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PATHOGENIC MICRO-ORGANISMS

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MACNEAL



# PATHOGENIC MICRO-ORGANISMS

A TEXT-BOOK OF  
MICROBIOLOGY FOR PHYSICIANS  
AND STUDENTS OF MEDICINE

BY

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IN THE NEW YORK POST-GRADUATE MEDICAL SCHOOL AND HOSPITAL, NEW YORK

(Based Upon Williams' Bacteriology)

WITH 221 ILLUSTRATIONS

SECOND EDITION, REVISED AND ENLARGED

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## PREFACE TO THE SECOND EDITION

In the preparation of this second edition the text has been revised throughout, but the changes have been in the nature of minor corrections and the addition of new matter to bring the text up to date. The general plan of the book has been preserved, keeping in mind its purpose as an introduction to the study of pathogenic micro-organisms.

Subjects in controversy, such as the pathogenic rôle of *Bacillus influenzae* or of *Bacillus typhi-exanthematici*, have received only brief mention, but some references to the literature have been given for the possible use of more advanced students. On the other hand more conclusive advances in microbiology, such as the recent studies on botulism, yellow fever, rat-bite fever and trench fever have been included in the text. Several new illustrations have been added, the credit for which is indicated in each instance.

The index and the table of contents have been made quite full in order to render the text readily accessible and to present to the student a skeleton outline for the review of each chapter.

My thanks are again due to Mrs. MacNeal for assistance, especially in the preparation of the index.

W. J. MACNEAL

NEW YORK



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## INTRODUCTION

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**Bacteriology and Microbiology.**—The science of Bacteriology occupies a somewhat peculiar position among the natural sciences, partly because of its recent development and partly because of the overshadowing importance of its practical applications. As bacteria are microscopic plants, some have considered bacteriology as a minor division of botany; but the methods of work and the practical applications of bacteriology have little in common with those of the more ancient science. Indeed were it not for the importance of these little organisms to the chemist, the pathologist, the physician and the agriculturist, we should hear little about them.

The foundations of the science were laid by Pasteur (1858) by the introduction of media and methods for artificial culture of bacteria and the separation of mixtures into pure culture by the laborious and uncertain but nevertheless successful method of dilution in fluid media, thus making possible the accurate experimental study of microbes. Robert Koch (1872-1882) contributed much to the establishment of the new science by introducing the use of solid media and the method of plating for the isolation of pure cultures and especially by his wonderful achievements in investigation of the pathogenic bacteria by his new methods. Koch used potatoes, and aqueous humor and blood serum rendered solid by the addition of gelatin. He first employed the anilin dyes in staining bacteria (1877), microphotography of bacteria (1877), homogeneous immersion objectives and the Abbé illuminating apparatus (1878). Much of our modern technic has been devised by his pupils and colleagues. The commonly used meat-water-pepton-gelatin was introduced by Löffler; agar by Frau Hesse.

The development of bacteriology has been promoted by the work of biologists, botanists, chemists, pathologists and agronomists, many of whom have been willing to include bacteriology as a subdivision of their own field. The practical importance of bacteriology to these various fields is becoming progressively more evident. The relation to pathology and medicine is perhaps most clearly recognized, although the importance of bacteria in chemical technology and in agriculture is no longer questioned. The relationships to general biology have not been so completely developed as yet, partly because these have seemed to offer less promise of immediate practical application, and partly because few well-trained zoologists or botanists have devoted serious attention to bacteriology.

As a matter of fact, bacteriology must be ranked as a distinct science, especially because of its peculiar technic and because of the peculiarly critical thought necessary in the interpretation of bacteriological observations and experiments. The importance of these can be fully appreciated only after actual experience in handling microbes. Here is a science in which skepticism is a necessary safeguard, a skepticism which will become convinced only when overwhelming evidence compels conviction; and while regarding other conclusions with interest or even with enthusiasm, still carefully reserves final judgment as long as the observed phenomena are open to more than one interpretation.

These methods of thinking and of working have been applied to organisms other than the bacteria, on the one hand to the unicellular animals, the protozoa, on the other to more complex plant-forms such as the yeasts and molds; and more especially to the study of the still undefined types of living things known as filterable viruses or more vulgarly as the ultramicroscopic microbes. Inasmuch as many of these live as parasites and some are important in the causation of disease, they are commonly considered along with the pathogenic bacteria. The terms microbe and micro-organism properly include these as well as the bacteria. There is thus an evident tendency to extend the field

of bacteriology so that it becomes microbiology or the science of micro-organisms. There are many reasons why this is desirable. It is certainly essential that the microbes included among the protozoa and the filterable viruses should receive more attention in the future, both from beginning students and from trained investigators. Until separate instruction in these subjects is provided for medical students, they may perhaps best be studied along with bacteriology.

