-borne epidemics of dysentery have been reported. It is stated that in Japan in the rural districts the mortality due to dysentery, resulting mainly from the use of human feces as a fer-

tilizer and the frequent infection of the small streams and wells, is over 20 per cent.

Immunity. — Bacteriolysins and agglutinins are abundantly produced in the immune serum of both human beings and animals. Since there are so many strains of dysentery bacilli it is customary

FERMENTATION REACTIONS OF THE PRINCIPAL MEMBERS OF THE COLON-TYPHOID GROUP

Organism	Dextrose	Levulose	Mannite	Maltose	Lactose	Saccharose	Litmus Milk	Indol
B. coli communis . . . . . m	++	++	++	++	++	—	C + C	+
B. coli communior . . . . m	++	++	++	++	++	++	C + C	+
B. acidi lactici . . . . . —	++	++	++	++	++	—	C + C	+
B. mucosus capsulatus . . . —	++	++	++	++	++	++	+	+
B. enteritidis . . . . . m	++	++	++	++	—	—	—	—
B. paratyphosus A . . . . m	++	++	++	++	—	—	—	—
B. paratyphosus B . . . . m	++	++	++	++	—	—	—	—
B. typhosus . . . . . m	+	+	+	+	—	—	—	—
B. dysenteriæ, Shiga . . . —	+	+	—	—	—	—	—	—
B. dysenteriæ, Park . . . —	+	+	+	—	—	—	—	—
B. dysenteriæ, Flexner . . . —	+	+	+	+	—	—	—	—
B. dysenteriæ, Wollstein-Harris —	+	+	+	+	—	+	—	—

m = motility
++ = acid and gas production
+ = acid production only
C = coagulation

to inject animals with an individual strain and so produce a "monovalent" serum or to inject them with mixed strains and so give rise to a "polyvalent" serum, the former for therapeutic use when the type of organism causing the disease has been determined and the latter when the strain is unknown. In mild cases doses from 10 c.c. to 30 c.c. are given twice daily according to the weight of the patient; in severe cases as much as 100 c.c. may be given.

The reduction in mortality by the use of the serum is estimated at about 20 per cent.

Vaccines. — Dysentery vaccines have been employed with moderately good results. The fact that there are so many strains lessens their value unless it is known to which group the invading organisms belong.

Bacteriological Diagnosis. — Isolation from the feces is the surest method of identifying the strain causing infection. The methods employed are exactly the same as those employed in typhoid and paratyphoid infection except that crystal violet should be omitted from the Conradi medium on account of the inhibitory effect of the anilin dyes on many of the dysentery strains. After isolation the organism should be tested with the specific agglutinating serum and confirmatory evidence gained by growing it on the different sugar media.

CHAPTER XXI

BACILLUS ANTHRACIS. BACILLUS MALLEI. BACILLUS PYOCYANEUS. BACILLUS PROTEUS.

B. Anthracis. — Anthrax or splenic fever is a disease occurring especially in sheep and cattle, although many of the lower animals are susceptible. Infection occasionally appears in human beings, but it is never transmitted from man to man; it is always contracted directly or indirectly from animals.

Anthrax has undoubtedly occurred among cattle from the earliest times. It was the first infectious disease shown to be caused by a specific microörganism and consequently it has been one of the most studied of all bacterial diseases. Pollender in 1849 described rod-shaped bodies which were contained in the blood of infected animals and suggested that they might be the cause of the disease. In 1863 Davaine demonstrated by inoculation experiments that blood containing these bodies invariably produced anthrax. He therefore concluded they were bacteria and suggested the name B. anthracis. Later, in 1877, Koch confirmed Davaine's work by isolating the organism, growing it in pure culture, and with the pure culture producing the characteristic disease. Koch's observations explained many apparent paradoxes that had greatly puzzled previous workers. Blood from infected animals which appeared to be free from bacteria had been found to produce the specific disease when inoculated into susceptible animals and also the disease was sometimes found to appear without any known means of infection. Koch discovered that very soon after blood is drawn the anthrax bacillus forms spores which are highly refractive and seen with difficulty, and that consequently they must have been present but not

noticed in the supposedly germ-free blood. Further research showed that animals might become infected by feeding them with spores. This fact together with a knowledge of the prolonged vitality of these bodies in the soil explained the persistence of the disease in certain localities and its reappearance in once-infected pastures after many years.

Morphology and Staining. — The bacillus is one of the largest of the pathogenic bacteria; it ranges from 2 to 20 μ in length and 1 to 1.2 μ in width. In stained film preparations the organisms may appear singly or joined end to end in chains of varying length. The free ends of the rods are rounded, while those coming in contact with one another are square or slightly swollen and concave, the latter giving the chain somewhat the appearance of a bamboo rod. In preparations from albuminous material a thin capsule may be seen surrounding the cell. (Fig. 31.)

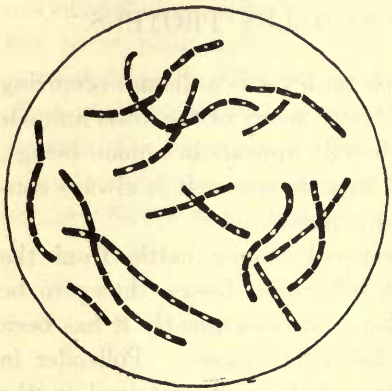


FIG. 31. — Anthrax Bacilli.

Spores are formed only in the presence of free oxygen; hence they do not develop in the blood while it remains in the body. They are oval in shape and appear in the center of the rod. As the spore develops it occupies more and more of the parent cell until the latter appears as a thin envelope which finally ruptures and sets the spore free. Sporeless strains of B. anthracis have been produced by growing the organisms on media containing antiseptics or by cultivation at their maximum temperature (43° C.). The bacilli stain with the usual dyes and are Gram positive.

Cultivation. — The organism grows well on ordinary culture media under aërobic conditions; vegetative forms are facultative anaërobes. Development will occur between 14° C. and 43° C., the optimum being about 34° C.; under the minimum and above the maximum temperature sporulation does not take place.

In gelatin stab cultures growth occurs along the track of the needle as a delicate white thread from which irregular projections soon extend, giving the culture the appearance of an inverted tree; at the end of two or three days liquefaction commences at the top. In broth after twenty-four hours' incubation growth appears as a flaky sediment which is deposited at the bottom of the tube. The colonies on agar or gelatin plates are particularly characteristic. At first they appear as small, white, opaque points; later long, wavy filaments project from each colony in all directions, which when examined through the microscope are seen to be composed of bacteria joined end to end.

Resistance. — Anthrax spores retain their vitality and virulence for years under favorable conditions. Exposed to dry heat, a temperature of 140° C. for three hours is required to kill them; moist heat has a much more rapid effect, a temperature of 100° C. being sufficient to destroy them in five minutes. They have been found to retain their vitality after thirty-six days' exposure to a 5 per cent solution of carbolic acid at room temperature. In a similar solution, however, they were destroyed after half an hour's exposure at 55° C. In the vegetative form B. anthracis has comparatively low resistance.

Pathogenesis. — Cattle and sheep, except the Algerian race, are the most frequently infected of all animals. In European countries an outbreak is apt to occur from time to time; in France the animal mortality among sheep was formerly about 10 per cent. An animal may suddenly show symptoms of collapse and death ensue within a few minutes; or in milder cases, bloody mucus is seen about the mouth and nose and in the feces, pulse and respiration are increased, and chills are followed by high temperature. In such cases death may occur in from twelve to forty-eight hours. In still less severe cases edema and often ulceration and necrosis of the neck lymph glands occur. On autopsy the spleen is found to be soft and of a dark red color and two or three times its natural size. Tissue sections show the capillaries both of the spleen and liver packed with bacilli; the blood is usually fluid but tar-like, and of a dark color.

In man one of three forms of infection may occur: entrance of the bacilli may be through a cut or an abrasion of the skin, resulting in a *malignant pustule;* through the lungs by inhalation of the spores, *wool sorter's disease;* or through the alimentary tract, *intestinal anthrax.*

When infection takes place through the skin a small red papule appears on the exposed surface in about one to three days. Very soon it becomes vesicular and contains clear or blood-stained fluid. The area surrounding it becomes greatly inflamed and within thirty-six hours the center begins to show signs of necrosis. If the pustule is not excised the disease spreads. Invasion of the blood stream by the bacilli is most likely to happen, and death from septicemia result in from three to five days. Occasionally instead of the typical pustule an extensive edematous area appears which may be so intense as to result in gangrene. Such cases are usually fatal. Skin infections develop chiefly among shepherds and butchers or those who work among hides.

The pulmonic form of anthrax, "wool sorter's disease," is contracted by the inhalation of spores during the sorting and cleansing of wool from infected animals. The symptoms are those of pneumonia, often with edema in the cutaneous tissue over the neck and chest. Recovery may occur or the disease may be fatal in from two to seven days.

Intestinal anthrax, although the usual form in cattle, rarely occurs in man. The few instances on record have been caused by the ingestion of spore-infected food or accidentally amongst laboratory workers. The symptoms produced are those of intense poisoning, chills, vomiting and diarrhea, and a moderate degree of fever.

Immunity. — With the hope of producing protective immunity Pasteur, in 1880–1882, devised a method whereby a mild attack of the disease could be produced by means of inoculation with attenuated cultures. Other methods have since been suggested, but that employed by Pasteur, Chamberland, and Roux is the one still most generally used.

Two vaccines are prepared: No. 1 is a broth culture so attenu-

ated by cultivation at 42° to 43° C. that it no longer affects guinea pigs but is still fatal for mice; No. 2 is sufficiently virulent to kill guinea pigs but not rabbits. The animal to be immunized is inoculated subcutaneously on the inner side of the thigh with five drops of vaccine No. 1; twelve days later a similar dose of vaccine No. 2 is injected; fourteen days later virulent organisms can be injected without any ill results. It is estimated that in about 40 per cent of all the animals vaccinated immunity disappears within a year and that to insure permanent protection revaccination would be necessary every year. Nevertheless, the system has done much to diminish the mortality from the disease. In France statistics show that during twelve years the mortality from anthrax amongst vaccinated sheep was less than 1 per cent as compared with 10 per cent in flocks not thus protected.

A moderately protective serum has been obtained from actively immunized animals, but the nature of its protection is not definitely known; it is thought to be largely due to the presence of opsonins. A combination of the active and passive methods of immunization has been employed with the result that a single treatment is said to suffice.

B. Subtilis. — In the early days of bacteriology B. subtilis, a saprophytic organism found widely distributed in nature, was thought to be closely related to B. anthracis because of their almost identical morphological appearance. B. subtilis, commonly known as the " hay " bacillus, may be distinguished, however, by its motility and by its non-pathogenicity.

BACILLUS MALLEI

Glanders is an infectious disease primarily of horses, mules, and asses; occasionally it is transmitted to other animals and to man. Towards the end of 1882 Loeffler and Schutz demonstrated conclusively the causal relationship of B. mallei to the disease by isolating the organism from diseased tissues and experimentally producing the characteristic symptoms by inoculating it into animals.

Morphology and Staining. — The bacillus is a small rod straight or slightly curved with rounded or pointed ends, ranging from 1.5 to 5 μ in length and 0.25 to 0.5 μ in width; it usually occurs singly but may occasionally be seen in pairs or long filaments. The organism is non-motile and does not form spores; it does not stain readily with the ordinary aniline dyes and is Gram negative.

Cultivation. — Growth occurs on ordinary culture media between 22° and 43° C.; a slightly acid reaction to phenolphthalein and the addition of glycerin greatly favors development. Stroke cultures on agar or glycerin agar at 37° C. are somewhat transparent and of a grayish white color and a rather slimy consistency. On agar plates colonies appear as round transparent droplets; in broth a diffuse cloudiness appears which later collects at the bottom of the tube as a heavy viscous sediment. Growth on potato is particularly characteristic; about the third day a yellowish transparent layer, somewhat like clear honey, is visible; as growth continues the color deepens until by the seventh or eighth day it is of a chocolate-brown color while the surrounding potato has acquired a greenish yellow tint.

Resistance. — B. mallei like other vegetative forms is only feebly resistant to heat and antiseptics. It is killed by exposure to moist heat at 55° C. in ten minutes and in a 5 per cent solution of carbolic acid in from three to five minutes. It is somewhat resistant to drying. It has been found to retain its vitality for fourteen days in a dry condition.

Pathogenesis. — As already stated, glanders occurs chiefly among horses; sheep, goats, swine, and rabbits are relatively less susceptible, cattle are immune.

The disease occurs in man as a result of the direct contact of a wound or skin abrasion with the discharges or diseased tissues of an infected animal. Consequently only those who come directly in contact with horses are likely to be affected. In animals the lesions are of two types, usually spoken of as "glanders" and "farcy." In glanders proper the nasal mucous membranes become inflamed and a profuse catarrhal discharge appears. Very soon firm translucent nodules are formed which later soften

in the center, break down, and leave irregular ulcerated cavities. Similar lesions occur in the lungs, in the liver, and in the spleen. In " farcy " the infection usually takes place through an abrasion of the skin; the lymphatics near the wound become thickened and tense and are spoken of as " farcy pipes " or " farcy buds "; Suppuration usually follows, resulting in deep ulcers with ragged edges and frequently a purulent discharge.

In man the disease occurs either in an acute or a chronic form. In the acute form an inflammatory swelling appears at the point of infection, which is usually the hand or arm, and a redness spreads along the line of the lymphatics as in a poisoned wound. A pustular eruption soon appears which may be local or cover a large area; in addition suppurative foci may occur in the lungs and other internal organs. In about 60 per cent of all cases the disease ends fatally in from two to three weeks.

In the chronic form a local ulcer forms which tends to spread deeply and superficially; the disease may run a chronic course for years and recovery may eventually occur, or, on the other hand, it may at any time change into the acute form and rapidly become fatal.

The glanders nodule has been considered by some authorities to be structurally similar to that formed by the tubercle bacillus. It is generally agreed, however, that in glanders there is a more marked inflammatory reaction and leukocytic infiltration and that the tissue changes are of a degenerative rather than of a proliferative nature. Caseation, which is so marked in tuberculosis, does not occur in the same degree in glanders, nor are the typical giant cells formed.

The mode of infection amongst horses is not definitely known. Recent evidence tends to show that it takes place mainly by the way of the alimentary tract. Since the bacilli are numerous in the nasal discharge, the public drinking trough may in a measure be responsible for the spread of the disease. In man infection probably only occurs through a break in the skin.

Diagnosis. — Several methods have been devised which greatly facilitate the diagnosis of glanders; of these the mallein reaction,

the Straus reaction, and sero-diagnostic tests are the most frequently employed.

Mallein Reaction. — Mallein is a concentrated glycerin broth in which B. mallei has been cultivated and is prepared in exactly the same manner as tuberculin. After a subcutaneous injection a positive reaction in a glandered animal is manifested by a rise in temperature of about two degrees, a tender local swelling at the point of inoculation, and a general disturbance, while in healthy horses the temperature does not rise above one degree and the local swelling is slight and soon disappears.

It has been recently shown that mallein dropped into the conjunctival sac gives a similar reaction to the ophthalmic test in tuberculosis. Three drops of mallein dropped into the eye of a glandered animal will cause a swelling of the eyelid and a purulent discharge from the tested eye in from five to six hours. The test is so simple and gives such a reliable and quick result that it has been adopted as the Federal test for the interstate shipment of horses.

The Straus reaction consists in injecting into the peritoneal cavity of a male guinea pig some of the suspected material. If virulent glanders bacilli are present, enlargement of the testicles and pus formation occurs within two to five days. A positive reaction together with the presence of typical organisms in the lesion is proof positive of the disease. Failure on the part of the guinea pig to react, however, does not preclude the possibility of the disease.

Serum Reactions. — The serum of an infected horse possesses a very high power of agglutination ; a dilution of 1 to 1000 or higher will react positively. Since normal serum will agglutinate the bacilli in dilutions as high as 1 to 500, three dilutions of serum from a suspected animal are generally employed : 1 to 500, 1 to 800, and 1 to 1000. If agglutination occurs only with the first dilution the reaction is considered negative; with the first and second, doubtful; with the first, second, and third, positive.

Complement fixation tests give probably the most reliable results of all. According to certain workers a positive reaction is

obtained in about 97 per cent of all positive cases of the disease. The test is conducted as described in Chapter XIII, save that several strains of B. mallei are used together as an antigen.

All attempts to produce artificial immunity against glanders have so far been unsuccessful.

BACILLUS PYOCYANEUS

The blue green color occasionally seen in the purulent discharge of wounds of long standing was shown by Gessard, in 1882, to be due to a chromogenic bacillus to which was given the name B. pyocyaneus.

Morphology and Staining. — The organism is a slender rod from 2 to 6 μ long and 0.3 to 1 μ broad; it possesses a single flagellum at one end and is actively motile. It stains with the ordinary aniline dyes, is Gram negative, and does not form spores.

Cultivation. — The bacillus is an aërobe and facultative anaërobe. It grows readily on all artificial culture media and gives to most of them a bright green color. On gelatin growth rapidly develops, imparting to the medium the characteristic hue; liquefaction commences about twenty-four hours after inoculation and soon the entire medium becomes fluid. On agar a wrinkled yellowish white surface growth appears, the agar itself being a brilliant green. In broth a heavy pellicle is formed and indol is produced; milk becomes curdled and shows an alkaline reaction.

The bacillus produces two pigments: one a fluorescent green which is soluble in water but not in chloroform and which is common to many bacteria; and another, pyocyanin, which is of a blue color and soluble in chloroform. Pigment production is most abundant in the presence of oxygen and at a temperature of about 22° C.

In addition to the ferment causing the liquefaction of gelatin another enzyme, pyocyanose, is produced, which acts on albumin and is able to dissolve bacteria. It has been applied locally in cases of diphtheria. The results, however, have not been markedly beneficial.

Pathogenesis. — B. pyocyaneus has been found in water, in the feces of many animals, and on the skin of healthy human beings, and for some time after its isolation it was regarded as a harmless saprophyte or at most of very limited pathogenic power. Later evidence has proved that not only does its presence in mixed infections retard the process of repair, but it has the power of producing suppuration itself. It has been found in pure cultures in cases of ophthalmia, broncho-pneumonia, and otitis media. Thus while only slightly pathogenic it may in cases of lowered vitality produce a serious infection.

BACILLUS PROTEUS

B. proteus, or rather the group of organisms known by that name, is abundantly found in soil and water and almost wherever putrefactive changes in organic matter are occurring. The organisms were discovered by Hauser in 1885. The number of varieties contained in the group has not been clearly defined. The following description, however, may be considered as typical.

Morphology and Staining. — The average length is about 1.2 μ and the width about 0.6 μ. The organism does not form spores. It possesses many peritrichal flagella, is actively motile, stains readily with the anilin dyes, and is Gram negative.