**Biological Relationships.**—Since the earliest times, the essential difference between living things and lifeless things, that is, the nature of life, has been an interesting subject of speculation. It was at first assumed as a matter of course that the transition from lifeless to living matter readily took place without the agency of preëxisting living matter. This speculative assumption is still not without its able supporters. The history of actual observations, however, is one long record of refutation of this assumption wherever the facts have been subjected to accurate observation. The ancient Greeks held that living beings arose spontaneously and even Aristotle (384 B.C.) asserted that animals were sometimes formed in this way. These ideas were disproved by more careful observation. A notable experiment was that of Francesco Redi (about 1650) who allowed meat to putrefy in a jar covered with fine wire gauze. The flies attracted by the odor deposited their eggs on the gauze and the maggots were hatched there. The assumption that the maggots arose *de novo* in putrefying meat was thus disproven. Harvey in 1650 made the famous statement, "*Omne animal ex ovo*" which was later extended to "*Omne vivum ex vivo.*"

When Anthony van Leeuwenhoek, the "Father of microscopy," discovered, described and figured bacteria in 1683, the assumption of spontaneous generation was at once applied to this group of organisms and, although rendered exceedingly doubtful by the experiments of Spallanzani (1777) and of Schulze (1836), it still continued to be accepted by many scientific men until it was combated by Pasteur, 1860 to 1872. After the accurate

observations of Pasteur upon fermentation and putrefaction and his successful defense of them through a long period of controversy, the assumption of spontaneous generation as applied to bacteria was discredited and has been very generally given up. Only a very few observers<sup>1</sup> still claim the existence of evidence in support of its application here. The more prominent advocates<sup>2</sup> of the assumption of spontaneous generation or abiogenesis seem inclined now to apply it to some group of living beings still beyond the limits of actual observation.

Closely related to the assumption of abiogenesis has been the assumption of heterogenesis among the bacteria, the notion that various kinds of microbes could readily be produced from one species. Although very successfully combated by Pasteur, this idea still persisted for many years in the early bacteriological literature, the observed new species of microbes actually resulting from faulty technic by which new germs had gained entrance to a previously pure culture. These observations are often repeated unwittingly by beginners in bacteriology. The validity of bacterial species is now unquestioned. On the other hand, the variability in the descendants of a single cell through a greater or less range, and the possibility of producing morphologically and physiologically different strains of the same species by appropriate environmental conditions are now well known, resulting again very largely from the pioneer work of Pasteur in the production of attenuated cultures of the germs of chicken cholera and of anthrax.

The systematic relationships and the classification of bacteria were first studied by O. F. Mueller (1786). Ehrenberg (1838) made the first serious attempt at a comprehensive classification and many modern systematists are inclined to return to his work to establish authoritative terminology for present use. He re-

<sup>1</sup> Bastian, *The evolution of Life*, London, 1907. *The Origin of Life*, London, 1913.

<sup>2</sup> Schäfer, *Nature, Origin and Maintenance of Life*, *Science*, 1912, Vol. XXXVI, pp. 289-312.

garded the bacteria as animals. Ferdinand Cohn (1872) recognized the nature of bacterial spores, showed the close relationship of bacteria to the algæ and established their classification in the plant kingdom. He distinguished six genera—micrococcus, bacterium, bacillus, vibrio, spirillum and spirochæta. Migula (1897) undertook an extensive revision of bacteriological nomenclature and classification, basing it upon morphological characters, and his system is doubtless the most satisfactory yet offered. The subject is still in a very unsettled state, nevertheless, and there is no system of classification generally accepted by bacteriologists. The problem presents so many difficulties and our knowledge of the bacteria is still so incomplete that many authorities seem prone to consign systematic classification to the future, and to employ names of sufficient historical prominence to insure their correct interpretation.

**Fermentation and Putrefaction.**—The relation of micro-organisms to the decomposition of organic matter, fermentation and putrefaction, was one of the first fields of applied bacteriology to be studied. Following the observation of bacteria in saliva by van Leeuwenhoek in 1683, micro-organisms were discovered in all sorts of decomposing material. At first, these organisms were regarded as unimportant for the chemical process and interest attached chiefly to the question of their origin, whether by spontaneous generation or from previously living cells. Needham (1745) directing his attention more particularly to this first question, boiled an infusion of meat and, keeping it free from contact with the air, nevertheless observed after some days the presence of “infusoria.” Spallanzani (1765) repeated Needham’s experiments, subjecting hermetically sealed flasks of meat infusion to the temperature of boiling water for one hour, and he found no subsequent development of life and no decomposition of the infusion as long as it remained sealed. While discussion continued concerning the discrepancy between the results of Needham and Spallanzani and concerning the relation which the subsequent exclusion of the air might bear to the absence

of life in the flasks, the method of heating was applied to the preservation of vinegar by Scheele (1782) and to the preservation of foods in general by Appert (1811). The method was quickly introduced into other countries and developed by various tradesmen, who attempted with more or less success to keep their processes secret. Success in preservation by canning remained somewhat uncertain, as a precise understanding of the underlying scientific principles was still lacking. Schulze (1836) showed that air might be admitted to flasks prepared by Spallanzani's method, without the development of life and without putrefaction, provided the air were first passed through a series of bulbs containing concentrated sulphuric acid. The subsequent work of Schröder and van Dusch (1853), who obtained similar success by filtering the air through cotton, of Pasteur and Tyndall (1860-62), who were able to preserve putrescible fluids directly in contact with air, provided the air were rendered perfectly free from dust, has established the fact that the decomposition ordinarily taking place after exposure to the air is due to the introduction of living germs into the previously sterile material.

The idea that specific kinds of fermentation are caused by specific kinds of microbes was first clearly put forward by Schwann and Cagniard-Latour (1837), who showed that yeast-cells were living organisms and claimed that the alcoholic fermentation of sugar solutions was due to their growth. The importance of this relationship received little recognition until Pasteur (1860-72), during his extensive and careful researches into the nature of fermentation and the causation of undesirable fermentation (diseases of wines and beers), demonstrated conclusively that the kind of decomposition of a fermentable substance depended upon the nature of the substance, the kind of microbes present and the environmental conditions, such as temperature and presence or exclusion of air. The mere introduction of a small number of unfavorable microbes was sufficient to change the whole nature and course of the fermentation. Furthermore, Van der Brock (1857) and Pasteur (1863) were able to collect such fermentable

materials as grape juice, wine, blood, tissues of plants and animals and preserve them free from decomposition and from all microbic life, merely by effectively avoiding contact with germs during collection and storage.

The agency of microbes in fermentation was ridiculed by Liebig, the most prominent chemist of the time, who steadfastly continued to regard decomposition of organic material as a purely chemical process uninfluenced by biological activity. His ideas prevailed for a time because of his prominent position. The correctness of Pasteur's contention is now universally accepted. Nevertheless it should not be forgotten that many organic substances are in themselves so unstable that even in the absence of microbic life they disintegrate, or become oxidized in the presence of the air. These changes are different from those ordinarily known as fermentation and putrefaction.

**Pathology and Hygiene.**—The history of the development of our ideas concerning the relation between microbes and disease is one of the most interesting and perhaps the most important chapter in the history of bacteriology. The customs and records of the ancients give evidence that they recognized the presence of an unseen agency in the body of the diseased individual capable of causing sickness in others. This was recognized by the ancient Persians as recorded by Herodotus. The isolation of lepers by the ancient Hebrews shows that the infectious character of this disease has long been known, though other affections than leprosy were probably confused with this disease. "He is unclean; he shall dwell alone; without the camp shall his habitation be." (Lev. XIII, 46.) There is, in fact, much in the laws of Moses that points to some knowledge of the nature of infection. "This is the law, when a man dieth in a tent all that come into the tent and all that is in the tent shall be unclean for seven days. And every open vessel that has no covering on it shall be unclean." (Numb. X IX, 14, 15.) "Everything that may abide the fire, ye shall make it go through the fire, and it shall

be clean." (Numb. XXXI, 23.) In Homer we read of Ulysses, that, having slain his wife's troublesome suitors:

"With fire and sulphur, cure of noxious fumes,  
He purged the walls and blood-polluted rooms." (Pope's *Odyssey*.)

These records certainly suggest a rather advanced state of knowledge concerning the nature of contagion. It may be that they record customs derived from a superior knowledge of some other ancient people, perhaps the ancient Egyptians. During the middle ages, as doubtless also before the dawn of history, epidemic disease was regarded as a visitation of Providence or attributed to the influence of gods, demons or other supernatural agencies. Epidemics were associated with the appearance of comets in the sky or with other evidences of divine wrath. These conceptions of disease have not altogether disappeared even at the present time.

Hippocrates (400 B. C.) denied the supernatural causation of disease and held that such doctrines were mere cloaks for helpless ignorance. He ascribed epidemic disease to a morbid secretion of the atmosphere, and later writers have expressed this idea of a morbid secretion by the word *miasm*, its exact nature remaining for centuries intangible and mysterious. There is here a conception different from that upon which the hygienic measures of the Persians and Hebrews were founded and the distinction was clearly expressed by Pettenkofer in the nineteenth century, who defined contagious diseases as those which are transmitted directly from man to man or through the agency of solid objects, while in miasmatic diseases the causative agent enters from the outside world where it may live naturally or where it must have undergone a ripening process since its escape from the body of the sick person. As will be seen later these ideas apply very well to certain diseases, for example, small-pox and syphilis as contagious diseases and yellow-fever and malaria as a miasmatic. The ancient Greeks recognized the contagiousness of several diseases and Galen classed plague, itch, ophthalmia, con-

sumption and rabies as contagious. Fracastorius (1546) during the period of the great epidemic of syphilis in Europe, published a book containing the first comprehensive discussion of the theory of contagion. He recognized contagion by contact, by fomites and at a distance. Soiled material of all kinds was included under fomites, as also those healthy individuals capable of transmitting disease, a phenomenon already recognized. Transmission by insects and animals was also included under this head. The transmission "per distans" was considered due to emanations from the patient diffusing to a distance through the atmosphere.

Kircher in 1658 claimed to have seen the living contagium in the body in the form of minute worms, and his observations were widely recognized. The objects he saw were not accurately described but it seems very certain that they were not bacteria. Probably they were the normal cells of the tissues.