Cultivation. — An aërobe and facultative anaërobe, it grows well on the ordinary culture media. Gelatin liquefaction commences at the end of ten or twelve hours. On agar slants a spreading, white, moist growth appears and on agar plates colonies tend to become confluent. Dextrose and saccharose are fermented with the production of acid and gas. In peptone solution indol and phenol are produced. In urine the organisms decompose urea into ammonium carbonate.

Pathogenesis. — Cultures injected subcutaneously into guinea pigs or rabbits cause purulent abscesses or death with symptoms of poisoning.

In man B. proteus has been found in a variety of pathological conditions: in purulent peritonitis, cystitis, and pyelonephritis.

Metchnikoff regarded it as the usual cause of infantile diarrhea. On nasal membranes it frequently occurs as a more or less harmless parasite, decomposing the secretions and giving rise to a putrefactive odor. Certain outbreaks of food poisoning have been attributed to the "ptomains" produced by the putrefactive action of B. proteus. In such cases the food is disagreeable both in taste and odor. Hence food poisoning of this type is less likely to occur than that due to the paratyphoid-enteritidis group or to B. botulinus, where there is little or no perceptible change.

CHAPTER XXII

(1) HEMOGLOBINOPHILIC GROUP. (2) HEMORRHAGIC SEPTICEMIA GROUP

(1) B. Influenzæ. B. of Koch-Weeks. B. Pertussis. B. of Soft Chancre

B. Influenzæ. — Influenza was described as early as the fifteenth century although in all probability it was known even earlier. It has appeared in all parts of the world sporadically in epidemics or in great pandemics. In 1889–1890 so widespread was the disease and so high the mortality that it was considered the most serious pandemic of modern times. During the two years following many attempts were made to discover the specific cause, and in January, 1892, Pfeiffer, Kitasato, and Canon simultaneously published a description of the organism now known as B. influenzæ. Pfeiffer's work was the most complete and to it is due most of the knowledge we possess of the organism. B. influenzæ has been definitely shown to be pathogenic and is generally accepted as the cause of the disease although the fact has not been absolutely proved.

Morphology and Staining. — The organism is one of the smallest pathogenic bacteria known. As seen in film preparations from sputum it averages from 0.5 to 1.5 μ in length and 0.2 to 0.3 μ in width. The ends of the rod are rounded and no capsule is formed. The organism is Gram negative and is colored rather faintly with the ordinary anilin dyes. Staining is best effected with a 1 in 10 solution of carbol fuchsin for five to ten minutes. It is non-motile and does not form spores.

Cultivation. — Pfeiffer succeeded in growing the organism in symbiosis with others on agar smeared with sputum, but all at-

tempts to cultivate it alone on plain agar or serum utterly failed. He then tried smearing the agar with drops of blood, and his efforts were completely rewarded. The necessary substance for their development seems to be hemoglobin, and for this reason the organism is spoken of as "hemoglobinophilic." On blood agar colonies appear at the end of eighteen hours as minute circular almost transparent dots. Even on blood culture medium the organisms very soon die. They will live indefinitely, however, if transplanted every three or four days. Grown in symbiosis with other organisms development is more rapid, and growth will occur for several generations on ordinary agar without the addition of hemoglobin. The organism is a strict aërobe and multiplies only at a temperature between 25° C. and 42° C.

Resistance. — The bacillus is extremely delicate and has only very feeble powers of resistance. In dried sputum it dies within twelve to forty-eight hours; in water it does not live more than two days; it cannot withstand boiling for one minute or a temperature of 60° C. for five minutes.

Pathogenesis. — B. influenzæ is only slightly virulent for experimental animals; the rabbit is moderately susceptible and the guinea pig even less. There is no satisfactory evidence that they ever contract the disease in a natural way.

In man the organisms appear in enormous numbers in the secretions of the nose, throat, and respiratory tract. Frequently they invade the lung tissue, and lobular pneumonia, purulent in character, results. The bronchioles become filled with leukocytes and in tissue sections the bacilli may be seen packed in between the epithelial and pus cells. The bacilli are rarely found in the blood. They may, however, be present in the lesions accompanying influenza. They have been found in inflammations of the middle ear, in meningitis, conjunctivitis, cystitis, and peritonitis.

Influenza may take a subacute form. Bacilli may remain latent or only slightly virulent in the lung tissue for many months, and then if by chance the body resistance is lowered they may become active.

Infection is undoubtedly transmitted directly from one indi-

vidual to another or indirectly by the use of objects contaminated with fresh secretions since the organism is so feebly resistant to influences outside of the body, and it is present in enormous numbers in the secretions of the respiratory tract. Carriers may be responsible for the cases which appear sporadically and for the commencement of epidemics.

Immunity. — No apparent immunity seems to be conferred by an attack of the disease; in fact, one attack seems to predispose to subsequent attacks. Nor has a serum been produced which could be used for the production of passive immunity; vaccine treatment has been said to have been of benefit. Its value, however, has not yet been fully confirmed.

KOCH-WEEKS BACILLUS

The organism was first observed by Koch in 1883 while in Egypt, and later in 1887 it was more fully described by Weeks in New York, who obtained it in cultures growing with B. xerosis from cases of " pink eye " or acute contagious conjunctivitis. The bacillus is closely similar to the influenza bacillus; the relationship has not yet, however, been determined. Recent studies indicate that the Koch-Weeks bacillus may be a strain of the influenza bacillus.

Several similar hemoglobinophilic organisms have been described in pathological conditions of the eye. In 1896 Morax and later Axenfeld isolated the **Morax-Axenfeld bacillus** from cases of subacute inflammation of the eye. Zur Nedden found a similar bacillus known by his name in certain ulcers of the cornea.

BACILLUS PERTUSSIS

Bordet and Gengou in 1906 were the first to describe an organism resembling the influenza bacillus which appears in enormous numbers in the sputum of cases of whooping cough and to which they gave the name B. pertussis.

Morphology and Staining. — The bacillus is a short, somewhat oval rod, usually appearing singly, but occasionally in pairs joined

end to end; it is non-motile. It stains faintly with the aniline dyes. Bi-polar bodies which stain more deeply than the center of the organism can sometimes be distinguished. It is decolorized by Gram's method.

Cultivation. — The organism grows feebly at first even on the medium especially recommended by Bordet and Gengou, consisting of 1 per cent glycerin mixed with macerated potato and an equal quantity of human or rabbit blood. After several generations it will grow moderately well on veal agar or broth. The organism is an aërobe and develops best at 37° C.

Pathogenesis. — A mild toxin is undoubtedly produced and absorbed, but of more importance is the mechanical disturbance caused by the bacilli in the respiratory tract. They have been described as being present in enormous numbers in the trachea, packed between and clinging to the cilia of the epithelial cells. This according to certain investigators constitutes the specific lesion of whooping cough. B. pertussis has not yet been definitely proved to be the specific cause of the disease, although much evidence has been advanced in favor of the theory. Its strongest claim to recognition is the fact that in the serum of convalescents a specific antibody is produced which gives a positive complement fixation reaction with the organism.

BACILLUS OF SOFT CHANCRE

The bacillus of soft chancre or chancroid was obtained in pure culture by Ducrey in 1889 from the purulent discharge of an ulcerated surface.

Morphology and Staining. — The organism is exceedingly small, measuring about 1.5 μ in length and 0.5 μ in width. In tissue sections it usually appears attached in long chains or grouped together in masses. It is non-motile and does not form spores and is Gram negative. Stained with carbol fuchsin deeply colored bi-polar bodies can often be distinguished.

Cultivation. — The best medium for cultivation has been found to be a mixture of agar and rabbit's blood in the proportion of

two parts of agar to one part of blood. In fact, on no other culture medium so far employed has cultivation been possible. If pus obtained from a lesion is smeared over the medium minute gray transparent colonies will usually appear after forty-eight hours incubation.

Pathogenesis. — The disease appears as an acute inflammation followed in one or two days by pustule formation. The pustule soon ruptures and a small depressed ulcer remains, around which other pustules and ulcers develop and necrosis spreads rapidly. The lymphatics in the groin become swollen, and later " buboes " or abscesses result. The lesions are usually upon the genitals and infection is most frequently conveyed from one person to another by direct contact. The chancre differs from that of syphilis in that there is no induration.

So far animal inoculations have been without result except in monkeys. The causal relationship of the organism to the disease, however, was established by Tomasczewski, who produced the lesions by the injection of a pure culture into a human body.

(2) HEMORRHAGIC SEPTICEMIA GROUP

A group of bacilli have been described all of which have similar characteristics and which give rise in the lower animals to an acute septicemia usually with hemorrhagic areas in the subcutaneous tissues and internal organs. The bacilli are short, non-motile, Gram negative, and they do not form spores; growth is scanty on gelatin and the medium is not liquefied. They have been found in rabbit septicemia, chicken cholera, swine plague, and a similar infection in cattle.

Morphologically and culturally the organisms isolated from the different sources appear identical. They vary, however, in their degree of virulence for the different animal species. **B. Avisepticus** produces a septicemia rapidly fatal in fowls but much less severe in pigs, sheep, and horses. **B. Suisepticus** is moderately pathogenic for fowls, but in young pigs it produces a broncho-pneumonia, to which they usually quickly succumb. Evidence points to a close

relationship between the members of the group. So far as is known none of the organisms affecting the lower animals are pathogenic for man except a very similar organism, **B. Pestis**, which is responsible for the much-dreaded bubonic plague or "black death."

B. Pestis. — Records of the ravages of the bubonic plague have been handed down through the centuries. At intervals it has appeared in vast epidemics and so great has been the infectiousness and mortality of the disease that in congested districts whole populations have succumbed. The "Great Plague" of the fourteenth century spread over all Europe, and so frightful was its severity that one quarter of the population or about 25,000,000 persons perished. Commerce was suspended and people fled panic stricken from the towns to the open fields for safety. During the last two centuries Western Europe has been practically free from the disease. It still occurs, however, in all its horror in India, the annual mortality averaging about 500,000. It is thought that the disease made its first appearance in America in 1899 at Santos, Brazil; since then other cases have been reported in San Francisco, Mexico, and Central America.

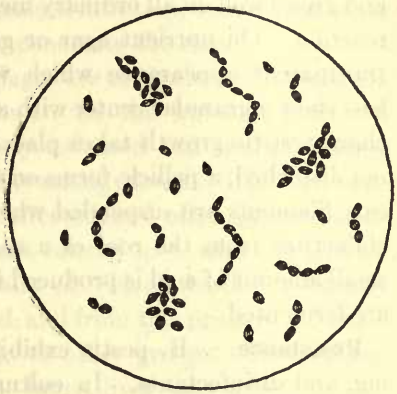


FIG. 32. — Bacillus Pestis.

The causal agent of the disease, B. pestis, was discovered simultaneously by Kitasato and Yersin in 1894 during an epidemic in China. A number of accidental infections with pure cultures have established its specificity.

Morphology and Staining. — In film preparations from infected tissues the bacilli appear as short, thick rods with rounded ends about 1.6 μ in length and 0.6 μ in width. In body fluids they may be seen singly or in pairs and rarely in chains; in broth cultures, on the contrary, they remain attached, and the individual organisms are so short and thick as to give almost the appearance

of streptococci. Many varieties in form are seen in smears of material from old lesions. Swollen involution forms with clubbed ends, short, round forms, and long rods may all be found, most of which stain with difficulty. (Fig. 32.)

Young forms stain well with methylene blue, which brings out clearly the deeper stained bi-polar bodies; they are decolorized by Gram's method. The organism is non-motile and does not form spores.

Cultivation. — The optimum temperature for B. pestis is somewhat lower than that of most pathogenic organisms; growth occurs better at 25° C. to 30° C. than at 37° C. The bacillus is an aërobe and grows well on all ordinary media which have a slightly alkaline reaction. On nutrient agar or gelatin, colonies present a delicate transparent appearance which when seen under the low-power lens show a granular center with a thin, uneven margin. The most characteristic growth takes place in broth, where, if the tubes are not disturbed, a pellicle forms on the surface, and from it long, delicate filaments are suspended which hang down into the broth like stalactites from the roof of a cave. Gelatin is not liquefied; a small amount of acid is produced from dextrose, but no other sugars are fermented.

Resistance. — B. pestis exhibits little resistance to heat, drying, and disinfectants. In cultures protected from light and air they have been found alive after ten years.

Pathogenesis. — Plague is primarily a disease of rodents transmissible to man. In all probability these animals are responsible for the maintenance of foci from which the great epidemics spring. Of the lower animals rats, mice, guinea pigs, and squirrels are particularly susceptible; dogs, swine, cattle, and horses do not contract the disease naturally, but may be infected by inoculation with large amounts of cultures. Mice and guinea pigs are usually employed for experimental purposes. After inoculation a local inflammatory swelling appears which follows the line of the lymphatics and terminates in a general infection and death in a few days. On autopsy the internal organs present a congested appearance accompanied by extensive hemorrhages. The liver and spleen

are enlarged and show a characteristic granular or mottled appearance, sometimes with abscess formation and necrosis. Rats and mice may also be infected by feeding them with pure cultures or the dead carcasses of their infected comrades. In such cases it is thought that infection takes place through the mucous membranes of the mouth rather than by the intestinal canal. The fact that infection may take place through slight abrasions of the skin serves a useful purpose in diagnosis. Often rats submitted for examination are decidedly decomposed. If, however, the suspected material is rubbed on to the freshly shaved abdomen of a guinea pig the plague bacilli enter through the slight scarification due to shaving and give rise to a general infection, whereas the other organisms present are not able to penetrate the skin.

In man three clinical types of plague are recognized: (1) bubonic, (2) pneumonic, and (3) septicemic. In the bubonic form the lymphatic glands become intensely inflamed and swollen, ending in necrotic softening if the patient lives long enough. The surrounding connective tissue is similarly affected, and often subcutaneous hemorrhages occur, causing the dark colored spots which originated in the middle ages the popular name of " black death." Usually one group of glands is first affected, and from this primary " bubo " the swelling and necrosis extends to other groups. Hemorrhage and necrosis may also occur in the lungs, liver, and spleen.

In the pulmonary form the disease appears as a broncho-pneumonia often attended by hemorrhages; there is usually a large amount of frothy, blood-tinted sputum in which the bacilli are present in enormous numbers. In this form the disease is extremely infective and almost always fatal. The mortality is estimated to be 90 per cent or more.

In plague septicemia there is a slight general enlargement of the lymphatic glands, but no primary bubo is discoverable. A case commencing in the bubonic form may, however, terminate with septicemia. In the bubonic and the septicemic types the bacillus remains in the diseased organs and the blood stream and is not eliminated in the excretions. These forms of the disease therefore are not contagious in the usual sense of the word, but are spread

mainly through the agency of the flea. On the other hand, the pneumonic form is frequently transmitted from one person to another by careless disposal of the sputum or droplet infection. The mode by which the bacilli enter the body does not necessarily determine the type of infection which will ensue.

The above three types of the disease are usually classified as *pestis major;* milder forms occur known as *pestis minor,* which bear somewhat the same relationship to the severer form that " walking typhoid " does to typhoid fever.

Modes of Infection. — When first it was noticed that an epidemic of plague was accompanied by an increased mortality amongst rats a supposition at once arose that there must be some relationship between rat plague and human plague, and the possible mode of the conveyance of the disease from rat to man or from rat to other rodent became a question of prime interest. As a result of experiments it soon became evident that rat fleas were responsible for the transfer of the infection. It was noticed that infected animals might be placed in a cage with healthy animals without the latter contracting the disease if fleas were excluded; on the other hand, healthy animals placed near enough to flea-infested plague rats invariably contracted the disease. It was also found that the common rat flea infests and bites human beings; large numbers were found on the legs of men who entered for a short time plague-infected houses. The ease with which bacilli may enter the tissues was also demonstrated by allowing a non-infected flea to bite a rat and then placing a drop of culture over the bite after which infection resulted. Since the blood of an infected rat may contain as many as 100,000,000 bacilli per c.c. an enormous number may be present even in the amount of blood sucked by a flea. The organisms multiply further in the stomach of the insect, which may remain infected for one or two months. Transference of the organism to man probably takes place by means of the feces expelled by the insect while feeding or the regurgitation of infected blood previously taken into the stomach. Once deposited on the skin it is an apparently simple matter for the bacilli to enter the skin through the minute opening made by the flea

and infect the surrounding tissues. The pneumonic types of plague may develop after infection by a flea if the organisms entering the blood stream happen to become localized in the lungs.

Immunity. — One attack of plague as a rule confers immunity. A method of protective inoculation with "Haffkine's prophylactic" is extensively practiced in India which has given very good results. The "prophylactic" is prepared by inoculating broth cultures with B. pestis, and as soon as the stalactite formation appears the tube is thoroughly shaken until the growth falls to the bottom. The tube is then reinoculated with fresh growth and the shaking and reinoculation process is repeated five or six times. After about six weeks the culture is killed by heating for one hour at 65° C. The dose administered is about 3 to 3.5 c.c., an amount equivalent to about 500 million bacteria. Vaccination gives a relatively short immunity but is sufficiently marked to warrant its use in case of exposure to infection. In the Punjab during the plague season 1902–1903, of those inoculated the mortality was only 23.9 per cent as compared with 60.1 per cent among the uninoculated.

A serum prepared by Yersin by injecting horses first with killed cultures and later with living cultures of B. pestis is reported by certain investigators to have a curative action; others, however, have failed to secure any favorable result from its use.

CHAPTER XXIII

PATHOGENIC ANAËROBIC BACILLI

B. Tetani. — The association of tetanus with wounds and soil was early recognized, but for centuries all attempts to find the exciting cause failed. In 1884 Nicolaier succeeded in infecting mice and rabbits by inoculating them with garden soil, but by none of the ordinary methods was he able to isolate an organism which would give rise to the disease. In 1889 Kitasato succeeded in isolating from lesions in mice which had been inoculated with material from a human case a bacillus which when injected in pure cultures into animals produced the characteristic symptoms. He further demonstrated that the cause of the earlier failures to obtain the organism was due to the fact that it could only grow alone in the absence of oxygen.

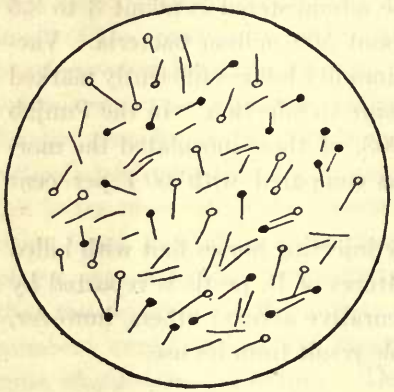


FIG. 33. — Tetanus Bacilli.