The discovery of bacteria by van Leeuwenhoek (1683) was not immediately recognized as of importance for the germ theory. Leeuwenhoek himself considered it impossible for his "animalcula" to penetrate into the blood because of the compactness of the epithelial tissues.

Almost a century later, Plenciz (1762) maintained that each infectious disease must have its own specific cause. Reimarus (1794) also expressed the same opinion and considered these living organisms to be of the order of infusoria or perhaps still smaller beings not yet visible with the microscope. These ideas were not supported by objective evidence and received only passing attention. They were soon thrust aside by other interesting if less valuable speculations.

The development of general knowledge of the animalcules in the early part of the nineteenth century, already referred to in the discussion of the biological relationships and of fermentation, was preparing the way for progress in the problem of disease. In 1834 the *contagium vivum* of itch, the itch mite (*Sarcoptes scabiei*), a fairly large mite to be sure, was rediscovered and its relation to the disease made evident. In 1837, the same year

in which Cagniard-Latour and Schwann established the relation of living yeast to alcoholic fermentation, Donné described vibri-ones (bacteria) in syphilitic ulcers, and Audouin amplified the discovery of Bassis that *muscardine*, a disease of the silkworm, was caused by a mold (*Botrytis bassiana*) which was transmitted from the sick to the healthy worms by contact or by air currents. These discoveries furnished a great impulse to further investigation.

Henle (1840) reviewed the evidence then at hand and concluded in a very logical way that the causes of contagious diseases were to be sought for among the minute living micro-organisms. He recognized that no human disease had yet been shown to be caused by a micro-organism and he formulated the requirements to be fulfilled in order to prove such a relation, namely, that the microbe must be constantly present in the disease, must be isolated from the infectious material, and must then alone be capable of producing the disease.

During the next twenty years, the attempts to discover the cause of an infectious disease and to satisfy the postulates of Henle were successful in several diseases due to molds, Favus (*Achorion Schoenleinii*) 1839, similar skin diseases known as trichophytosis and pityriasis and especially thrush, shown to be caused by *Oidium albicans* by Robin in 1847; but in all the more important diseases only failure resulted. The reawakened interest in *contagium vivum* therefore again gradually faded away.

During this time Pollender and Davaine and Rayer (1850) had discovered the minute rods in the blood of animals sick with anthrax, and in 1863 Davaine had proved the almost constant presence of these rods in the disease and the possibility of transmission by inoculation from one animal to another.

Pasteur from 1865 to 1868 investigated the fatal disease of silk-worms known as pébrine, discovered the microsporidium (*Nosema bombycis*) which occurs in the sick worms and in the eggs, and devised a successful method of eradicating the disease.

In 1870-71 the presence of bacteria in wounds and in the

internal purulent collections in pyemia and septicemia was first definitely recognized by Rindfleisch (1870), but more especially by Klebs in a large number of cases at the military hospital at Karlsruhe. The latter observed spherical bacteria arranged in groups or as a rosary, to which he gave the name *Microsporon septicum*. His observations were quickly confirmed by other competent pathologists. Similar organisms were soon found in a great many wounds and other inflammatory processes. Specific causal relationship was still unproven.

In 1873 Obermeier described the slender but actively motile spirochetes seen by him in the blood in relapsing fever as early as 1868.

In 1874 Billroth concluded that there was still no disease in which the causal relationship of micro-organisms had been conclusively proven. The skin diseases due to molds were relatively unimportant and had not been recently studied. The microbes found in other diseases might just as reasonably be regarded as a product of the disease or as only incidental to it. Even in anthrax, where the evidence seemed strongest, there were cases of the disease without the presence of the peculiar rod-like bodies in the blood, and indeed these rods might be crystals and not living organisms at all.

Since 1867 Lister, stimulated by the investigations of Pasteur on fermentation and putrefaction, had been developing and applying an antiseptic method to the treatment of wounds, which consisted of the use of carbolic acid. The results of this method published in 1875 were so remarkably favorable that it was quickly adopted throughout the world, and its success did much to prepare the way for the recognition of the rôle of microbes in suppuration, if it did not in itself convince.

Robert Koch, 1876-1881, first satisfied the postulates laid down by Henle, and again formulated by himself, in the bacterial disease, Anthrax. The presence of the bacilli in the blood of animals suffering from anthrax had been established by a large number of previous workers, and the transmissibility of the dis-

ease by inoculation with blood of diseased animals was already known. Koch was able to grow the bacillus in pure culture in a test tube, using the aqueous humor of the ox's eye as a medium. He was able to observe growth and division and the formation and germination of spores under the microscope. Finally with these cultures, which had been propagated a long time in the culture medium, he was able again to cause anthrax by injecting them into susceptible animals. The demonstration of the causation of disease by bacteria had been achieved.

The introduction by Koch in 1881 of the plate method of separating bacteria paved the way for rapid advances in bacteriology, and during the next ten years the bacterial causes of several diseases were discovered and proven by thorough test, and since then the number of diseases known to be due to bacteria has gradually increased.

The history of immunity extends far back into ancient times. For many diseases it was recognized that those who recovered could associate with the sick without danger to themselves. Recognizing this, people sometimes exposed themselves purposely in order to have the disease at a convenient time. Artificial inoculation to cause small-pox was introduced into Europe from the Orient in 1721. The use of cowpox, vaccination, was discovered by Jenner in 1797. Artificial immunization by inoculation with altered bacterial cultures was first successfully demonstrated by Pasteur in chicken cholera and in anthrax in 1881. The practice of inoculation with dead bacterial cultures has become almost universal in the armies of the world since 1914, for the prevention of enteric fevers. Analogous methods have been devised for many other diseases. The discovery of the antitoxic property of the blood serum of animals immunized to tetanus and to diphtheria was made by von Behring and Kitasato (1891).

With the discovery of amebæ in the stools in tropical dysentery by Loesch (1875) and of the malarial plasmodium in the blood by Laveran (1880) the relationship of protozoa to important diseases was suggested. An enormous number of protozoal para-

sites are now known, many of them associated with important diseases. The strict proof of causal relationship to the disease has presented greater difficulties here, especially the step of artificial culture. However, the causal relationship of bacteria having been demonstrated, the probable causal relationship of the protozoa has found more ready acceptance. Cultures of ameba have been obtained by many workers but the successful cultivation of a pathogenic ameba is still questionable. Pure cultures of trypanosomes were obtained by Novy and his pupils (1903-04) and the infections again produced by inoculation with these cultures.

The transmission of protozoal diseases by insects, first demonstrated by Salmon and Smith in Texas fever, has developed into a subject of prime importance. Malaria and the insect, *Anopheles*, sleeping sickness and tsetse fly, *Glossina*, are important examples of this relationship.

Obermeier (1873) described a motile spiral organism in the blood of relapsing fever, the first known parasitic member of a group of very great importance. Very many pathogenic spiral organisms of this general type are now known. Their systematic relationships have not been fully worked out and further knowledge is necessary before they can be finally classed with either the bacteria or the protozoa. Artificial culture of these organisms has revealed a close relationship to anaerobic bacteria, as far as nutritive requirements are concerned, the most successful culture methods being those devised by Noguchi (1912). Many of these parasites are transmitted by insects and they pass through a somewhat obscure development in the insect carriers, the forms developed being extremely minute (Nuttall, 1912). These facts suggest a possible relationship of this group of organisms to the filterable viruses.

Nocard (1899) discovered that the virus of pleuro-pneumonia of cattle would pass through filters impervious to bacteria. The number of recognized filterable viruses has grown appreciably since then and among them are the causes of several very im-

portant diseases, such as yellow-fever, dengue fever, poliomyelitis, measles, typhus fever, small-pox, rabies and hog cholera. Knowledge of this group of organisms is still relatively meagre and many features are still obscure or in controversy. Microscopic methods of defining their form and structure are still poorly developed but they cannot with justice be regarded as wholly in the realm of the unknown.

**Agriculture.**—The importance of microbes in soil fertility and agriculture has a relatively short history. Duclaux, 1885, showed that plants could not well utilize complex organic matter as food in the absence of microbic life. In addition to ordinary decomposition of organic matter, bacteria also bear an important relation to the nitrogen metabolism of plants. Hellriegel and Wilfarth (1886-88) showed the infectious nature of the nitrogen-fixing root tubercles of legumes, and the organism *B. radicicola* was isolated by Beyerinck in 1888. The importance for agriculture of other living elements in the soil, such as amebæ and nematodes, has been more recently recognized.

Although it is well to recognize the many important applications of bacteriology, a word of caution may not be amiss, lest we follow too eagerly the alluring applications and neglect the secure foundation of scientific knowledge of the biology and biological relationships of micro-organisms, the proper training in logical thinking concerning these beings and in the technic of dealing with them.

# PART I

## BACTERIOLOGICAL TECHNIC

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### CHAPTER I

#### THE MICROSCOPE AND MICROSCOPIC METHODS

The development of bacteriology has depended especially upon the development of new methods of scientific study, and in a very important way upon the improvements in construction of the microscope and in methods of preparing objects for study under the microscope. Knowledge of the construction of a microscope is not an essential part of bacteriology but the demands of modern microscopical methods require a skill in manipulation of the instrument which is best acquired after the principal structural features of the microscope are understood.