Morphology and Staining. — The tetanus bacilli appear as slender rods with rounded ends about 4 μ in length and 0.4 μ in diameter. They readily form spores which are round and have a diameter usually much larger than the thickness of the bacilli; the spore develops at one end of the organism, giving it an appearance somewhat like a drumstick (Fig. 33). The bacilli are slightly motile in the vegetative form and possess a large number of peritrichal flagella. They stain with the ordinary anilin dyes and are not decolorized by Gram's method.

Cultivation. — At 20° to 24° C. growth occurs slowly and spores are produced in six to ten days; at 37° C. development is much more rapid and spore formation begins within twenty-four to thirty hours. Under ordinary conditions the tetanus bacillus is a strict anaërobe. If, however, aërobic bacilli are growing with it in culture medium it will develop even when air is admitted. Presumably the air is used up by the associated aërobes. Growth occurs abundantly on gelatin or agar containing 1 to 2 per cent glucose; the colonies have a fleecy thread-like margin radiating from a heavier central portion. In gelatin stabs growth appears along the needle track and from it outgrowths extend, giving the appearance of an inverted fir tree; liquefaction takes place slowly, generally with the production of a gas of characteristic and disagreeable odor.

As the tetanus bacillus is almost always associated with other organisms the most successful method of obtaining a pure culture is by taking advantage of the resistance of its spores to heat. The material containing the organism may be inoculated into a tube of glucose broth and incubated at 37° C. for two days. By that time sporulation will have occurred and the culture may then be heated to 80° C. for three quarters of an hour in order to destroy the associated vegetative forms. From the heated culture agar anaërobic plates are made, and if the tetanus bacilli are the only spore-bearing anaërobic organisms present a pure culture may be readily obtained.

Resistance. — In the vegetative form the tetanus bacillus is destroyed by the same agencies that kill spore-free bacteria. On the other hand, few forms of life are more resistant than tetanus spores; in a dried condition they may retain their vitality for years. In 5 per cent carbolic acid they are killed in ten hours. The addition of 0.5 per cent hydrochloric acid hastens the germicidal effect and destroys them in two hours. Bichloride of mercury 1 to 1000 kills them in three hours and in thirty minutes if 0.5 per cent hydrochloric acid be added to the solution. The spores are completely destroyed when exposed to dry heat at 160° C. for one hour or to steam at 120° C. for twenty minutes.

Pathogenesis. — Tetanus under ordinary conditions affects only man and horses; it can be produced, however, in other animals by the injection of pure cultures on their toxin.

The normal habitat of the tetanus bacilli is the intestinal tract of herbivorous animals. In their dejecta the organisms find their way into the soil and in the pulverized soil they are scattered with the dust practically everywhere. Tetanus bacilli may be found almost wherever man and domesticated animals have been. In some localities they are much more numerous than in others, and in certain parts of Long Island and New Jersey an unusual number of cases have developed as a result of small wounds.

When tetanus bacilli enter the body by way of the mouth they are quite harmless. Passing into the intestines, they find ideal anaërobic conditions and a temperature suitable to their development, and they are able to multiply there without causing any harm to their host. It is exceedingly curious that the horse, which may almost be regarded as a tetanus "carrier," is the most susceptible of all animals to tetanus toxin. Rats and birds are only slightly susceptible and fowls scarcely at all. It is estimated that an amount of tetanus toxin sufficient to kill a hen would kill five hundred horses.

Tetanus appears in man almost always as a wound complication, although the presence of tetanus spores in a wound does not necessarily result in infection. It has been found by animal experimentation that in a clean wound a few spores free from their toxin do not give rise to the disease; they are in all probability disposed of by the phagocytes. On the other hand, a lacerated or contused wound made by a dirty, blunt instrument is a much more favorable ground for their development, but even in such wounds if mixed infection is prevented by prompt disinfection and consequent killing of the associated bacteria, tetanus spores if present in all probability will not develop. Symbiosis is perhaps the main factor in determining whether or not the spores will be able to germinate. The presence of other organisms, and especially the pyogenic cocci, seem to facilitate their development.

Not only traumatic tetanus but all other forms are now known

to result from infection with B. tetani. In tetanus neonatorum the organisms gain entrance through the umbilical wound; in puerperal cases through the inner surface of the uterus. Contamination of vaccine and sera used in human therapy have unfortunately occurred. In St. Louis in 1901 diphtheria antitoxin taken from a horse during the period of incubation of tetanus was administered to seven children, all of whom died of tetanus. Bacteriological examination showed the serum to be sterile, but it contained large amounts of tetanus toxin. A law has since been passed requiring all sera and vaccines sold in interstate traffic to be controlled by animal tests.

Tetanus is a local infection resulting in general toxemia. Clinically it is characterized by a gradually increasing stiffness and spasmodic contractions of the voluntary muscles, commencing with those of the jaw and the back of the neck. The spasms are tonic in character, and as the disease progresses succeed each other with only a slight intermission. The bacilli never gain access to the blood and consequently are never distributed to the internal organs but remain localized at the site of infection, where they produce one of the most powerful toxins known, which when absorbed gives rise to the main symptoms and lesions of the infection. Therefore while the blood of a tetanus patient contains no bacilli it is laden with the toxin which is responsible for the disease.

The most important feature of tetanus toxin is its strong affinity for nerve tissue. It is rapidly absorbed from the local site of infection into the blood and lymph streams, is distributed to the muscles, and it is thought by many investigators reaches the spinal cord and brain indirectly through absorption by the end plates of the motor nerves.

Regardless of the amount of infection there is always an incubation period during which the bacilli multiply and produce the toxin. Generally speaking, the severer the infection the shorter will be the period of incubation and the greater the probability that the case will have a fatal ending. The toxin is produced and may be absorbed during or soon after the first twenty-four hours follow-

ing infection; hence the necessity for the immediate administration of antitoxin. For, once the toxin has entered into a firm union with the nerve cells it cannot be displaced. Antitoxin in sufficient amounts will neutralize the toxin as quickly as it is produced and thus protect the nerve tissue until the leukocytes and other body cells have destroyed the bacilli and spores. Unfortunately treatment is often deferred until symptoms have appeared. In such cases all the antitoxin can do is to combine with the free toxin and prevent further damage. As a therapeutic measure injections are usually made intravenously or by means of a lumbar puncture in order that the effect may be as speedy as possible. In acute cases 50,000 to 100,000 units are administered during the first few days. A prophylactic dose is usually 1000 units injected subcutaneously or intramuscularly.

The method of preparing tetanus antitoxin for the purpose of passive immunization is described in Chapter II.

Bacillus Welchii (B. Aërogenes Capsulatus). — The name applies rather to a group than to an individual organism. Welch and Mittal in 1892 were the first to isolate and describe minutely a member of the group, and they gave to the organism the name *B. aërogenes capsulatus*. It is frequently spoken of, however, as the *Welch bacillus*. Strains of bacilli closely related to and probably identical with the Welch bacillus have been described, among which are *B. phlegmonis emphysematosi*, *B. perfringens*, *B. enteritidis sporogenes*, and a number of others. During the recent war the bacilli have been recognized as the most important agents in the production of gas gangrene in infected wounds.

Morphology and Staining. — B. Welchii is a comparatively large bacillus measuring from 4 to 6 μ in length and relatively thick. In preparations from tissues or body fluids a distinct capsule is seen surrounding it; hence its original name. Spores are formed by some strains and are most likely to appear when blood serum is used as a medium.

Cultivation. — On agar the colonies are round with smooth margins and no outgrowths. The bacillus ferments glucose, saccharose, lactose, and maltose with the production of acid and

gas. It does not liquefy gelatin. In milk its growth is especially characteristic: acid is rapidly formed, coagulation occurs, and soon the clot becomes torn apart by gas bubbles, so-called "stormy fermentation." Ultimately it forms an irregular firm mass in the comparatively clear whey. The organism is a strict anaërobe. Growth will occur at room temperature, but is most abundant at 37° C.

Pathogenesis. — In the lower animals infection seldom occurs. In man the organism has been observed in diseases of the gastro-intestinal and genito-urinary tracts. When such cases end fatally the bacilli frequently pass into the blood stream at the time of death and produce gas cavities in the various internal organs; the so-called "foamy organs" observed at autopsy. The bacillus is of special interest in that it has been by far the most important cause of gas gangrene in war wounds. Laceration of the muscle tissue with an object contaminated with soil containing the bacillus is usually the starting point of infection. The remarkable feature of the disease is the rapidity with which it spreads. Cases have been recorded in which emphysematous swelling and gangrene of a limb has ended fatally within twenty-four hours.

BACILLUS OF MALIGNANT EDEMA

The organism was first discovered by Pasteur in 1877 in putrid flesh. He found that by introducing such flesh into rabbits an edematous condition of the tissues and degenerative changes in the various organs were produced. Koch and Gaffky in 1881 isolated the organism, carefully studied it, and gave to it the name *B. edematis maligni*. The organism is found in the intestinal tract of the higher animals, in the upper layer of the soil in putrefying substances, and in polluted water. During the recent war it was found in putrid wounds and cases of gas gangrene next in order of frequency to *B. Welchii*.

Morphology and Staining. — The bacilli vary in length from 2 to 10 μ and 0.8 to 1 μ in width. They are usually seen in pairs joined end to end; occasionally they occur in long chains. The

organism is motile and forms an oval spore situated in the center of the bacillus. It is stained readily by the ordinary dyes. Young cultures are usually Gram positive; older ones appear to be less able to retain the stain.

Cultivation. — The organism is a strict anaërobe. Growth occurs at 20° C., but is more rapid and abundant at 37° C. On glucose agar colonies appear of a dull white color with an irregular margin. It is able to ferment glucose, lactose, and maltose and to liquefy gelatin.

Pathogenesis. — A number of animals are susceptible to inoculation with the bacilli. Subcutaneous injections produce a spreading gelatinous edema and exudation of a blood-stained fluid, while the underlying muscles become soft and partly necrosed. Attempts to produce the disease by feeding animals with the bacilli or by injecting them intravenously have so far failed. In man practically all infections appear to have started from fractured bones or deep wounds.

BACILLUS CHAUVEI

Symptomatic anthrax, popularly known as "black leg" or "quarter evil," is an infectious disease occurring chiefly among cattle and sheep; so far as is known infection has never occurred in man. The disease was formerly confused with true anthrax. The microörganisms which give rise to the two diseases, however, are totally different. Morphologically and culturally B. chauvei closely resembles the bacilli of malignant edema. The disease is usually rapidly fatal. Edematous swelling on the thigh or shoulder appears, in a few hours a considerable quantity of gas collects in the tissues, and the affected muscles become almost black. Death usually occurs in from one to two days. The disease is usually due to wound infection with soil containing the bacilli.

Differentiation of Wound Anaërobes. — Practically all of the anaërobes concerned in wound infections come from the soil and originally from animal feces. The group appears to be closely related. The following characteristic points of each, however, are relatively constant and afford a means of differentiation.

B. Tetani presents a drumstick appearance due to large terminal spores; in glucose stab cultures growth resembles an inverted tree.

B. Welchii. — One of the surest tests for differentiating B. Welchii from the other anaërobes was devised by Welch and Mittal. Bacilli are injected into the ear vein of a rabbit, which is killed after a few minutes by a blow on the head. The body is incubated and within twenty-four hours it becomes tensely distended with gas, and at autopsy gas bubbles are found in all the organs.

B. Edematis may be distinguished from B. Welchii by the fact that it is motile and the lesions it produces are edematous rather than emphysematous.

B. Chauvei. — Injection of pure cultures into rabbits is the best means of differentiating B. chauvei from B. edematis and B. welchii. Rabbits are immune to the former and susceptible to the two latter organisms.

BACILLUS BOTULINUS

As already stated the term *meat poisoning* is applied to different conditions produced by different agents. The relation of certain members of the colon-typhoid group to so-called ptomain poisoning has already been noted. Another and totally different type of food poisoning is due to the toxin generated by B. botulinus during its growth on nitrogenous substances *outside* of the body. The bacillus is a parasite and does not multiply within the body. Fortunately it requires time for it to develop and produce its toxin, and for this reason fresh foodstuffs are not apt to be dangerous so far as botulism is concerned. Sausage, canned meat, and fish have been found mainly responsible for the conveyance of the poison to human beings. It is of singular importance that meat may contain numbers of the bacilli and relatively large amounts of the poison without any visible sign of decomposition. In 1896 van Ermengen isolated B. botulismus from a sample of ham which had been eaten raw and which had caused a number of cases of poisoning, some of which had ended fatally.

Morphology and Staining. — The organism is a large bacillus measuring from 4 to 9 μ in length and .9 to 1.2 in width. It forms large terminal spores, is motile, and Gram positive.

Cultivation. — Colonies are yellow and coarsely granular, gelatin is liquefied and glucose is fermented with the production of acid and gas. The organism is a strict anaërobe growing best at 22 to 24° C.; it does not multiply at a temperature above 35° C. Hence growth does not take place in the body.

Pathogenesis. — The poison produced by B. botulinus is a true toxin in the same sense as that of diphtheria or tetanus. It is, however, entirely produced outside of the body. Thus the bacillus occupies an unique position in that it forms its poison only on dead nitrogenous substances.

Unlike the toxin of diphtheria or tetanus it is poisonous when taken by mouth, absorption evidently taking place from the intestinal canal. Symptoms of botulism appear after ingestion of the poison in from twenty-four to forty-eight hours and are chiefly the effect of the toxin on the cranial nerves. Dilated pupils, dysphagia, and sometimes aphagia and aphonia, profuse secretion from the mouth and nose, constipation and retention of urine, and more or less derangement of the cardiac and respiratory centers are apt to occur.

The toxin is pathogenic for several of the lower animals. It is readily destroyed by heat; it also deteriorates rather quickly in sunlight.

Botulism differs mainly from the "meat-poisoning" due to members of the colon-typhoid group in that the poison produced is a true toxin and causes the production of an antitoxin. The causal agent cannot multiply in the body or in the presence of oxygen. Few or no lesions occur in the intestinal canal.

B. FUSIFORMIS

The organism has been observed to be constantly present in large numbers in pseudomembranous conditions of the mouth and throat known as "ulceromembranous angina" or "Vincent's

angina," and because of this fact it is strongly suspected as the etiological cause of the disease. The bacillus is a long, slender organism, slightly curved, pointed at both ends, and somewhat swollen in the center, presenting thus a spindle-shaped or fusiform appearance (Fig. 34). It is non-motile and Gram negative. Grown under anaërobic conditions it forms a whitish sediment in broth; on agar the colonies have characteristic filamentous outgrowths.

FIG. 34. — Fusiform Bacilli and Spirochetes.

In film preparations made directly from a lesion long spirilla seem to be almost as numerous as the fusiform bacilli. When, however, culture medium is inoculated only fusiform bacilli can be found. The fact has given rise to much speculation as to the relation of the two forms. Many investigators have considered the two organisms as distinct, but as having a symbiotic affinity; others have advanced the theory that the two forms are only different phases in the life history of one organism.

Fusiform bacilli associated with spirilla have also been found in gangrene, noma, gingivitis, and dental caries.

TYPHUS FEVER

The disease, said by various writers to be one of the most highly contagious of all febrile diseases, occurs principally under conditions of overcrowding and filth. Formerly it appeared in epidemic form. Improved sanitation, however, has greatly diminished its occurrence; and in some localities it has practically disappeared. Infection is characterized by an incubation period of from five to ten days, followed by a high temperature and petechial rash. The body louse seems to be mainly responsible for its transmis-

sion and in all probability the head louse too, a fact which justifies its classification as a filth disease. Typhus fever still occurs in certain parts of Europe and in North and South America. In Mexico it appears epidemically under the name of Tarbardillo. An infection known as Brill's disease and thought by Brill to be a new malady has recently been shown to be a mild form of typhus fever.

For a long time the disease has been attributed to a filtrable virus, and some observers still hold the opinion. Many extensive investigations have been made as to its etiology, and various organisms have been described as the probable causal agent, but until quite recently all attempts to obtain cultures failed. In 1914 Plotz obtained from the blood of cases of Brill's disease an organism which he succeeded in cultivating anaërobically. In a great many subsequent cases Plotz and his co-workers have found the same organism and have demonstrated the presence of agglutinins and complement-fixing antibodies in the blood of convalescents. Much of the evidence so far obtained is in favor of the bacillus as the causal agent of typhus fever.

CHAPTER XXIV

THE CHOLERA SPIRILLUM AND ALLIED ORGANISMS

In the Delta of the Ganges Asiatic cholera has been known for centuries. Not until the nineteenth century, however, did it appear in Europe and America. Traveling along the trade route it reached Europe in 1830, and in 1832 it arrived in America by way of New York and Quebec and spread west as far as the Mississippi. By 1849 it had traveled with the searchers for gold as far as California.

Prior to 1883 nothing was known regarding the causal agent; in that year Koch discovered in the feces of cholera patients a curved organism now generally known as the "comma bacillus" or "cholera spirillum." Soon other observers obtained comma-shaped organisms from many other sources, and a great deal of controversy arose as to their classification. With the advent of serological tests, however, all of these organisms except the vibrio of Koch were shown to be unaffected by the serum of animals immunized to cholera and hence in no way connected with the disease.

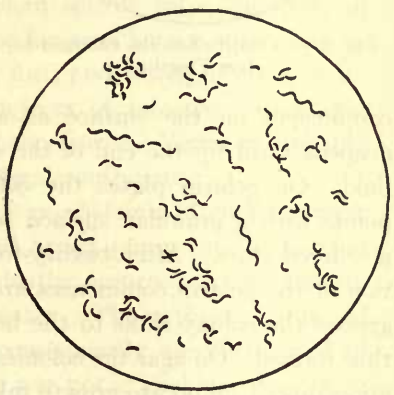


Fig. 35. — Cholera Spirilla.

Morphology and Staining. — The cholera spirillum appears in stained preparations as a curved rod about 1.5 μ in length and 0.4 μ in width. A single organism may appear slightly curved like a comma. Two organisms remaining attached and curved in an opposite direction may produce a resemblance to the letter S, while adherent in greater numbers they may appear as a long, spiral filament (Fig. 35). The organism is actively motile although

it possesses only a single long fine flagellum attached to one end (Fig. 36). Small highly refractile bodies are sometimes noted, but true spores are not formed. Staining is best accomplished with Loeffler's methylene blue or a weak solution of carbol fuchsin. The organisms are Gram negative.

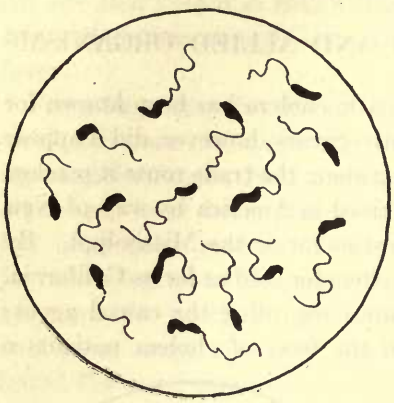


FIG. 36. — Cholera Spirilla, enlarged to show Flagella.