**The Development of the Microscope.**—Roger Bacon, in 1276, seems to have been the first to recognize the peculiar properties of a lens. Spectacles began to be used about the same time and are said to have been invented by d'Armato.<sup>1</sup> Galileo (1610) probably made the first record of the use of the compound microscope. It was a lens maker, Anton van Leeuwenhoek, who first saw bacteria in 1683. A method of correcting chromatic aberration was discovered by Marzoli in 1811, but became generally known through the work of Chevalier in 1825. The correction of the color defects was accomplished by the combination of two kinds of glass, crown glass and flint glass, in the objective lens system, and made possible the construction of achromatic objectives, perhaps the most important advance ever

<sup>1</sup> *Jour. A. M. A.*, Nov. 9, 1912, Vol. LIX, p. 1721.

made in the construction of the microscope. Abbé (about 1880) introduced his substage condenser which made possible the intense illumination of the microscopic field. In collaboration with Zeiss, Abbé (1886) devised an objective lens system with more perfect chromatic correction than had been previously attained. These objectives are constructed of several different kinds of glass and have in addition one lens composed of fluorite. They are called apochromatic objectives. Siedentopf and Zsigmondi (1903) devised a method of illuminating the microscopic preparation by horizontal beams and so brought to view exceedingly minute refractive particles as luminous points on a dark field. The various dark-field condensers introduced in recent years

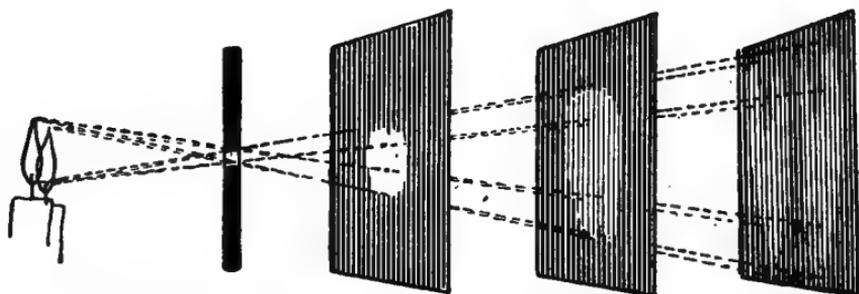


FIG. 1.—The formation of an image by means of a simple pin-point aperture.  
(After A. E. Wright.)

(1906) utilize similar principles, the object being illuminated by oblique light. Recently, Gordon has devised the tandem microscope, an instrument which has demonstrated the possibility of achieving greater microscopic resolution than has previously been attained and even suggests that there is no necessarily final limit to the degree of magnification at which satisfactory definition and resolution may be achieved.

**Principle of the Microscope.**—The formation of an image by means of a simple pin-point aperture is illustrated in Fig. 1. It will be noted that the magnification achieved is the quotient of aperture-image distance divided by object-aperture distance;

also that the sharpness of outline of the image increases and the brilliancy diminishes as the size of the aperture is decreased.

If the simple aperture be replaced by a convex lens and the object and the screen be set at the conjugate foci of the lens, it will be seen that magnification is again the quotient of the aper-



FIG. 2.—Image formation by a single lens. Note that the image, at the right is  $\frac{2}{5}$  the size of the object, in proportion to their respective distances from the lens; the opening angle being  $\frac{3}{5}$  the size of the closing angle.

ture-image distance divided by the object-aperture distance. The sharpness of outline, however, depends now upon the quality of the lens and the accurate adjustment of the distance, and brilliancy is not seriously impaired in attaining definition.



FIG. 3.—Image formation by two lenses in series without magnification. Note that the opening angle of the beam proceeding from the object, at the left, is equal to the closing angle of the beam forming the image at the right.

Image formation in the human eye is an example of the working of the lens-armed aperture. The rays of light are brought to a focus on the retina and the image produced here is inverted and actually much smaller than the object, the reduction (minification) being again measured by the quotient of the lens retina distance divided by the object-lens distance. The longer the antero-posterior diameter of the eye, the larger will be the retinal

image. Our subjective interpretation of the stimulation of the retina (*i.e.*, what we see) is influenced by other psychological elements and especially by the memory of things seen before.

When two lenses are disposed in series so that the rays of light coming from a point in the object pass through both lenses



FIG. 4.—Image formation by two lenses in series, with magnification of two diameters. Note that the opening angle of the beam is twice as large as the closing angle.

before coming to a focus, we find the possibilities shown in Figs. 3, 4 and 5. In the figures it will be seen that the image produced when the first lens is in position so as to render the rays parallel (Fig. 4), is just five times as large as that produced when it is



FIG. 5.—Image formation by two lenses in series, with magnification of three diameters. Note that the opening angle of the beam is three times as large as the closing angle.

left out (Fig. 2), assuming that the second lens is capable of change so as to focus upon the same screen slightly divergent rays proceeding from the object. It will further be perceived that the sine of the angle of divergence of the beam proceeding from the object varies directly with the magnification achieved, and further

that the magnification in any such system is equal to the quotient of the sine<sup>1</sup> of the angle of divergence of the beam proceeding from the object, divided by the sine of the angle of convergence of the beam to form the image. This is capable of mathematical proof and is illustrated in the four figures. From these it is evident that magnification is a function of the relation of these two angles of the opening and closing limbs of the beam, and that the intermediate course of the rays, whether parallel, convergent or divergent, is negligible in this computation. If the second lens be that of the eye and an image is to be formed on the retina, then the rays proceeding from a point must be rendered parallel, or approximately so, by the first lens. This is the arrangement which exists in the simple microscope or in the ordinary reading glass. The magnification achieved by such a simple microscope is measured by the relation between the magnitude of the image on the retina when the lens is employed, and the size of such an image when the lens is left out of the path of the light. The value of the reading glass, entirely aside from considerations of magnification, in conditions of hyperopia and presbyopia is also evident from these figures, as it of course renders the rays coming from a near point more nearly parallel, and thus enables the refracting media of the presbyopic eye to bring them to a focus.

So far we have been employing in our discussion the ideal lens, one which refracts all light equally and brings to a focus in one plane all rays proceeding from one plane in the object. As a matter of fact the ideal lens in this sense does not exist. The simple convex lens has many serious optical defects.

Points in the same plane in the object are imaged by the simple lens on a curved surface, the segment of a spherical surface. This defect is known as spherical aberration. It is diminished to some extent by combining convex and concave lenses and the

<sup>1</sup> In the figures, as drawn, this statement actually applies to the tangents of the angles designated, rather than the sines. However, for very small angles the sine and tangent are approximately equal. The use of the term sine finds its complete justification in the fact that the plane at which the rays are bent is not flat but is the segment of a sphere or its optical equivalent.

correction may be changed by altering the distance between these component lenses, as, for example, in an objective equipped with a correction collar. Objectives corrected in respect to spherical aberration are designated as aplanatic. Restriction of the size of the field is also an important factor in making it appear flat.

Light of different wave lengths (different colors) is refracted to a different degree by the simple lens, so that, for example, the violet rays are brought to a focus earlier than the red rays, with the remainder of the spectrum spread out between. This defect is known as chromatic aberration. It is corrected to a very considerable extent by combining biconvex lenses of crown glass



FIG. 6.—Microscope objectives showing the component parts of the objective lens system.

with plano-concave lenses of flint glass (achromatic objectives), to a still nicer degree by combinations of lenses of several different kinds of glass together with a lens of fluorite (apochromatic objectives); and finally, when desired, chromatic aberration may be wholly avoided by employing mono-chromatic light.

A third defect of lenses is known as diffraction, which is a phenomenon giving rise to a whole group of less luminous secondary images around the principal image. The influence of diffraction is most evident when the surfaces of the lens are roughened by scratches or by presence of dust, but even the most perfect lens systems are not wholly free from diffraction phenomena.



Some of these defects will require brief consideration in our discussion of the compound microscope.

In the modern compound microscope the beam of light proceeding from a point in the object is refracted by the lens system of the objective (Fig. 6) so as to render the rays slightly convergent. Near the upper end of the tube of the microscope these rays are further refracted by the lower lens of the eye-piece and are

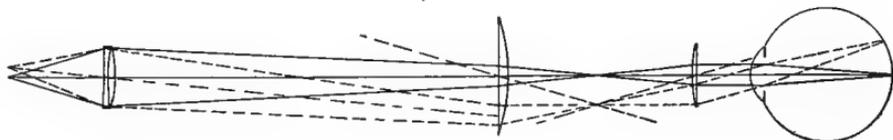


FIG. 8.—Image formation in the compound microscope. Compare with Fig. 9.

converged and brought to a focus in the interior of the eye-piece. A screen placed at this level would show a real image, and any pattern (for example an eye-piece micrometer) inserted in the eye-piece at this level is readily fused with the microscopic field. Continuing in a straight line the rays diverge from this focus to reach the upper lens of the eye-piece. In traversing this lens



FIG. 9.—Image formation in the compound microscope with an eye-piece of higher power. Observe that the increased magnification is accomplished by narrowing the beam of light which enters the eye and so diminishing the size of the closing angle. Compare with Fig. 8.

they are again refracted and made parallel so that they will enter the eye and be brought to a focus on the retina. The paths of two beams of light, one proceeding from the center of the microscopic field and one from its periphery, are illustrated in Fig. 8. Fig. 9 shows the change which is introduced by the use of an eye-piece of higher magnifying power.

It will be noted that the objective and lower lens of the eye-

piece bring the beam to a focus forming a real image, and that the rays diverging again from this image are again brought to a focus on the retina by the upper lens of the eye-piece and the optical structures of the eye. The magnification represented in the first image is the quotient of the sine of the angle of the opening limb of the beam divided by the sine of the closing angle. The subsequent magnification between this and the eye is the quotient of the sine of the opening angle of the rays proceeding from this image divided by the sine of the closing angle of the rays approaching the retina. The closing angle at the formation of the first image and the opening angle of the beam proceeding from it are obviously equal, so that the total magnification equals the sine of the first opening angle divided by the sine of the last closing angle in the system. It will be noted that the eye-piece of higher power narrows the beam and decreases the closing angle.

By placing the eye about two feet above the eye-piece, one may measure the width of the beam as it emerges from the latter. Now by changing to an eye-piece of higher power he can readily demonstrate the consequent narrowing of the beam. This narrowing of the beam makes more important the obscuration caused by any defect in the optical system of the eye itself. Microscopic vision is disturbed by floating shadows and hazy definition due to such partial obscuration especially when the beam has been too much narrowed. For ordinary work, therefore, an eye-piece of low power will prove more satisfactory.