Cultivation. — The cholera spirillum is an aërobe and grows on all ordinary media provided the reaction is distinctly alkaline to litmus. Development is most luxuriant at 37° C. although multiplication will occur at a temperature as low as 16° C. In gelatin stab cultures growth appears along the needle tract, and in about twenty-four hours liquefaction commences on the surface as a small bell-shaped area which deepens until by the end of the week the entire medium may be fluid. On gelatin plates the colonies appear as minute whitish points with a granular surface somewhat resembling a layer of powdered glass. After twenty-four to forty-eight hours liquefaction of the gelatin commences around each colony and as it progresses the colony sinks to the bottom of the cup-like depression thus formed. On agar the colonies have a characteristic opalescent appearance; on potato growth takes place only at 37° C. and then it appears as a dirty moist layer. Acid is rapidly produced from glucose, saccharose, and maltose; coagulated blood serum is liquefied. In milk growth takes place without producing any visible change.

Indol is produced in peptone water medium by all true cholera spirilla; certain other organisms have the same ability, but they are comparatively few. The cholera spirillum produces nitrites at the same time as indol, so that in applying the test it is only necessary to add a few drops of sulphuric acid to a peptone water

culture which has been incubated twenty-four to forty-eight hours. When the growth is that of a cholera spirillum a reddish pink color appears, the so-called " cholera red reaction."

Resistance. — The cholera spirillum has much the same degree of resistance that other spore-free organisms possess. It is killed by exposure to moist heat at 60° C. in ten minutes and at 100° C. in one minute. Chemical disinfectants are speedily effective. The organisms are especially sensitive to drying, hence it is unlikely they are ever carried in a living condition in the dust particles in the air. In the dejecta of cholera patients they remain alive usually from one to three days, although occasionally they have been found after a much longer period. In running water they persist for six or seven days, and in stagnant water for about eighteen days. In milk the acidity produced by other bacteria soon destroys them.

Modes of Transmission. — Cholera spirilla leave the body of infected patients in the feces and so far as is known enter only by way of the mouth. Consequently, food and water, directly or indirectly contaminated with the dejecta of infected individuals, are the main cause of the spread of the disease. Water is probably solely responsible for the great epidemic outbursts.

The earliest authentic account of an epidemic of cholera traceable to polluted water is the Broad Street pump case in London in 1854. Within five weeks 616 deaths occurred in the vicinity amongst people who drank of the water. The outbreak happened before the days of bacteriology, consequently absolute proof of the presence of the cholera spirillum was not available. Inspection of the premises, however, revealed a cesspool with defective brickwork from which fluid material was constantly percolating into the well.

In Hamburg in 1892 a similar explosive outbreak occurred. Cholera was taken to the city by immigrants and the water of the Elbe was infected with their discharges. The sewers of Hamburg emptied into the river near the water intake from which the city received its supply for drinking purposes. The adjoining city of Altona received its water from the same source, but purified it by

sand filtration before use. Throughout the period of the epidemic houses situated on one side of a street and supplied with Hamburg water developed many cases of cholera, while those on the other side of the street receiving their supply from Altona remained uninfected.

Epidemics occasionally occur which are more difficult to trace to their source. Cholera spirilla have been found in the feces of healthy individuals and of people suffering from slight intestinal disturbances. "Cholera carriers" therefore are a possible foci of infection. Investigations have shown that the spirilla tend to disappear from the feces in from four to fourteen days. They have been known, however, to persist for sixty-nine days and longer. Contact infection plays an important rôle where persons live together under uncleanly conditions. The careless handling of dejecta and soiled linen is especially liable to result in infection. Haffkine, in India, found that sterilized milk if left in open jars to which flies had access might become contaminated with cholera organisms in a cholera-infected locality.

Pathogenesis. — Asiatic cholera appears to be a disease peculiar to man; none of the lower animals have been known to contract it naturally. Intraperitoneal injections of the organisms into a guinea pig may be speedily fatal, but intestinal lesions are rarely seen. Koch succeeded in producing the disease in much the same form as it appears in man by first neutralizing the gastric juice with a solution of carbonate of soda and inhibiting peristalsis by an injection of tincture of opium and then introducing a culture of the cholera spirilla into the intestinal tract by means of a catheter. Metchnikoff obtained similar results with new-born rabbits by rubbing a small amount of culture on the teats of the mother rabbit.

In man the lesions are primarily intestinal. The short incubation period, which is usually one to two days and rarely over five, is indicative of the rapid multiplication of the organisms once they have gained entrance to the alimentary tract, and to the production of a speedily effective toxin. The lower part of the small intestine is the part most affected. Penetrating the surface

of the mucosa the organisms loosen the epithelial cells, which are shed in flakes and give to the stools their characteristic rice-water appearance. In the more chronic forms of the disease extensive necrosis of the intestinal wall and the formation of a false membrane may occur together with a considerable amount of hemorrhage. It is generally thought that the organisms never invade the blood stream and internal organs.

The causal relation of the " comma bacillus " of Koch to Asiatic cholera has been fully established by a number of laboratory experiments and accidents. In 1884 a student in Koch's laboratory in Berlin suddenly developed a severe attack of cholera, and infection could have come in no other way than through the cholera cultures with which the man had been working. In another German laboratory Pettenkoffer and Emmerich experimented upon themselves by swallowing a small quantity of a fresh cholera culture. Pettenkoffer developed a mild attack of the disease, but Emmerich became seriously ill. In both cases numerous cholera spirilla were found in the stools. Dr. Oergal, an assistant in the Hamburg Hygienic Institute, became accidentally infected while experimenting with the peritoneal fluid of an injected guinea pig. After a few days he died of typical cholera, although there were no other cases in the city at the time.

On the other hand, many similar experimental cases have given negative results. This, however, may be explained as due to different degrees of susceptibility. The positive cases have been sufficiently well marked to warrant the acceptance of the organism as the causal agent of the disease.

Immunity. — One attack of cholera produces a moderate degree of immunity of rather short duration. Prophylactic vaccination affords a certain degree of protection in case of exposure to the disease.

Bacteriological Diagnosis. — A stained film preparation or a hanging drop is made from the feces and examined microscopically. In some cases the spirilla are so numerous and present a picture so unique that a microscopic examination is sufficient for diagnosis during an epidemic. For the detection of carriers or in case of

the first appearance of a cholera-like disease other tests are applied. Usually peptone water is inoculated with a small amount of feces, and at the end of six to twelve hours a hanging drop is made from the surface growth. If the organisms are sufficiently numerous agglutination tests are made with the serum of an immunized animal. Control tests are made at the same time with a known cholera strain. A speedy method for the detection of suspected cases when a number of examinations must be made is the inoculation with feces of saccharose peptone water to which an indicator has been added. As the cholera vibrio has the ability to ferment saccharose, decolorization occurs in from five to eight hours. The tubes not decolorized may be discarded, and no further examination is necessary since the cholera spirillum is not present. Because of the presence of sugar the decolorized cultures are unsuitable for agglutination tests. The difficulty, however, is avoided and time saved if duplicate inoculations are made, one in saccharose medium and the other in plain peptone water. In this way the peptone culture corresponding to the decolorized saccharose culture is used for the agglutination test as confirmatory evidence. By this method a great many unnecessary microscopic tests can be eliminated and a diagnosis made of a large number of cases in a few hours.

Allied Spirilla. — El Tor Vibrios. — Six different strains of spirilla were isolated by Gotschlich at El Tor from the bodies of pilgrims on the way to Mecca, who had died with dysenteric symptoms although there were no cases of cholera in the vicinity. These El Tor strains appear identical with the cholera spirilla morphologically and culturally and in their serological relation, but in addition they produce a strong hemolysin. There is still a difference of opinion as to whether they should be classed with the true cholera or regarded as a distinct species.

Spirillum Metchnikovii. — The organism was first obtained by Gamaléia from a cholera-like disease of fowls epidemic in Odessa. Morphologically and culturally it is identical with the cholera spirillum save that colonies on agar have a brownish tinge. It can readily be distinguished from the latter, however, by serum

reaction and by pigeon inoculations. A minute amount of a culture of S. Metchnikovii injected subcutaneously into a pigeon gives rise to a rapidly fatal septicemia; the same quantity of a cholera culture produced practically no effect.

S. Massaval and S. Finkler-Prior, both isolated from feces, and **S. Deneke** isolated from cheese, all closely resemble the cholera bacillus, but they do not give a specific reaction with cholera-immune serum.

SPIROCHETES

Because the structure of certain spiral organisms appears to be more complicated than that of many bacterial forms, and because several observers have found structural similarities between them and the protozoa, they have come to be regarded by many bacteriologists as members of the latter group, or as a separate genus intermediate between bacteria and protozoa. Their classification, however, is still undecided. The discovery that a spirochete was the cause of syphilis brought the organisms into great prominence, and since then many varieties have been isolated and studied. The diseases produced by them fall into two main groups: one usually transmitted by contact and in which infection is primarily of the tissues, as in syphilis and yaws; and the second, a blood infection accompanied by fever and transmitted by an animal parasite.

Treponema Pallidum (Spirocheta pallida).

Schaudinn and Hoffman, working together in 1905, found in the fresh exudates of syphilitic lesions a spirochete which they thought might be the cause of the disease and to which they gave the name Spirocheta pallida. Later they decided that the organism was sufficiently distinctive to be placed in a separate genus and they changed the name to *Treponema pallidum*.

Morphology and Staining. — The organism appears as a long, slender spiral averaging about 10 μ in length and 0.3 μ in diameter and with three to twenty small, sharp, regular curves. The ends are pointed and at each is a fine flagellum (Fig. 37). Movement may be of gliding to-and-fro nature, rotation on the long axis, or

bending of the entire body. It is thought by some observers that division takes place in a longitudinal rather than a transverse direction, as among many of the protozoa. The organisms are seen with difficulty in unstained preparations by ordinary microscopic methods. Their presence in smears is best demonstrated by the Indian ink method and in tissue sections by the silver impregnation method.

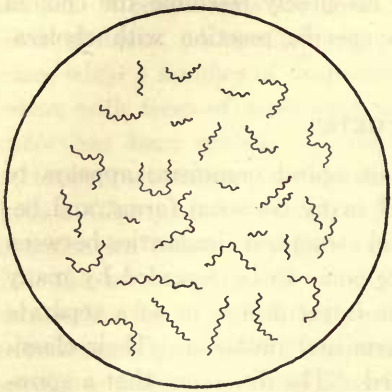


Fig. 37.— Treponema Pallidum.

Cultivation. — Schereschewsky was the first to cultivate T. pallidum artificially, although never in pure cultures. In 1911 Noguchi obtained the organisms from syphilitic lesions and succeeded in cultivating them in the following manner. The medium employed consisted of one part of ascitic or hydrocele fluid and two parts of 2 per cent agar to which was added a small piece of sterile rabbit kidney or other organ. The medium was covered by a deep layer of paraffin oil in order that strict anaërobic conditions might be maintained, and a stab inoculation with the material containing the spirochetes was made through the oil into the medium beneath. After ten days' incubation the spirochetes were found to have grown out into the surrounding medium, while most of the associated organisms remained along the needle track. Subcultures made from the outgrowths finally resulted in a pure culture of T. pallidum.

Pathogenesis. — Infection, so far as known, never occurs among the lower animals except as a result of inoculation with pure cultures or material containing the human virus. By this means investigators have recently succeeded in producing the disease both in rabbits and monkeys.

In man the disease is acquired by direct contact with infected persons or things. It runs a chronic course, usually divided into three stages, primary, secondary, and tertiary. The initial or

primary lesion appears two or three weeks after infection, first as a papule which develops into an ulcer with a hardened base, the so-called chancre, and at the same time there is a marked swelling of the nearest lymph nodes. The symptoms subside and six or seven weeks later secondary lesions appear as an eruption on the skin and mucous membranes, accompanied by general constitutional disturbances. In the tertiary stage masses of new tissue, spoken of as gummata, are formed through the viscera and in the periosteum. The organisms may usually be found in great numbers in the primary sore and in the papules and mucous patches which appear during the secondary stage. The latter fact explains the infectiousness of the saliva. They have been found also in the liver, spleen, and kidneys. In tertiary lesions they appear to be much less numerous. As a sequelæ to the tertiary stage, such conditions as general paresis, arteriosclerosis, and locomotor ataxia frequently result. Recently Noguchi and Moore have discovered the spirochete in the brain of a certain number of paralytic insane cases.

Immunity. — Immunity in syphilis appears to be somewhat different to that produced in other infectious diseases. All attempts to produce active immunity artificially have so far failed, nor is passive immunity conferred by the injection of serum from an animal in whom the disease has been produced. On the other hand, man, as a rule, is not susceptible to reinfection during the active stage of the disease. According to Colles's law a mother who gives birth to a syphilitic infant may not herself contract the disease, but may develop such a degree of immunity that she can nurse the infant without becoming infected even though it has venereal ulcers of the lips and tongue; whereas the child would infect the healthiest nurse even if she only handled and dressed it. The converse condition is stated in Profeta's law; namely, an infant showing no taint but born of a syphilitic woman may with impunity be suckled by its mother. Exceptions to both laws have, however, been recorded.

Microscopic Examination. — Because of its low refractive index T. pallidum is best seen with the dark-stage illumination. Material

may be obtained by first washing the lesion with sterile water and drying it with sterile gauze. Part of the base of the ulcer is then scraped with a curette until the superficial tissue is removed and blood appears. The blood is wiped off with sterile gauze until clear serum begins to ooze. A drop of the serum mixed with a drop of distilled water is placed on a coverslip, which is then inverted over a hollow slide as a hanging drop. Examined with the dark-stage illumination the organisms may be distinctly seen as brightly illumined objects on a dark background. Films may also be prepared and stained as already described.

Luetin. — Noguchi has prepared an extract from pure cultures of T. pallidum to which he has given the name of " luetin," which gives a characteristic reaction in syphilitic individuals. The reaction is analogous to the tuberculosis reaction in tuberculosis.

Wassermann Reaction. — The complement fixation test devised by Wassermann, Neisser, and Bruck, whereby the presence of specific antibodies in serum of syphilitic individuals may be detected, is described in Chapter XIV. The test is widely used and gives a positive reaction in over 90 per cent of active cases. It is practically always present during the second stage and tends to disappear as the disease becomes latent or is cured.

T. PERTENUE

A spiral organism was found by Castellani in 1906 in frambesia or " yaws," a disease occurring in tropical countries. The lesions seem to be analogous to those of syphilis, and by some writers the diseases are considered identical; other observers have found that the antibodies produced against the two organisms differ, and that consequently they are distinct species, and yaws cannot on this account be considered as a mild form of syphilis.

S. ICTEROHEMORRHAGIÆ

In 1915 Inada, Ido, and other Japanese workers demonstrated the presence of a spirochete, to which they gave the name S. ictero-

hemorrhagiæ in cases of infectious jaundice or Weil's disease. The disease is characterized by irregular fever, often severe jaundice and hemorrhagic herpes. The organisms appear both in the blood and the internal organs. Thus the disease seems to be intermediate between the two classes of spirochetal infections. The relation of rats to the spread of the disease has been established in Japan and during the recent war in the trenches. It has been found that the proportion of infected rats is sometimes as high as 30 per cent; the spirochetes are passed in large numbers in the urine of infected animals, and in this way the soil and various articles become contaminated.

S. OBERMEIERI (S. RECURRENTIS)

Obermeier discovered in 1873 an organism in the blood of patients suffering from relapsing fever which is usually known as Spirocheta obermeieri. He described its microscopical appearance and he noted its presence in the blood during the time of fever, its disappearance about the time of the crisis, and its reappearance during relapses.

Morphology and Staining. — The organisms are seen as long, delicate filaments from 16 to 40 μ in length and about 0.5 μ in width. The coils are somewhat wide and irregular. They possess a single flagellum at one end and move in a partly undulating, partly twisting fashion. They stain faintly with the anilin dyes and much better with the Romanowsky stains. They are Gram negative.

Cultivation. — All early attempts to cultivate the organisms on artificial culture media were unsuccessful. One investigator succeeded in keeping them alive for several generations by placing them in celloidin capsules in the peritoneum of a rat. By Noguchi's method, however, they can be readily cultivated.

Pathogenesis. — Rats, mice, and monkeys appear to be the only animals susceptible to the disease. In man the symptoms commence with severe frontal headache and a rapid rise of temperature, which remains high for five to seven days and returns to

normal by crisis. About a week later a relapse occurs, but on this occasion the fever lasts a shorter time before suddenly disappearing; a second and sometimes a third relapse occurs after about the same interval of time. The disease is usually benign, the mortality varying from 2 to 10 per cent.

Immunity. — Active immunity follows recovery from an attack of the disease, and the blood of immunized animals will confer passive immunity. Metchnikoff observed that during the fever the spirochetes were rarely taken up by the leukocytes in the circulating blood, but that at the time of the crisis the organisms disappearing from the blood accumulated in the spleen and were there ingested in large numbers by the leukocytes. These observations suggested the theory that the immunity produced during the first period of fever is of short duration and does not last until all the organisms are destroyed, and that with the disappearance of immunity the survivors escape from the internal organs and appear again in the blood stream. The second attack is less severe and is of shorter duration than the first, and with each succeeding attack the period of immunity is lengthened until finally it lasts long enough to permit all the organisms to be killed.

Varieties. — Several distinct diseases are caused by spirochetes similar to S. recurrentis. West African tick fever has been shown to be due to S. duttoni, a spirochete twice as long as S. recurrentis and possessing a number of flagella. East African tick fever is caused by a third variety, S. kochi, and relapsing fever as it occurs in India is thought to be caused by still another form. The West African type of the disease was shown by Dutton to be transmitted by a species of tick. Infected insects may harbor the parasites for several months, and of equal importance as regards the spread of the disease is the fact that the spirochete is transmitted to the offspring of the infected tick and may even appear in the third generation. Strong evidence has been produced for believing that head and body lice are the usual agents of transmission of European relapsing fever.

Miscellanecus Spirochetes. — In addition to the spirochetes causing syphilis, frambesia, and the various forms of relapsing

fever, other species have been described as occurring in the normal intestinal tract of human beings and mosquitoes, in various gangrenous processes in man, and in the blood of fowls suffering from a disease resembling relapsing fever. Several non-pathogenic forms are commonly found in normal mouths.

CHAPTER XXV

PATHOGENIC TRICHOMYCETES. MOLDS. YEASTS

THE trichobacteria appear to hold a position intermediate between the lower forms of bacteria and the molds. Structurally and functionally they are more complex than the former and much simpler than the latter. Certain authorities consider they should all be grouped together under the name of **streptothrix** and placed with the molds; other authorities hold a different view. Hence their classification is still undecided.

FIG. 38. — Trichomycetes.

The characteristics of the group are (1) an irregular, thread-like growth of interdependent segments, one end of which may be free while the other remains attached to an object, and (2) the development of a special portion of the organism for the purpose of reproduction and a tendency to branching true or false (Fig. 38). In false branching two terminal cells are developed from a parent cell, one of which is pushed aside but remains partly attached to the main stem. As the two cells continue to develop the appearance of branching is produced. In certain forms, when reproduction is about to take place, small, rounded cells known as conidia or spores appear either at the free end of the organism or at intervals along the filament, from which new individuals develop. Such spores are more closely related to those of the molds

and should be differentiated from the spores of the lower bacterial forms since they do not possess the same high resistance and serve only to reproduce the species. The forms known to be pathogenic for man may be arranged in four main groups:

1. Leptothrix. Almost straight thread-like growth. No branching.
2. Cladothrix. False branching.
3. Nocardia. True branching. Reproductive elements.
4. Actinomyces. True branching. Characteristic wreath-like growth in tissues.

Leptothrix. — Suppurative conditions of the mouth have been reported as due to a member of this class. Investigators have considered that Leptothrix buccalis, a form frequently found in normal mouths, may under certain conditions become pathogenic. Since the organisms have been so little studied, however, confirmatory evidence has not yet been obtained.

Cladothrix. — Several infections have been reported as due to this group, but some difficulty has been experienced in deciding whether the organisms should be classed as cladothrix or nocardia, since the only morphological difference existing between the two forms is that of true or false branching. An organism isolated at autopsy by Eppinger from a chronic cerebral abscess which had resulted in purulent meningitis was considered to show false branching and given the name *cladothrix asteroides*. On artificial media it developed a delicate fungoid growth, and in rabbits and guinea pigs it produced an infection similar to that observed in a number of lung infections usually designated " pseudotuberculosis."

Nocardia. — The group is perhaps more frequently named streptothrix, although according to the rules of nomenclature the name is not applicable since it was given as early as 1839 to a species of mold. Trevisan in 1889 suggested the name *nocardia* in its place for the organism discovered by Nocard in *farcin des bœufs*. Nocardia have been found in brain abscesses, meningitis, wound infections, and pneumonic conditions. Consolidated areas in the lungs and nodular formations have been found which clini-

cally so closely resembled tuberculosis that no means of distinguishing the two forms of disease could be devised save that of finding the causative organism.

Subcutaneous injections into rabbits cause the formation of abscesses, which when incised are found to contain a thick mucilaginous fluid; intravenous injections produce a rapidly fatal infection.

In smears made from cultures the organisms have a fine slender appearance, — the branching is unsymmetrical and almost at right angles to the stem. When properly stained a distinct beading of the protoplasm may be observed; stained by Gram's method they retain the violet color. In broth cultures growth appears as minute white fluffy tufts clinging to the sides of the tube when left undisturbed. Small colonies appear on Loeffler's serum after three to five days' incubation at 37° C.

Actinomyces. — Actinomycosis has been the most studied of the group of diseases caused by the higher bacteria. It occurs chiefly in cattle, but occasionally in other animals and in man. The disease was described early in the nineteenth century under the name of *osteosarcoma*. Later in 1877 Bollinger discovered the specific parasite, and the botanist Harz, who studied the organism, gave to it the name **Actinomyces** or **ray fungus**, on account of the ray-like formation of its growth in the tissues.

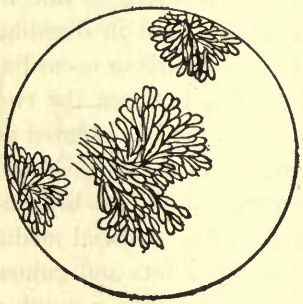


FIG. 39. — Actinomyces.

Actinomycosis is an inflammatory condition characterized by the presence of " granules," which are small round masses or colonies of the parasite. To the naked eye the largest appear as yellow or greenish points about the size of a small pin's head. When suppuration has occurred they appear free in the pus, otherwise they may be found embedded in the granulation tissue. According to their age and their structure they may appear as a whitish yellow, a green black, or more rarely red. Microscopically

each granule is found to consist of one or many rosettes of club-shaped organisms arranged in the definite radial manner which suggested their name. Each rosette is composed of threads which radiate from a center and terminate in glistening club-like endings closely packed together (Fig. 39). The clubs are especially found in lesions where the tissue appears to be displaying a degree of resistance to the growth of the parasite. Consequently they have been thought to represent a means of defense on the part of the parasites against the action of the phagocytes. Other observers have considered them degenerative portions caused by contact with the body fluids. The central threads show true branching and in the older colonies a tendency to segmentation which gives to them the appearance of a chain of cocci. It has been suggested that the coccus-like bodies may be spores or conidia. The view, however, is not generally held. The threads stain readily with the anilin dyes and are Gram positive, while the clubbed ends lose the color and take on the counter-stain.

Cultivation. — The organism is regarded by most authorities as a strict anaërobe. On agar or glycerin agar at 37° C. growth is visible after several days as small yellowish points which after becoming confluent resemble somewhat a culture of tubercle bacilli. The organisms penetrate into the medium, making the growth difficult to remove; in broth a sediment is deposited at the bottom of the tube in the form of solid white granules. In order to obtain a pure culture the following method has been recommended: granules are removed from a lesion, thoroughly washed in sterile water to remove extraneous organisms, and then crushed between two sterile coverslips. A microscopic examination is made to be sure a filamentous mass is present; otherwise, especially in bovine material, no development will take place. If filaments are seen a portion of the crushed granule is transferred with a platinum loop to tubes of melted 1 per cent glucose agar cooled to 40° C. and thoroughly distributed through the medium. At the same time a number of the granules after washing are placed on the side of a sterile test tube and allowed to dry at room temperature in the dark. In this way all contaminating organisms will be killed

by drying, and should first cultures be unsuccessful a second attempt may be made by using the dried granules in the same manner as the fresh ones. If after several days' incubation growth has occurred in the inoculated tubes colonies appear as opaque white nodules, most numerous about 7 or 8 mm. below the surface. A characteristic colony may be cut out of the agar by means of a platinum wire and transplanted into a fresh tube of melted glucose agar.

Resistance. — The organisms show considerable resistance to drying. On the walls of a test tube they may be found alive after seven weeks and in cultures for a year or more.

Pathogenesis. — Only slight local lesions can be produced by the inoculation of pure cultures into the smaller animals such as guinea pigs and rabbits; cattle, however, are very susceptible to the disease and in a less degree horses and swine also. In cattle there is usually an abundant growth of granulation tissue which results in large tumor-like masses. The disease may remain local or spread by continuity; it usually appears in the head or neck and produces the condition known as "lumpy jaw." Lesions may occur in the lungs, subcutaneous tissue, skin, liver, and other organs. Death resulting from actinomycosis is due rather to the mechanical action of the tumor in pressing upon or occluding the respiratory or alimentary tract rather than to any toxic effect.

In man the disease manifests itself in a similar manner save that there is generally less production of new tissue and more extensive suppuration. It may terminate fatally in a short time through a secondary infection or it may take a chronic course for years. Treatment with potassium iodide has effected cures both in man and cattle although its method of action is still unknown.

Mode of Infection. — Transmission by direct contact has not been satisfactorily proven. Many cases in human beings have been reported, in which so far as could be discovered no contact with a previously existing case had occurred. The frequent localization of the disease in the head and jaw has led to the supposition that the organism enters the body by way of the mouth,

probably around a decayed tooth, or the crypts of the tonsils, or some slight abrasion. Both in cattle and in pigs fragments of grain have been found in the soft tissues of the mouth embedded in an actinomycotic growth, and since there is a certain amount of evidence that grain is the natural habitat of the organism, infection has been thought to occur from this source. Other authorities maintain that the parasite is present in normal mouths and that penetration depends only on a damaged mucous surface and a certain degree of susceptibility on the part of the host.

Mycetoma (Madura Foot). — The disease resembles actinomycosis both as regards the character of the lesions and the occurrence of the parasite in the form of granules. They are nevertheless undoubtedly distinct. Mycetoma usually appears as a purulent inflammation of the foot, occasionally of the hand or other part of the body. A small swelling first appears which gradually enlarges, and in the center of the new tissue there occurs a purulent softening followed by ulceration. Enlargement and distortion of the affected part and frequently necrosis of the bones occur. Within the softened tissue the small granular bodies may be seen, yellowish pink in color or almost black like grains of gunpowder. It is thought by some observers that the yellow form is actinomycosis and that the black variety is caused by a member of the hyphomycetes group. Clinically the two forms of the disease are identical.

HYPHOMYCETES

The molds and trichobacteria closely resemble each other in that both have a branching, thread-like growth. The life history of the former, however, is much more complicated than that of the latter.

The growth of the hyphomycetes is characterized by a mass of tubular, branched filaments termed *hypha*, which interlace one within the other, forming a more or less web-like structure known as the mycelium. In the lower forms, the *phycomycetes*, each hypha is a single sometimes branched cell except when reproductive organs occur, whereas in the higher forms, the *mycomycetes*, the

hyphæ are segmented by transverse walls, each filament consisting of cells placed end to end.

Among the *phycomycetes*, of which Mucor mucedo, the white cottony mold which grows in damp bread, is a familiar example, reproduction may take place asexually or sexually; the former method is the most usual. The end of a hypha becomes shut off by a transverse wall and the extremity then swells into a globular sac or sporangium within which numerous oval spores develop. The swelling of the gelatinous mass in which the spores are embedded ruptures the thin cell wall and the spores thus escape.

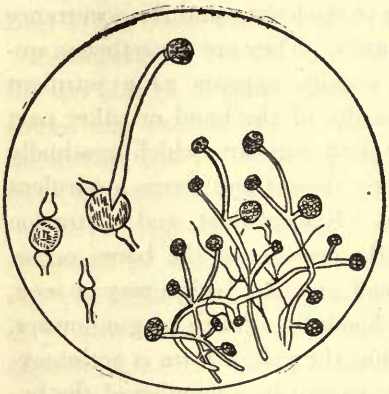


Fig. 40. — Mucor Mucedo.

Other forms of asexual reproduction occur amongst these lower forms, one of which is the production of thick-walled spores termed *chlamydospores*, which possess a much higher degree of resistance than the thin-walled variety. Under certain conditions the conjugation of two cells precedes spore formation (sexual reproduction). So-called *gametophores* occur as outgrowths in neighboring hyphæ, and when the tips of the two gametophores come in contact they fuse, transverse septa are formed, and a zygospore is the result. From the matured zygospore a germ tube arises which may begin to function at once in the usual manner (Fig. 40).

The *mycomycetes* reproduce almost invariably by asexual spore formation. Two main groups are recognized on the basis of their method of forming spores. In one series a cell or *ascus* is formed at the end of a hypha and within it is produced a number of spores constant to the species. The number is always a multiple of two, usually eight. The group having *asci* is known as *ascomycetes*. In the second series no spore sac is formed. The terminal portion of a hypha segments into germinating branches known as conidiophores. These conidiophores divide into two or three branches,

the sterigmata. From these other sterigmata may be produced, at the end of which a single chain of constricted, bead-like spores or conidia are formed. Penicillium, the common blue mold, is a familiar example of this group (Fig. 41).

Molds have claimed attention probably more because of their ability to spoil fruit preserves and other food substances than their tendency to produce disease. Their spores are practically ubiquitous and are more numerous in ordinary air than bacteria. Mold infection of plants, such for example as potato rot, often results in serious economic loss; other infected plants may if ingested have a disastrous effect upon the body cells. The mold Claviceps purpurea which infects rye and other grain has been found to cause a condition of poisoning known as ergotism.

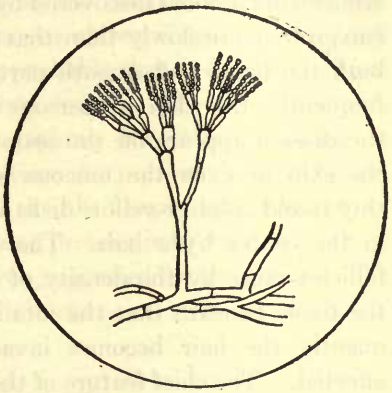


Fig. 41. — Penicillium.

Fortunately, comparatively few varieties are pathogenic for man. Pigeons are extremely susceptible to the genus Aspergillus, which gives rise to a form of pseudotuberculosis. A number of cases of the disease have been reported in human beings, especially amongst bird fanciers. A case of mold infection has been reported which at autopsy showed multiple abscesses in the brain, lungs, intestines, and peritoneum, and in all the lesions a species of *mucor* was found. Eye and ear infections have also been attributed to the same organisms.

Ringworm. — Ringworm is probably the most frequently met with of the diseases due to hypomycetic growth. It is contagious in that it may be communicated from one individual to another or it may be contracted from domestic animals. The disease is caused by at least two different members of the species Trichophyta of the *fungi imperfecti* group, Tinea circinata affecting the body and Tinea tonsurans the head. The fungi are more parasitic

than certain other forms in that they penetrate to the underlying tissue instead of vegetating on the surface layer of the skin. The infection probably commences first in the epidermis surrounding the hair bulb and from thence it spreads into the bulb and up into the hair substance. The organisms may be readily seen by removing a hair with the bulb attached and placing it in a drop of sodium or potassium hydroxide solution under a coverslip. Examined with the microscope, enormous numbers of interlaced threads and spores may be seen lying within the bulb.

Favus. — The disease is contagious and is produced by Achorion schoenleinii, a mold discovered by Schoenlein in 1839. The organism grows more slowly than that producing ringworm. It affects both the hairy and smooth parts of the body and attacks most frequently the skin of persons whose vitality is low. Usually the disease appears on the scalp, but may attack any portion of the skin or even the mucous membranes. Growth appears as tiny round sulphur-yellow disks with a cup-like depression pierced in the center by a hair. The spores penetrating into the hair follicles cause by the density of their growth such pressure upon the tissue beneath that the vitality of the hair is impaired. Frequently the hair becomes invaded, the shaft especially being affected. The chief feature of the disease, however, is the destruction of the epithelial cells of the hair follicles, resulting when recovery takes place in the formation of cicatricial tissue.

Pityriasis Versicolor. — The organism giving rise to this condition was discovered by Eichstedt in 1846 and later named *Microsporon furfur*. It appears to be less parasitic than the fungus of ringworm and flavus and attacks only the superficial layer of the skin. The growth appears as a scaly eruption, varying in color from a creamy yellow to a reddish brown. It occurs chiefly in persons living under uncleanly conditions or in those who have a tendency to profuse perspiration. Several of the lower animals are susceptible, especially the cat.

Sporotrichosis. — The disease, which is characterized by a swelling of the lymphatics and a chronic ulcerative condition, was shown by Schenk in 1898 to be due to another member of the *fungi*

imperfecti group. The initial infection usually takes place through some slight abrasion of the skin and from thence spreads along the line of the lymphatics. Lesions have been recorded in the larynx, pharynx, and muscle and bone tissues. Sporotrichosis appears in the past to have been frequently confused with syphilis because the condition readily yields to treatment with iodin compounds; mercury salts have no curative effect.

Thrush. — Infection, which is most frequent in young infants, occurs as white patches of fungoid growth on the tongue and fauces and may extend into the esophagus. General infection has been reported, with abscess formation in the various internal organs. In lesions and in cultures the fungus shows characteristics both of the molds and yeasts, and it is probable that it occupies a position intermediate between the two forms. The yeast-like portions are oval in form, averaging about 5.5 μ long and 4 μ in diameter, while the thread formations vary greatly in length and thickness. Several varieties of thrush fungus are thought to exist, although their classification at present is by no means clear. The name *monilia candida* has been suggested for the group. The term in common usage is Oidium albicans.

BLASTOMYCETES

Yeasts, like molds, have been studied in the past mainly because of their economic importance; for many centuries their rôle in the brewing and baking industries has been recognized. The main characteristics of the yeasts is their mode of reproduction by budding, hence their name of blastomycetes or "budding fungi" in contrast to that of molds, hyphomycetes, or "thread fungi." Nevertheless no strict separating line can be drawn between the two groups since, as already stated, forms exist which possess characteristics of both groups.

Ordinarily yeast cells are oval in shape, each cell possessing a more or less definite nucleus and surrounding wall of cellulose; they vary in size from 1 μ in diameter in old cultures to giant cells which may have a width of 40 μ. During the process of

budding the nucleus moves to the edge of the cell and commences to divide. Soon a protrusion appears which rapidly develops into a daughter cell of the same shape and size as the mother cell. Under certain conditions many yeasts are able to reproduce by spore formation; the nucleus divides usually into four portions, each of which becomes the center of a new cell lying within the parent cell (Fig. 42).

FIG. 42.— Yeast Cells.

Pathogenic Yeasts. — Busse in 1894 was the first to report the pathogenicity of a yeast to which was given the name Saccharomyces busse. In the case he studied the first lesion appeared in the form of an abscess on the tibial bone. Thirteen months later the patient died from a generalized yeast infection, and on autopsy the yeast was found in lesions in the ulna, lung, kidney, and spleen.

Since the discovery by Busse many similar infections have been reported. In most cases a small papule first appears with a moderate indurated area surrounding it; later a pustule forms which discharges yellowish pus. The lesion spreads slowly, and as it invades fresh tissue the older areas show a tendency to heal.

The organisms may be readily demonstrated in film preparations made from pus in the usual manner and stained with methylene blue. For their cultivation glucose agar is the most suitable medium. They are isolated with difficulty from material in which bacteria are growing because they develop more slowly than the latter. Repeated plating and the use of high dilutions is generally necessary for their isolation.

The tumor-like growths in some forms of blastomycosis has led certain observers to assume a relationship between these organisms and cancerous growths. The assumption has not yet been supported by satisfactory evidence.

CHAPTER XXVI

THE PATHOGENIC PROTOZOA. AMEBÆ. FLAGELLATA

THE lowest forms in the animal kingdom, the *protozoa*, are characterized by the simplicity of their structure as compared with the higher animals, the **metozoa**. For the most part each organism consists of a single cell composed of cytoplasm and nuclear substance. Nevertheless, although unicellular and of such simple morphology the protozoa are much more complete than bacteria both in form and in their life cycle.

Morphology. — The cytoplasm of the cell consists usually of an outer, dense portion, the ectoplasm, and an inner, more fluid portion, the endoplasm, which surrounds one or more nuclei as well as various granules and vacuoles. Certain of the latter appear to act as digestive organs; others show periodic contractile movements and serve to eject waste products from the cell body.

In many of the protozoa the chromatin substance of the nucleus is massed together in a deeply staining round body called the **karyosome**, and embedded in the karyosome is the centrosome, a small body always present in metazoön cells, which plays an important part in cell division. In certain forms still another definite portion of the nuclear chromatin, the **kinetic nucleus**, may form the root of a flagellum. The kinetic nucleus may be distinct or it may merge into another small body, the **blepharoplast**. Each of these four bodies, the **karyosome, centrosome, kinetic nucleus,** and **blepharoplast**, have their origin in the nucleus of the cell.

When conditions become unsuitable the organisms may protect themselves by forming a highly resistant enveloping membrane. Such an encysted form will withstand extremes of heat and cold and long periods of drying, and then as soon as conditions

become again suitable the organism absorbs water, the cyst wall ruptures, and ordinary development is recommenced.

Nutrition and Reproduction. — Many of the protozoa obtain their nourishment by the absorption of the fluid food directly through the cell wall; others are able to ingest solid particles, such as bacteria, through a suctorial tube or, as amongst the ameba, by extending a portion of their protoplasm and completely surrounding the food morsel. After the food is digested the waste substance may be excreted by osmosis or by means of a contractile vacuole.

The simplest form of reproduction is by transverse or longitudinal division. A more complex form, spoken of as multiplicative reproduction, or brood formation, occurs, in which the nucleus divides into several portions, each of which becomes surrounded with protoplasm and finally separates into as many daughter cells. Multiplicative reproduction without conjugation is spoken of as **schizogony** and the daughter cells are known as **merozoites**; when after fertilization such division takes place within a cyst it is spoken of as **sporogeny** and the resulting cells are called **sporozoites.**

Many forms multiply both sexually and asexually, some of which pass the sexual phase of their existence in one host and the asexual phase in another.

The forms already studied that are pathogenic for man are included in four main classes:

1. Rhizopodia. Movement by means of temporary protruding portions known as pseudopodia; reproduction by simple division or multiplication within a cyst; possession of one or more nuclei. Genus parasitic for man. **Entameba.**
2. Flagellata. Movement by means of flagella; possession by certain forms of nucleus, contractile vacuoles, and a small opening for food. Genera parasitic for man. **Trypanosoma, Leishmania.**
3. Sporozoa. May form pseudopodia; food ingested by osmosis; one or more nuclei; no contractile vacuole;

reproduction by spores. Genera pathogenic for man. **Coccidia, Sarcosporidia, Nosema, Babesia, Plasmodia.**

4. Ciliata. Movement by means of cilia; reproduction by transverse division. Germs parasitic for man. **Balantidium.**

AMEBÆ

The amebæ are characterized by their ability to project portions of their protoplasm into pseudopodia, or " false feet " which serve as organs of locomotion and nutrition. The pseudopodia may protrude from any portion of the cell or from different parts at the same time; they are quite irregular in form and are called forth only in response to some physical or chemical stimulus. When such a stimulating object may be used as food the pseudopodia flow around it and eventually absorb it into the cell protoplasm. All forms of ameba possess one or more nuclei and usually a contractile vacuole. Multiplication takes place by simple division or by encysted brood formation (Fig. 43).

FIG. 43.— Ameba.

Saprophytic forms are abundant in nature; they may be found wherever moisture and decaying vegetable matter exist. Yet, notwithstanding their common occurrence, little is known of their life history. Not all forms of protozoa showing ameboid movement can be classified as rhizopodia until most of their life history is known, since members of other classes may pass through an ameboid stage. Some flagellates, for example, during one period of their existence develop blunt pseudopods and crawl along as typical ameba.

Three forms of ameba have been described as parasitic in man: Entameba histolytica, E. coli, and E. gingivalis.

E. Histolytica. — As early as 1860 Lambl of Prague discovered amebæ in the stools of a severe case of dysentery. Very soon other investigators reported their presence both in dysenteric and in normal stools, with the result that a number of parasitic forms were thought to exist. Schaudinn in 1903 clearly showed that many of the forms described represented different stages in the development of one organism and that practically only two intestinal forms had been discovered, which he renamed E. histolytica and E. coli. The latter he regarded as a harmless parasite and the former as the inciter of amebic dysentery.

Amebic dysentery differs from bacillary dysentery in that it is a chronic infection of the colon which starts insidiously and is characterized by relapses and recurrences. It occurs sporadically or in endemic form in the tropics and not unfrequently in the temperate zones and in about 20 per cent of cases is complicated by liver abscesses. Emetin administered hypodermically has proved of such therapeutic value that it is accepted as a specific. Bacillary dysentery, on the other hand, is an infection of the small intestine, has an acute onset, marked symptoms of toxemia, occurs in epidemic form, usually has no sequelæ, and is not influenced by emetin.

Amebic dysentery runs an irregular course over a period of a few weeks to several years. In severe forms the stools are watery and contain varying amounts of blood and mucus; they vary in number from twenty to fifty in twenty-four hours. The amebæ penetrate between the epithelial cells to the submucosa, multiply there, and by their presence irritate the tissues. At first there is an edematous local swelling; soon the mucous membrane becomes ulcerated, and gangrenous sloughs result. The ulcers thus formed have an irregular overhanging border with a much larger cavity in the submucosa than the opening into the mucous membrane indicates.

When liver abscesses occur as a complication they are usually single and of a large size, or occasionally numerous small ones may be seen. The contents usually consist of a gelatinous pink fluid containing necrosed tissue, blood, and amebæ. It not

infrequently happens that such abscesses rupture through the diaphragm into the lungs or into the peritoneal cavity.

E. histolytica has been shown to be pathogenic for cats, dogs, and monkeys, provided infection takes place either by feeding them with material containing cysts or by rectal inoculation of vegetative forms.

The source of pathogenic amebic infection is not definitely known. In Manila the water supply is considered responsible for its transmission. Recent experiments on Filipinos who acted as volunteers tend to show that E. histolytica is a strict parasite, and consequently infection can only come from an individual harboring the organism in the intestines. Since the organism always enters the body by the mouth and leaves in the feces, transmission evidently occurs through the ingestion of substances contaminated with infected excretions.

Examination of Feces. — In fresh specimens E. histolytica appears as a large, round or oval body 20 to 30 μ in diameter; the nucleus is pale and not readily seen. Usually there are numerous digestive vacuoles in which red blood cells may be seen. Often in unstained preparations the amebæ appear to be of a greenish color, due, it is thought, to the hemoglobin liberated from the ingested red cells. When in the cyst form they are small and round and show four distinct spherical nuclei.

The feces from a suspected case should be examined as soon as possible after being passed; a loopful of the slimy portion is diluted with physiological salt solution and examined in a hanging drop, preferably on a warm stage. By this means the kind of movement and the nature of the vacuoles may be observed. The addition of a drop of 1 to 500 neutral red solution will stain the amebæ a pale pink color.

Cultivation. — After many unsuccessful attempts to cultivate amebæ, Musgrave and Clegg devised the following ingenious method. Agar medium was poured into sterile Petri dishes, and after hardening several rings of a pure culture of dead bacteria were spread around the medium. A loopful of the material containing the amebæ was placed in the center of the dish. It was

found that as the amebæ multiplied they traveled toward the edge, and in passing the rings of bacteria they deposited the living organisms with which they started and took up the dead ones. Thus after forty-eight to seventy-two hours active amebæ free from living bacteria were found at the periphery. It has recently been found that certain strains of intestinal amebæ will grow in pure culture when inoculated on sterile tissue such as brain, liver, or kidney placed in nutrient agar.

E. Coli. — Various investigators have reported the presence of E. coli in from 20 to 60 per cent of all normal stools examined regardless of locality. The organisms vary from 20 to 40 μ in diameter. As a rule they are less actively motile than E. histolytica and contain more and larger vacuoles, which usually are filled with bacteria and rarely with the red blood cells so palatable to the latter. Another distinguishing characteristic is their division in the encysted form. E. histolytica usually produces only four daughter cells, while the normal number for E. coli is eight. Experiments have demonstrated that parasitism may be brought about by feeding human beings with E. coli cysts. No disease, however, results, though the amebæ may be present in the intestines for years.

Entameba Gingivalis. — The organism is almost invariably present in pyorrhea alveolaris, but it is also present in normal mouths, and as yet no experimental evidence has established its relation to the disease. The organism can readily be found in tartar scraped from the teeth near the gum margin. It measures from 12 to 20 μ in diameter, contains a nucleus and many food vacuoles. Multiplication occurs only in the vegetative stage and then by simple division. Cyst formation does occur, but it is purely a protective stage and not one of reproduction.

FLAGELLATA

The flagellata, as their name implies, are characterized by the possession of one or more flagella. They are divided into several subclasses. Those pathogenic to man belong chiefly to the genera Trypanosoma and Leishmania.

Trypanosomes. — The structure of the trypanosomes while varying in detail is more or less uniform for the entire genus. The body is long and flexible, tapering anteriorly to a fine point; the posterior extremity is always less sharp and often quite blunt. The nucleus is usually situated in the center of the cell and behind it, often near the posterior end, is the kinetic nucleus. The flagellum rises from the blepharoplast, which is located near to or within the kinetic nucleus, and reaching the surface, turns forward and forms the edge of a thin fluted fold of ectoplasm, the *undulating membrane*, which runs the entire length of the cell, and then continues forward as a thin, thread-like filament; a smaller flagellum occasionally is formed which is directed backwards and acts as a rudder. During life the constant wave-like motion of the undulating membrane and the lashing of the flagellum enables the organism to move with great rapidity.

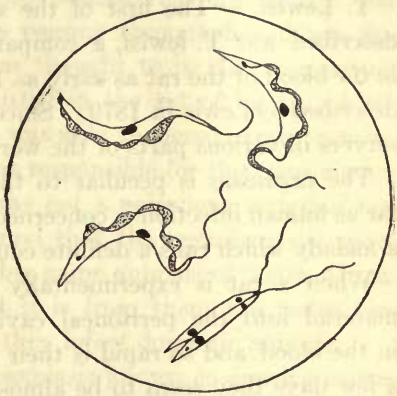


FIG. 44. — Trypanosomes.

The average length is about 30 μ and the width 1.5 to 3 μ. Usually one to several contractile vacuoles as well as food vacuoles may be seen. Occasionally there is a definite grooved opening or cytosome for the entrance of food. Pseudopodia may develop during one phase of existence, but they are transitory.

Multiplication ordinarily occurs by longitudinal division; in certain forms reproduction takes place within a cyst after fertilization.

Cultivation of the trypanosomes outside of the body was first accomplished by Novy and MacNeal in 1903. They prepared a medium consisting of equal parts of nutrient agar and defibrinated rabbit's blood. On such medium at the end of several days a fairly good growth may be obtained.

The presence of the organism may usually be demonstrated

in a hanging drop preparation of freshly drawn blood. If trypanosomes are present attention is soon attracted to their neighborhood by a disturbance amongst the red blood corpuscles, and soon the rapidly undulating organisms come into view. Blood films may also be made and colored with Romanowsky stain.

With the exception of dourine, a disease occurring amongst horses, the trypanosomes are transmitted from one animal to another through the agency of blood-sucking insects or leeches. The organism undoubtedly passes through a definite cycle of development within its invertebrate host, the details of which, however, are not yet known.

T. Lewisi. — The first of the species to be more or less fully described was T. lewisi, a comparatively non-virulent form seen in the blood of the rat as early as 1845, but more fully studied and described by Lewis in 1879. Since then it has been noted by observers in various parts of the world.

The organism is peculiar to the rat and of no importance so far as human infection is concerned, but in that animal it produces a malady which runs a definite course although it is rarely fatal.

When a rat is experimentally infected by injecting infective material into the peritoneal cavity the organisms soon appear in the blood, and so rapid is their multiplication there that within a few days they seem to be almost as numerous as the red blood cells. The infection continues for about two months, during which time the animal may show no symptoms of disease. At the end of that period the parasites gradually disappear and the rat is immune against further infection. The serum of such a rat has highly protective properties and a marked agglutinative capacity, causing the trypanosomes to gather together in a rosette formation in which the flagella are directed outward.

The rat flea is responsible for the transmission of the parasite. It becomes infective about a week after biting a diseased animal and remains so for the rest of its life, passing the trypanosomes in its dejecta. The organism may enter through the puncture made by the flea in the act of sucking or may be swallowed by the rat when licking its fur.

T. Evansi. — A disease of horses known as *surra,* which is very prevalent in India, was shown by Evans to be due to a trypanosome. Experiments have shown that the disease is transmitted by flies.

T. Brucei. — Nagana or tsetse fly disease is prevalent in South Africa and especially in Zululand, where it affects chiefly horses, cattle, and dogs. In 1894 Bruce discovered that the blood-infected animals swarmed with the trypanosomes now known by his name. The disease, which is similar to the surra of India, is characterized by a watery discharge from the eyes and nose, swellings on the surface of the abdomen and legs, and an increasing emaciated and anemic condition which generally results in death after several weeks. It had been noticed that the disease was frequently contracted by horses passing through hot, damp, fly-infested areas, and the cause was thought to be due to a poison secreted by the fly and transmitted in the act of biting. After a number of experiments Bruce was able to demonstrate conclusively that while the tsetse fly was responsible for the transmission of the causal agent the latter was not a poisonous secretion but living trypanosomes. Bruce found that the organisms are more or less harmless parasites of the big game animals of South Africa. Consequently he concluded that it is from them the tsetse flies derive the parasites with which they infect domestic animals.

T. Equiperdum. — A trypanosomiasis known as dourine occurs among horses in various parts of Europe, and has occasionally developed in some of the northern states of America and western Canada amongst imported horses. A characteristic of the disease is that so far as is known it is transmitted by coitus and not by biting insects. The disease is usually of a chronic nature; the animal becomes paralyzed and death occurs as a rule in from two to ten months.

T. Gambiense. — Within comparatively recent years the important discovery was made that the terrible disease known as *sleeping sickness,* which affects the natives of South Africa and also Europeans, is a form of human trypanosomiasis. The parasites found by the first investigators are grouped together under the name T. gambiense. They closely resemble T. equinum and T.

brucei and are also conveyed by the bite of a tsetse fly, *Glossina palpalis*, belonging to the same genus as the fly responsible for the transmission of nagana.

The symptoms of the disease are divided into two stages. In the first they are of a mild character, the pulse and respirations are quickened; there is an irregular elevation of temperature and enlargement of the lymph nodes. The trypanosomes are found in small numbers both in the enlarged glands and in the blood. After several months and in some cases years the second stage of the disease commences. The fever becomes hectic, the patient is listless and apathetic, neuralgic pains, trembling of the muscles, and gradually increasing emaciation and lethargy develop, until finally a comatose condition occurs and death ensues. The duration of the second stage is from four to eight months, during which period the parasites may always be found in the spinal fluid. So far as is known the disease is invariably fatal, and so prevalent is it in certain parts of South Africa that in some villages from 30 to 50 per cent of the inhabitants have been found to be infected.

Treatment with atoxyl and arsenic compound is said to have been of benefit in animal trypanosomiasis. In human cases, however, the results have been more uncertain.

T. Rhodesiense. — Another trypanosome, T. rhodesiense, isolated in 1911 from cases of sleeping sickness in Rhodesia, differs morphologically from T. gambiense and is also more virulent. It is conveyed by the tsetse fly (Glossina morsitans) and is a harmless parasite of certain wild South African animals.

Still another form of human trypanosomiasis transmitted by a bug occurs in Brazil, where it is known as " Chagas disease."

LEISHMANIA-DONOVANI

Leishman discovered in 1900 in the blood of a number of soldiers suffering from a febrile disease, who had been invalided home from Dum-Dum, an unhealthy section of India, peculiar bodies which he thought resembled degenerating forms of T. brucei. In 1903 he published his observations and suggested that the cachexial

fever so prevalent in India might be a form of trypanosomiasis. Later in the same year Donovan, working independently in India, confirmed Leishman's findings, and the organisms became known as the Leishman-Donovan bodies. The disease to which they give rise has been called by a variety of names in different sections of the tropics, *e.g.* dumdum fever, non-malarial cachexia, and kala-azar. Recent investigators have reported cases in many parts of India, China, Turkestan, Algiers, Egypt, Italy, and Greece.

Kala-azar is characterized by fever of an irregular type, a peculiar dark earthy pallor of the skin, progressive anemia and emaciation with enlargement of the spleen and liver, and frequently edematous swellings and ulcers of the skin. The disease is chronic, continuing for several years and having in about 80 per cent of cases a fatal ending.

In a film preparation made from the spleen the characteristic bodies appear round or oval and usually 2.5 to 3.5 μ in diameter. Within the protoplasm two intensely stained bodies can be distinguished: one, large, round or heart-shaped, situated near the periphery; the other, rod-shaped and generally distinct from the larger body. The organisms may be seen free or packed within phagocytic cells.

In the body the parasite multiplies ordinarily by simple division. Sometimes, however, multiplicative reproduction occurs, the nucleus dividing several times within the cytoplasm and giving rise to a corresponding number of new organisms.

Cultivated outside of the body on Novy and MacNeal's medium the organism develops into a flagellate form. The cell lengthens to about 20 μ. The smaller nucleus moves near to one end and from it arises a long, fine flagellum. The whole development occupies about ninety-six hours. It was not until the organisms were cultivated on artificial medium that their relationship to the flagellates was established.

Nothing definite is known as to the manner in which the disease is spread. Bedbugs fed on kala-azar patients have been found to harbor flagellates, but since a variety of similar organisms

may normally inhabit the intestines of the insects the evidence is not generally accepted as conclusive.

Leishmania Infantum. — In the southern parts of France, Portugal, Greece, and Italy a disease apparently identical with kala-azar occurs, affecting children between two and five years of age. The fact that in the regions in which it occurs the infection is confined to young children has given rise to the name *Leishmania infantum*. A similar disease occurs in dogs in the same regions, and the view has been advanced that the infection of children may be through the agency of dog fleas.

Leishmania Tropica. — In the tropics and also in subtropical regions a chronic ulceration of the skin is widely prevalent which is known by various names, such as tropical ulcer, Delhi sore, Aleppo boil, etc. From these ulcers bodies have been obtained which appear to be identical with Leishmania-donovani. The close similarity of the organisms isolated from the three forms of disease suggests that they may be of the same species. Knowledge of their relationship, however, is as yet incomplete.

CHAPTER XXVII

SPOROZOA. CILIATA

THE sporozoa, as their name implies, are characterized by their method of reproduction through spore formation. They are strict parasites and are for the most part harmless; only a few species are known to be pathogenic. The organisms vary greatly in size, in structure, and in development. Certain forms are so small that several may be contained within a single red blood corpuscle, while others are large enough to be seen with the naked eye. Some members of the group show ameboid movements during certain phases of their existence, but the pseudopodia serve only as a means of locomotion and not of nutrition. Their life history is more or less complicated, one period being passed in one host and the other period within the body of another species; or different phases of development may take place in different tissues of a single host.

The known pathogenic forms occur among five different genera: **Coccidia, Sarcosporidia, Nosema, Babesia,** and **Plasmodia.**

Coccidia. — A disease of rabbits occurs in epidemic form due to **Coccidium cuniculi**; a few cases of human infection have been reported.

Sarcosporidia. — Organisms belonging to this group affect mainly swine and cattle; a case of human sarcosporidiosis was reported in 1909 in Panama.

Nosema. — So far as is known no infection attributable to the nosema has occurred in man. One member, however, is of interest in that it is the cause of pebrine, the infectious disease of silkworms, to which Pasteur devoted several years of study.

Babesia (Piroplasma). — Babes was the first to observe the parasites in the blood of Roumanian cattle. Later, in 1893,

Theobald Smith described them more fully and established their relationship to the disease of cattle known by various names, such as Texas fever, tick fever, hemoglobinuria, and red-water fever. The disease is characterized especially by destruction of the red blood corpuscles and infection of the spleen and liver. So far as is known ticks are solely responsible for its spread. After fertilization the female gorges herself with blood, then drops to the ground and there lays about 2000 eggs, depositing within the shell of each sufficient blood to serve the embryo as food. The insect dies within a few days after the egg laying has been completed. The eggs hatch in about three weeks, and the larvæ, containing within their bodies some of the blood of their mother, crawl about until they die or have the opportunity of attaching themselves to another animal. If the larvæ are the progeny of an infected mother they thus become the means of further disseminating the disease.

Similar infections affecting other animals have been shown to be due to Babesia-like parasites.

Plasmodia. — For many centuries malaria has appeared in certain regions as a veritable scourge, yet nothing definite was discovered as to its cause until the latter part of the nineteenth century. The fact that it remained prevalent in certain areas and not in others led to the supposition that atmospheric conditions were in some way responsible and to its being termed " malaria " or " bad air."

With the establishment of the theory that each infectious disease is caused by a specific infecting organism the study of malaria was taken up with enthusiasm. Several investigators described bacterial forms which they thought might be the causal agents, but their findings were disproved. In 1880 Laveran, a French military surgeon stationed in Algiers, announced that he had discovered a parasite in the blood of malarial patients which was found to be a protozoön of the class Sporozoa. His discovery was confirmed by the independent researches of two Italian investigators, Marchiafava and Celli, who gave the organism the name of *Plasmodium malariæ*. The term Hemameba has been suggested as more appropriate and is frequently employed,

yet according to the rules of zoölogical nomenclature the first given name must be retained.

In 1896 Ross found, while studying a form of malaria affecting birds, that the parasite was transmitted from infected to healthy birds by the bite of the common mosquito, Culex. Reasoning from this fact, he concluded that the malarial parasite giving rise to the disease in human beings might be conveyed in a similar manner. After a long series of experiments he found that the latter could not develop in the body of Culex, but that it did develop in another variety, Anopheles. He observed rounded, pigmented bodies in the stomach wall of Anopheles that had been fed with the blood of malarial patients, and he was able to trace all the stages of development from the time the organism entered the stomach with the blood until it settled in the poison-salivary gland of the insect ready to be transferred again to a human host.

Strikingly conclusive evidence of the ability of mosquitoes to transmit infection was afforded by Manson in London. About forty mosquitoes were shipped from Rome after sucking blood from a case of tertian malaria, and Manson's son allowed himself to be bitten by them, with the result that an attack of tertian fever followed and the parasites were found in the patient's blood.

Reproduction. — Both man and mosquito are necessary to complete the life cycle of the parasite. Since the sexual phase is passed within the mosquito the latter is considered the definitive host and the former the intermediate host. It is, however, more convenient to consider the asexual cycle first.

Asexual Phase (Schizogony). — At the time the parasite is conveyed by the bite of the mosquito it appears as a small ring-like body, and on entering the blood stream of its human host it attaches itself at once to a red blood corpuscle and commences to send out distinct pseudopodia into the cell substance. As it approaches maturity segmentation begins, giving to it the appearance of a rosette within the corpuscle. Very soon the segments separate into small, rounded forms or *merozoites*, the cell wall of the red blood corpuscle ruptures, and the young merozoites thus liberated into the blood stream at once fasten themselves on to

fresh corpuscles and begin anew their cycle of development. The typical malarial chill appears just at the time of the freeing of the merozoites (Fig. 45).

Not all of the full-grown parasites segment and produce merozoites; a certain number become *gametocytes* or sexual forms. The female cells, the macrogametocytes, are the larger, measuring about 12 μ, and containing coarse grains of pigment; the male cells, microgametocytes, are smaller and stain more faintly. In

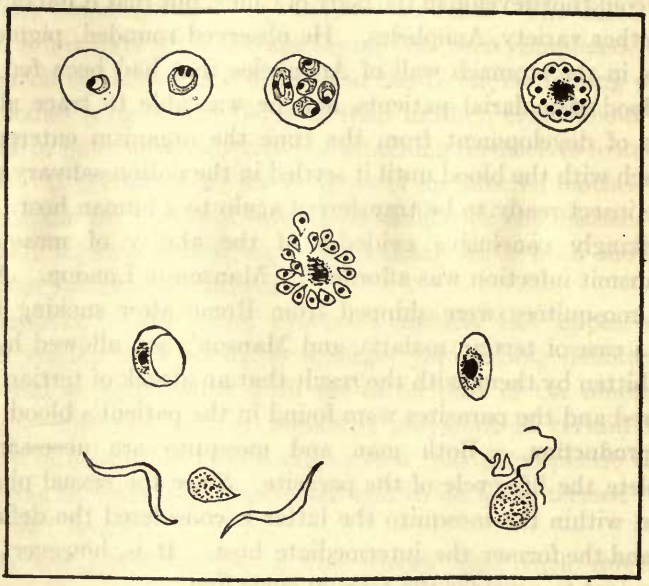


FIG. 45. — Malarial Parasite. *A*, Development of Merozoites; *B*, Gametocytes.

the circulating blood of human beings the gametocytes show no further change, but when the blood is shed or after it is swallowed by a mosquito an interesting series of changes may be observed.

Sexual Phase (Sporogeny). — The same phenomena which occur within the body of a mosquito may be observed in freshly drawn infected blood examined under the microscope. In the rounded male cell a vibratory movement of the chromatic granules can be seen and very soon four to eight long flagella-like appendages emerge. These delicate filaments, which represent the sperm cells

of the parasite, are extremely active, soon become detached, and move away into the surrounding fluid in search of the female cells. The latter project a small portion of protoplasm on to their surface and into this one of the flagellate sperm cells enters, the protoplasm is instantly retracted, and the fertilized female cell is then spoken of as a *zygote* or *oökinete*. In the stomach of a mosquito the zygotes penetrate the stomach wall and settle between the muscle fibers, where on the second day after the ingestion of infected blood they may be seen as small, rounded cells about 8 μ in diameter, containing masses of pigment. Around each zygote a membrane, termed a sporocyst, develops, and as the organisms increase in size they project into the stomach cavity, giving to it an irregular beaded appearance. Meanwhile numerous spherical bodies (sporoblasts) have been formed within the interior of the zygote and these again divide into a large number of thread-like cells (sporozoites). The full development of the sporocyst takes from eight to ten days, after which it bursts, and the liberated sporozoites are carried by the lymphatics to all parts of the body of the mosquito. They settle especially within a large gland which is a combination of a poison and salivary gland and which also is in close connection with the biting apparatus of the mosquito. Hence when an infected mosquito bites a human subject the sporozoites readily pass into the puncture with the gland secretions and the cycle within the human host begins. Since only the female mosquito sucks blood it alone is responsible for the spread of the disease.

It will be noted that in the mosquito the parasite passes through one cycle only, while in the human host it passes through an indefinite number which recur at regular periods.

So far as is known the parasites of human malaria do not invade any other mammalians nor does any other species of mosquito other than Anopheles harbor them. Anopheles may be distinguished from the more common Culex by the following characteristics: it appears usually after sunset, while Culex is a day-flying variety; when at rest its body stands off at an acute angle from the surface of the object on which it is reposing, whereas the body of Culex appears almost parallel with the surface. Many species

of Anopheles may be distinguished by their spotted wings. Another distinction exists in that in the female Anopheles the palpi are as long as the proboscis, whereas in the female Culex they are always shorter. The eggs and larvæ of the two genera also differ; the eggs of Anopheles are single and are supported on the surface of the water by air cells, those of Culex are numerous and attached together in masses by a cementing substance. The breathing tube of the Anopheline larvæ is short and its angle with the body necessitates that the larvæ lie parallel with the surface of the water, while that of Culex permits the body to lie at an angle with the surface.

Varieties of Malarial Parasites. — Three distinct varieties of the malarial parasite have been described: *Plasmodium malariæ* — the cause of quartan fever; *Plasmodium vivax* — the cause of tertian fever; and *Plasmodium falciparum* — the cause of estivo-autumnal fever. Certain authorities are of the opinion that two varieties may be concerned in the latter condition. Only one, however, is definitely known. The tertian and quartan fevers are more prevalent in temperate countries and are rarely fatal; the estivo-autumnal type, the malignant or pernicious fever of the tropics, is of a much more serious nature (Fig. 46).

P. Malariæ. — The asexual cycle of development is completed in seventy-two hours, the typical chill and fever occurring every third day, or according to the Roman method of reckoning, every fourth day. Double or even triple infection may occur. In the latter case infection on three successive days would result in the liberation of a brood of merozoites and the consequent clinical symptoms every day. The young P. malariæ are less active than P. vivax; the pigment is of a darker color, more coarsely granular, and is arranged around the periphery of the parasite, while that in the tertian parasite is distributed throughout the protoplasm. P. malariæ arranges itself as a band across the infected corpuscle. When mature the adult forms do not exceed the size of the red blood corpuscles, and the segments, usually six to twelve in number, are arranged symmetrically around the central pigment, giving the parasite at this stage the so-called daisy appearance.

P. Vivax. — The parasite of tertian malaria completes its asexual development in forty-eight hours; the paroxysms accordingly occur on alternate days. The adult parasite is larger and shows

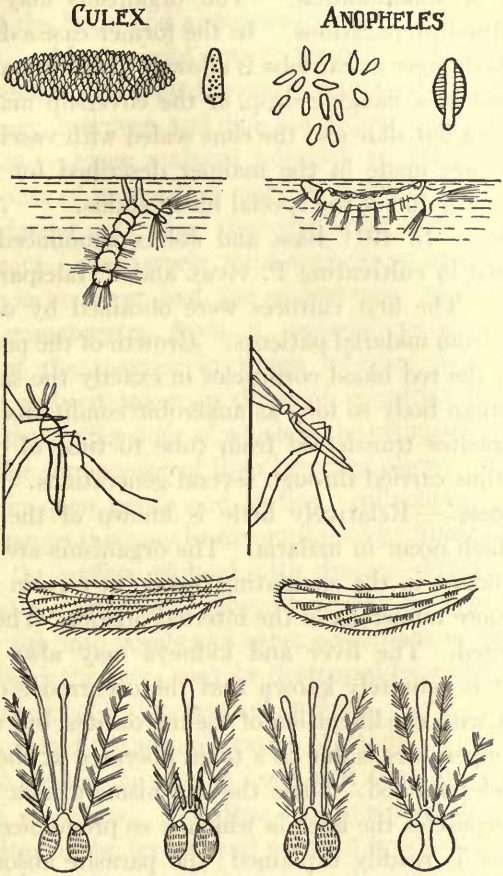


FIG. 46. — Comparison between Culex and Anopheles.

more active ameboid movements than the quartan form. The average number of merozoites produced is usually about sixteen.

P. Falciparum. — The developmental cycle in the human host occupies from twenty-four to forty-eight hours. The parasite is much smaller than the two other varieties and rarely reaches more

than half the diameter of a red blood corpuscle. The gametocytes differ also in that they are crescentic in shape when first liberated from the red blood cells.

Methods of Examination. — The organisms may be studied in fluid or dried preparations. In the former case a drop of blood from a pricked finger or ear lobe is allowed to fall upon a coverslip and examined in a hanging drop, or the coverslip may be gently inverted over a flat slide and the rims sealed with vaseline. Dried preparations are made in the manner described for blood films and stained with one of the special blood stains.

Cultivation. — In 1911 Bass and Johns announced that they had succeeded in cultivating P. vivax and P. falciparum outside of the body. The first cultures were obtained by defibrinating blood taken from malarial patients. Growth of the parasites took place within the red blood corpuscles in exactly the same manner as in the human body so long as anaërobic conditions were maintained. Parasites transferred from tube to tube of defibrinated blood were thus carried through several generations.

Pathogenesis. — Relatively little is known of the pathogenic processes which occur in malaria. The organisms are not always equally abundant in the circulating blood; at certain stages they tend to be more numerous in the internal organs. The spleen becomes enlarged. The liver and kidneys may also show some swelling. It is definitely known that the occurrence of the fever is coincident with the liberation of the merozoites, but whether the increased temperature is due to a toxin liberated at the same time is as yet undetermined. Since the organisms attack mainly the red blood corpuscles the anemia which is so pronounced a feature of the disease is readily explained: the parasite absorbs the cell pigment and thus destroys its function. During the paroxysms there is always a marked increase in the number of leukocytes, followed by a rapid decline. An interesting feature of the disease is the increased percentage of the mononuclear leukocytes, which sometimes even outnumber the polynuclears. The cells become particularly active phagocytes and engulf great numbers of pigmented parasites. In fact, the presence of excessive numbers of

pigmented mononuclear leukocytes has been taken as evidence of malaria by some workers, even though no parasites could be found in the blood. A striking feature in estivo-autumnal fever is the presence of enormous numbers of infected red blood cells in the capillaries of the brain and abdominal viscera.

Immunity. — Many mild cases recover without treatment hence it is evident that some immunity is produced by an infection. It is temporary, however, and does not protect against reinfection.

Prophylaxis. — Since malarial infection is transferred only by a certain species of mosquito a malarial patient can be considered a source of danger only when that particular variety of mosquito is in the vicinity. Conversely, the Anopheles mosquitoes are harmless if they have never had the opportunity of drinking blood containing gametocytes from a malarial patient. With the knowledge of the manner in which infection is spread active measures have been taken in different countries to control the disease, the most efficacious of which is the extirpation of the mosquitoes by the suppression of their breeding places. Since Anopheles breeds in open ponds and natural collections of water in fields and swamps this may be accomplished by filling up low places and drying the surface of land with drains. Where drainage is not practical the number of mosquitoes may be kept in check by introducing fish into ponds and other collections of water. Upon limited surfaces the larva may be destroyed by cutting off the air supply by means of a thin layer of coal oil.

Koch advocated the administration of quinine in order to destroy the parasite within the human body and thus prevent the infection of the mosquito. The drug has a remarkable effect upon the merozoites of the tertian and quartan types and great success has resulted from its use. A single large dose may be administered as the temperature begins to decline, that is, shortly after the young merozoites have been liberated into the blood stream; or in cases of double or triple infection where different broods come to maturity at different times smaller doses at definite intervals have given better results. Unfortunately, the gametocytes are quite resistant to the drug.

The use of quinine as a prophylactic on a large scale is a comparatively recent measure. The Italian Government in 1902 commenced its sale at cost price to those communities which agreed to distribute it gratuitously to individuals unable to purchase it. The result has been remarkable. During the ten years previous to 1902 the deaths from malaria averaged 14,048 annually, whereas during the ten years following the average fell to 3853.

The administration of quinine to healthy individuals does not prevent infection; it destroys the young parasites in the blood after infection has occurred. Its use is advantageous in that it is cheap and its action is prompt. It can, however, only be considered as a tentative measure and cannot supplant mosquito suppression.

BLACKWATER FEVER

The condition known as blackwater fever occurs especially amongst Europeans in tropical countries. It is characterized by fever, hemoglobinuria, and delirium, frequently ending in coma and death. The etiology of the disease is not at all clear. A few observers consider it an independent disease; the majority, however, believe it to be the terminal stage of a malarial infection. The fact that an attack is often precipitated by the administration of quinine has led to the suggestion that the drug may be the responsible agent for the marked destruction of red blood cells which characterizes the disease. In the great majority of cases, however, if the malarial organisms cannot be found in the blood there is evidence of the patient having suffered from repeated attacks of malaria.

CILIATA

The Ciliata are the highest type of protozoa. They may be distinguished from the other groups by the presence of cilia distributed over the cell, which serve as organs of locomotion and which give to the group its name. They possess special structures for the reception of food and also for excreting waste products.

Only one of the ciliates, *Balantidium coli*, has been found patho-

genic for man. The parasite was first described in 1857; it is frequently present in the intestinal tract of swine, and though usually harmless it may give rise in them to a subacute form of dysentery. Cases of human infection have been reported, most of which have been found to be suffering from chronic intestinal catarrh.

The organism has somewhat the form of an egg with a funnel-shaped mouth opening. The ectoplasm is covered with thick bands of cilia which give the organism a striated appearance. Multiplication usually takes place by binary division; conjugation also occurs.

CHLAMYDOZOA

A small group of minute coccus-like organisms have been described which have the power of enveloping themselves with a covering derived from the cell substance of the host. Certain authorities have created a special class named **chlamydozoa** for them (Greek stem, chlamys — a mantle) and include them with the protozoa. A number of observers believe that members of the group may be the causal agents of rabies, smallpox, scarlet fever, trachoma, and other diseases. No definite proof, however, has as yet been advanced.

CHAPTER XXVIII

DISEASES CAUSED BY FILTRABLE VIRUSES. DISEASES OF UNKNOWN ETIOLOGY

A NUMBER of infectious diseases are caused by organisms that are able to pass through a fine porcelain filter. That the causal agents are present in the filtrate has been proven by animal inoculations; yet in a certain number of cases the highest degree of magnification possible fails to reveal their presence. Such organisms evidently are either "ultramicroscopic" or they cannot be rendered visible by present methods. Just what determines the ability of an organism to pass through a filter is uncertain; plasticity may be as important a factor as minuteness. A few of the filtrable viruses, although exceedingly minute, are still within the range of visibility.

The groups apparently bear no close relationship one to the other. Their modes of transmission are also widely different. Certain forms, for example, are transmitted by biting insects, as in yellow fever; others by direct contact through a wound or abrasion, as in rabies; still others by contact, as in cattle plague. Eventually, certain members will in all probability be classified with the protozoa and others with the bacteria.

In order to be certain that an organism is filtrable several precautions must be observed in carrying out the filtration process. The integrity of the filter must first be tested with a culture of known infiltrable organisms, all of which must be retained and not pass into the filtrate; after which the filter must be sterilized to insure its freedom from all germs. The pressure or suction should only be moderate; and the filtration should be completed within two hours; otherwise certain bacteria might grow through

rather than pass through the pores. After all precautions have been taken it must be shown that the pathogenicity of the filtrate is due to a living organism and not a toxin. This may be determined by inoculating a series of animals with the filtrate and later inoculating another series of animals with a filtrate of material obtained from the first series.

Epidemic Poliomyelitis. — The disease occurs both sporadically and in epidemic form in all quarters of the world and affects chiefly children under five years of age. The mortality is low, but about 75 per cent of the survivors are permanently deformed. The chief symptoms of the disease are fever, sometimes accompanied by sore throat and followed after a few days by paresis and paralysis.

Nothing was definitely known of the etiology of the disease until, in 1909, Landsteiner and Popper in Vienna reported its transmission to apes by the intraperitoneal injection of an emulsion of the spinal cord of a child who had died of infantile paralysis on the fourth day of illness. In the same year Flexner and Lewis transmitted the disease to monkeys by intracerebral injections and found that the brain and cord of the inoculated animals were infective for other monkeys.

In 1911 Flexner and Noguchi announced that they had succeeded in cultivating the virus in human ascitic fluid to which had been added a small piece of fresh sterile rabbit kidney. Growth takes place at first only under anaërobic conditions. Fragments of infected brain or cord or a filtrate of nerve tissue may be used for inoculating the medium. After about five days' incubation at 37° C. growth appears as an opalescent haze upon the fragment of tissue. Film preparations treated with Giemsa stain show the organisms as minute bluish or violet round bodies, measuring about 0.2 μ in diameter and arranged in pairs, chains, or groups. They appear to have a special affinity for nerve tissue. In an infection they are contained in the brain, spinal cord, salivary glands, mucous membranes of the nasopharynx, and very rarely in the cerebrospinal fluid or the blood. They are not weakened by freezing and will withstand 1 per cent carbolic acid for at least five days.

They are, however, only moderately resistant to heat; exposure to 45° C. to 50° C. for half an hour destroys them.

Experimentally the disease may be induced in monkeys by intracranial inoculation or by rubbing the virus on the sound nasal membrane. In such cases the incubation period averages from eight to nine days, but may range from five to forty days. Monkeys which have recovered from an attack of the disease are immune to fresh inoculations; also it has been shown that the serum of such animals when mixed with infective material has the power of neutralizing the virus to a certain extent. Furthermore, the serum of recently recovered human cases when injected into new cases within the first forty-eight hours is capable of arresting paralysis.

Since the worst epidemics occur in summer and in rural rather than urban communities the view has been advanced that an insect may be responsible for its transmission. Experiments have shown that a blood-sucking fly when infected may transmit the virus to monkeys. It is doubtful, however, if the fly is responsible for the spread of the disease; there is more evidence at the present time that its dissemination is due to contact, direct or indirect, with the virus discharged in the secretions from mouth or nose. The existence of healthy carriers may perhaps explain the outbreak of the various epidemics.

Yellow Fever. — The disease is an acute infection occurring chiefly in tropical countries and characterized by fever, jaundice, and hemorrhages. Several investigations have been made concerning its etiology, the most extensive probably being that of the United States Commission. The members of the Commission, Reed, Carroll, Agramonte, and Lazear, began their work in 1901, and although they did not succeed in demonstrating the causal agent they discovered facts concerning the transmission of the disease that has led practically to its eradication in areas where the necessary precautions have been taken.

In Havana preventive measures were first enforced in 1901, and within ninety days the town was free of yellow fever. Several weeks later new cases appeared, but the same measures were applied and the disease was quickly stamped out.

The Commission demonstrated that two species of hosts are necessary for the life cycle of the parasite, human beings and mosquitoes, and that under natural conditions it is transmitted from infected to healthy individuals only by the bite of a small mosquito, *Ædes calopus*, first spoken of as *Stegomyia*. After being bitten a period of about five days elapses before the parasite appears in the blood of the individual and remains there only during the three succeeding days. Thus an infected mosquito must necessarily have sucked the blood of a patient during the first three days of his illness. An infected mosquito does not transmit the parasite until at least twelve days after it has bitten the first patient. Yellow fever can be produced in man under artificial conditions by injecting the blood of a patient taken during the first three days of illness or even by inoculation with the serum of such a patient after it has passed through a fine Berkefeld filter.

Noguchi has recently announced the visibility of the parasite by dark field illumination and the possibility of its culture.

Rabies or Hydrophobia. — The disease occurs occasionally amongst most carnivora. It is transmitted to man usually by the bite of a dog. Experiments have shown that the virus is contained in the saliva from twenty-four to forty-eight hours before symptoms appear. In man the incubation period after being bitten varies from fourteen days to seven months, the rapidity with which the disease develops being governed by the amount of virus introduced, the point of inoculation, and the individual degree of susceptibility. It has been frequently observed that in wounds inflicted where the skin is thick and the nerves are few, or where the clothing has afforded some degree of protection, the incubation period is relatively long, whereas in wounds in parts more abundantly supplied with nerves the incubation period is much shorter and the disease usually more virulent. The symptoms of rabies generally begin with pain in the wound, extending along the nerves in the limb bitten, followed by a stage of nervous irritability, difficulty in breathing and swallowing due to spasmodic contraction of the throat muscles, and a marked increase in the flow of saliva. Very soon the convulsive attacks become more or less general

over the whole body. Generally the patient has full consciousness between the attacks until the final stage of the disease. The convulsive period lasts from one to four days and may be followed by a paralytic stage lasting from two to eighteen hours. In the majority of cases death occurs on the third or fourth day after symptoms appear.

Despite repeated investigations all attempts to discover the causal agent of the disease were unsuccessful until in 1903 Negri described certain round or angular bodies lying within the large nerve cells or their processes, which he claimed were specific for rabies and in all probability protozoan in character. Negri's observations have been generally confirmed and the presence of the bodies is accepted as diagnostic.

Negri bodies may be detected in fresh tissue by means of the smear method. Small pieces of gray matter are removed from three different portions of the central nervous system: (1) from the cortex in the region of the crucial sulcus; (2) from Ammon's horn, and (3) from the gray matter of the cerebellum. Minute portions of the tissue are placed on well-cleaned slides, crushed into a thin layer under a coverslip, after which the coverslip is moved slowly and evenly along the slide, leaving a film of nerve cells in its train. The smears are dried in the air and stained with Giemsa's stain. When examined under the oil immersion lens the organisms appear pale blue and within their protoplasm one or several round or oval pink bodies may be seen. In addition, both within the protoplasm and the pink-stained bodies small red or violet granules occur singly or in clumps.

Experiments have shown that the virus is filtrable. It is unharmed by freezing. On the other hand, it is readily destroyed by drying and by direct sunlight and is rendered inert by exposure for one hour to 50° C. When protected from heat, sunlight, and air it retains its virulence for a long period.

Pasteur in 1880 made the important discovery that rabies may be prevented by immunization with gradually increasing doses of the attenuated virus. So successful was his method of treatment that with some modifications it is still used in all parts

of the world. The method is based upon the principle of stimulating the production of rabic antibodies by the injection of weakened virus during the period of incubation, so that the virus introduced into the wound may be destroyed. Starting with the idea that the virus as it occurs in rabid dogs under natural conditions (street virus) is of a more or less constant degree of virulence, he found that the potency of such a virus could be diminished within a certain limit by passage through monkeys and similarly increased by passage through rabbits. In the latter case the virulence could be so exalted for rabbits that the incubation period was lessened from twelve or fourteen days to six or seven days, but beyond that point it was impossible to go. A virus of such strength he termed "fixed." By drying fixed virus over caustic potash he was able to obtain different degrees of attenuation according to the length of time of exposure, the weakest virus being so modified that it could not produce the disease in man but yet was able to produce the specific antibodies.

"Fixed" virus is prepared by the subdural inoculation of street virus through a series of young rabbits. During from thirty to fifty passages the incubation period is reduced to six or seven days. Immediately on the death of the animal a piece of the floor of the fourth ventricle is emulsified in sterile broth and three or four drops of the emulsion are injected beneath the dura of a normal rabbit. In from six to seven days paralytic symptoms appear followed in from three to four days by death. The cord and brain are then removed under aseptic precautions and the brain is set aside for further animal inoculations. From the cord, which is severed just below the medulla, a fragment is cut off and dropped into sterile broth to test for purity and the remainder is then divided into two equal portions which are suspended by sterilized silk threads in a glass jar containing sticks of caustic potash. The jars with the suspended cords are kept in a dark room at a temperature of about 22° C. After a suitable period of drying small pieces of cord are emulsified and injected subcutaneously into patients under treatment. In the New York Board of Health Laboratories $\frac{1}{2}$ c.c. of the indicated cord is emulsified

in 3 c.c. of saline, and 2½ c.c. of this emulsion is administered. If the material is to be shipped, 20 per cent glycerin and 0.5 per cent carbolic acid is added.

The uniform dose of 2½ c.c. for adults, prepared as described above, is administered in a series of inoculations as follows:

Day	Age of Cord
First	8 and 7 and 6 days
Second	4 and 3 days
Third	5 and 4 days
Fourth	3 days
Fifth	3 days
Sixth	2 days
Seventh	2 days
Eighth	1 day
Ninth	5 days
Tenth	4 days
Eleventh	4 days
Twelfth	3 days
Thirteenth	3 days
Fourteenth	2 days
Fifteenth	2 days
Sixteenth	4 days
Seventeenth	3 days
Eighteenth	2 days
Nineteenth	3 days
Twentieth	2 days
Twenty-first	1 day

According to reliable statistics the mortality of rabies without the Pasteur treatment is about 16 per cent, with the treatment 0.46 per cent. Taking into consideration only those cases in which the diagnosis of rabies has been confirmed in the animal, the mortality of the cases treated at the Pasteur Institute in Paris for the past ten years, which number about 6000, has been only 0.6 per cent, an enormous reduction in the 16 per cent of untreated cases.

Once the symptoms of rabies have appeared the treatment is unavailing.

An antirabic serum has been produced by inoculating sheep or horses with "fixed" virus. Its use alone is of little effect. Favorable results have followed its administration in conjunction with the Pasteur treatment, its use enabling the injection of the virus to be condensed.

Foot and Mouth Disease. — The disease, which was probably the first shown to be due to a filtrable virus, is highly infectious and occurs chiefly among cattle. It may, however, be communicated to man by milk or milk products or by contact with infected animals. It is characterized by a vesicular eruption on the mucous membrane of the mouth and on the skin of the foot. No specific microörganism has been demonstrated. It has been found, however, that lymph from the vesicles passed through the finest porcelain filter is still infectious.

Dengue. — The disease is restricted to warm climates and in many respects resembles yellow fever. The virus is filtrable, is found in the blood of patients suffering from the disease on the third and fourth day, and is probably transmitted by a mosquito.

Trench Fever. — During the recent war the malady has become recognized as a distinct disease. It is characterized by a sudden onset, pains in the limbs, back, and behind the eyes, fever, headache and giddiness. After from three to six days the symptoms subside. Frequently, however, in three or four days there is a relapse of shorter duration and milder form than that of the original attack. In the majority of cases complete recovery occurs; occasionally chronic rheumatic pains and myalgia persist.

Extensive studies have shown that the parasite is present in the blood, urine, and sputum of infected individuals, and that it is at least in one stage able to pass through a moderately fine filter. It is somewhat resistant to drying and to sunlight, but is killed by exposure to a temperature of 70° C. for half an hour. It is transmitted by lice.

A number of other diseases affecting animals have been shown to be due to filtrable agents, among which are *cattle plague* (*Rinderpest*), *hog cholera*, *African horse sickness*, *chicken sarcoma*, and *contagious pleuropneumonia of cattle*. A plant disease, the *Mosaic disease of tobacco*, has also been shown to be due to a filtrable virus.

Smallpox. — Variola or smallpox has been one of the most studied of the infectious diseases, not only on account of the terrible havoc it formerly wrought, but also to furnish an explanation of

the active immunization resulting from the form of vaccination introduced by Jenner.

The origin of vaccination against smallpox is not definitely known. The method of introducing the virus from a smallpox patient into a healthy person through an abrasion of the skin in order to produce a mild form of the disease and protection against subsequent attacks was practiced by the Turks during the eighteenth century. In 1778 Lady Mary Montagu, the wife of the British Ambassador at Constantinople, observing this practice among the Turks, had her own son and daughter inoculated and by her influence was instrumental in introducing the practice in Europe. The disease thus induced was generally but not always mild. Occasionally a case of unexpected virulence developed which proved fatal to the individual inoculated and a starting point of infection among unprotected persons.

Edward Jenner, a physician practising in Gloucestershire, England, was much impressed with the popular belief that those who contracted cowpox from an affected animal were immune to subsequent infection from smallpox. He believed that a disease occurring amongst horses, known as horsepox, manifested by an inflammatory and ulcerative condition of the hocks, was transferred, by the hands of men who dressed the sores, to the teats of the cows later milked by them and gave rise to cowpox. From infected cows other milkers contracted a mild form of the disease which manifested itself in lesions similar to those in the cow; namely, slight fever, malaise, loss of appetite, and a local papular eruption, later becoming pustular and finally drying up, leaving cicatrices varying in depth and extent at their site. Fully convinced that such an infection gave rise to immunity against smallpox Jenner determined to make experimental tests. In May, 1796, he inoculated a boy with lymph from a cowpox lesion on the hand of a dairymaid and in July he inoculated the same boy with pus from a smallpox patient without resulting infection. In 1798 he furnished additional proof of the protection afforded by vaccination with cowpox virus by inoculating a child direct from the vesicle on the teat of a cow and from the resulting lesion inoculating another child, and

so on through a series of five children, after which all were inoculated with smallpox virus without a single case developing. So convincing were Jenner's experiments that within a year or two such vaccination became extensively practiced all over Europe. It is said to have been introduced into the United States in July, 1800, by Dr. Benjamin Waterhouse, Professor of Physic at Harvard University, who vaccinated his own children.

Jenner and his supporters met with bitter opposition, and even now, more than one hundred years later, there are still to be found opponents to cowpox vaccination, notwithstanding the fact that its systematic application would soon eradicate smallpox from the list of human diseases.

The relationship of smallpox (variola) to cowpox (vaccinia) has been the subject of a great deal of controversy since Jenner's time, yet no adequate explanation has been found. According to the general belief the smallpox virus, whatever it may be, is so modified by its passage through a lower animal that it loses forever its power of producing smallpox, yet it still retains the ability to provoke the production of antibodies protective against the disease.

In sections of skin from both variola and vaccinia microscopic cell inclusions were first described by Guarnieri in 1892. These "vaccine bodies" are thought by certain investigators to be protozoan in character and to be closely associated with the cause of both variola and vaccinia; other authorities regard them as degenerative products.

As yet all attempts to obtain growth of the virus on artificial culture media has been unsuccessful. A few investigators have reported that the virus is filtrable; others have failed after repeated efforts to obtain an infective filtrate.

During the early days of Jennerian vaccination it was customary to inoculate with the material taken from the pustules of those previously vaccinated with cowpox lymph. The procedure served the purpose of immunization but it had several drawbacks, the chief of which was the danger of transmitting syphilis. For many years now it has been the custom to employ only vaccine

obtained directly from healthy animals, the production of which can be carefully controlled and tested.

The virus used for vaccinating the animals (seed virus) may be prepared in several ways. That usually employed by the New York City Health Department is obtained by first passing an emulsion of crusts obtained from healthy children about nineteen days after vaccination through a calf and subsequently through rabbits. The pulp obtained from the rabbit lesions is emulsified in a solution of glycerin and serves as seed for inoculating calves for the regular supply of vaccine.

Young female calves from two to four months old that are certified to be free from disease are prepared for vaccination. The posterior abdomen and inner surface of the thighs are shaved, washed with soap and water, then with sterile water and alcohol, and dried with a sterile towel. Over this area a number of long incisions about a quarter of an inch apart are made into which the seed virus is rubbed.

After vaccination the calves are kept in specially constructed stables with concrete floors and walls; they stand upon raised racks of galvanized iron and are fed upon milk.

On the fifth day the inoculated area is washed with sterile water and sterile cotton and the crusts are removed. The soft pulpy mass remaining is scraped with a sterile curette into a sterilized container and mixed with four times its weight of glycerin and water (glycerin 50 per cent, water 49 per cent, carbolic *acid* 1 per cent). The diluted pulp is passed through a fine meshed sieve, after which it is tested for purity by (1) plating on agar each week for five weeks and counting the colonies (usually by the end of three weeks no growth occurs, the glycerin and carbolic acid having killed off all the contaminating organisms); (2) animal inoculations to test for streptococci and tetanus bacilli.

After the product has been found to be free from all extraneous organisms its efficiency is tested by inoculating fifteen previously unvaccinated children, all of which must show a perfect " take " in order that the vaccine may be passed as up to standard. After it has been issued for general use a clinical test is made every two

weeks during the period for which its potency is guaranteed, and if one of these tests fail the vaccine is called in.

The immunity produced by successful vaccination is of relatively long duration, — it may last from ten to fifteen years. Nevertheless it is well, when liable to exposure, to revaccinate at the end of a year.

DISEASES OF UNKNOWN ETIOLOGY

Measles. — Cell inclusions, bacilli, and cocci have all been described by different investigators as possibly associated with the etiology of measles. The reports, however, have not been confirmed and as yet the causal agent of the disease is unknown. Hektoen in 1905 succeeded in experimentally producing measles in two medical students by the subcutaneous injection of blood taken from a measles patient during an early stage of the disease. Anderson and Goldberger in 1911 reported having produced the disease in monkeys by means of a filtrate of measles blood. All efforts to cultivate the virus have failed.

Scarlet Fever. — The causal agent of the disease is still unknown. Streptococci have been repeatedly found in large numbers in the throats of scarlet fever patients and for this reason have been considered by certain authorities as the possible inciters of the disease. Other workers regard them merely as secondary invaders.

Mumps. — Although an infectious disease mumps has been little studied; serum taken from recovered cases has been shown to contain protective bodies. The organism giving rise to the disease, however, has not yet been demonstrated.

Rocky Mountain Spotted Fever. — The disease is characterized by fever and an hemorrhagic eruption. Diplococcoid bodies have been described as present in the blood of infected patients. Similar bodies have also been found in the glands of ticks who have fed upon such patients. Numbers of these supposed organisms may also be found in the larvæ of infected female ticks; but their causal relationship to the disease is not proved.

Chickenpox. — No specific organism has been demonstrated in connection with the disease. It has been claimed that a degree of immunity may be conferred by vaccination with the clear contents of the vesicles.

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