In the above discussion, the refractive index of the vitreous humor has been disregarded. This is not the same as that of air (in reality it is about 1.3) and the peripheral beam is therefore bent toward the axis of the eye instead of proceeding in its former direction, the magnification being thereby reduced by precisely the fraction

$$\frac{\text{refractive index of air}}{\text{refractive index of vitreous}} \text{ or } \frac{1}{1.3}.$$

This brings us to a definition of numerical aperture. The numer-

ical aperture of the closing limb (n.a.) is the sine of half the angle of the converging beam multiplied by the refractive index of the medium (in this instance the vitreous humor). This is commonly designated as n.a. The numerical aperture of the opening limb of the beam (N.A.), proceeding from a point in the object to the objective, is the sine of half the angle of this beam multiplied by

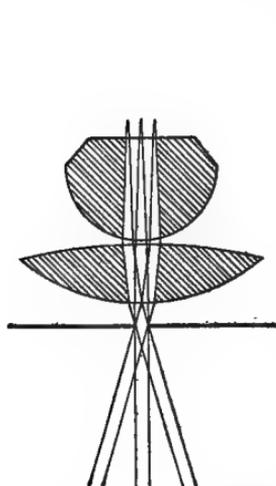


FIG. 10.—Central illumination by a narrow beam. Three beams of parallel rays, such as might come from a large white cloud, are represented. Note that these rays reach the object as almost vertical rays, varying from the vertical by only a narrow angle. Compare with Fig. 15.

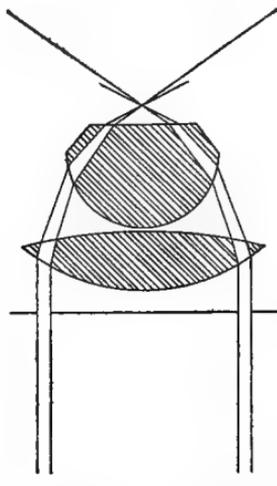


FIG. 11.—Illumination by a hollow cone of light converging upon the object at a wide angle, by use of the central spot stop. Compare with Fig. 14, and with Fig. 16.

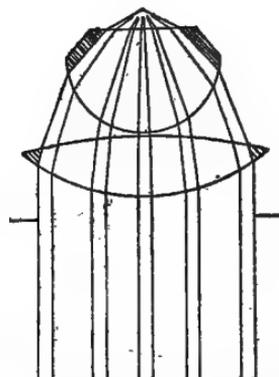


FIG. 12.—Illumination by a broad beam converging upon the object at a wide angle. Only a few beams of parallel rays from a distant point source of light are represented in the figure. Compare with Fig. 17.

the refractive index of the medium through which it passes. This is commonly designated as N.A. Many desirable properties of objectives, other than magnification, such as brilliancy of illumination, definition, and resolution in depth, also depend upon the numerical aperture, which is therefore perhaps the most important single feature of objectives of high power.

Another important optical part of the bacteriological micro-

scope is the substage illuminating apparatus, consisting of the mirror, the iris diaphragm and the condenser. These are necessary to illuminate minute objects so that they may be satisfactorily studied at high magnifications. By the use of the iris diaphragm and of the central spot stop, the ordinary condenser may be made to furnish three different kinds of illumination, (1) central illumination by a narrow beam, (2) illumination by a hollow cone of light converging on the object at a wide angle, an example of dark-field illumination, and (3) intense illumination by a broad beam converging at a wide angle upon the object. These possibilities are illustrated in Figs. 10, 11 and 12. Dark-field illumination is obtained in

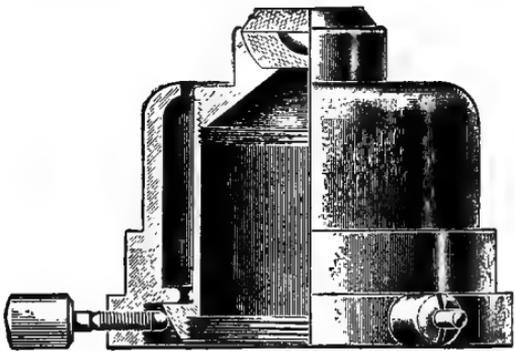


FIG. 13.—Dark-field condenser showing optical parts and centering mechanism.

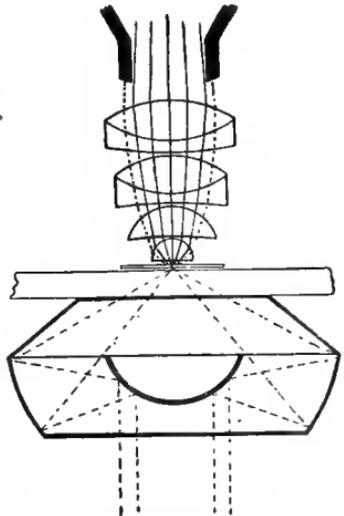


FIG. 14.—Optical parts of the dark-field condenser with object slide and microscope objective with funnel stop in position. The path of light rays is indicated by the dotted lines.

a more satisfactory manner by employing a special condenser made for the purpose, illustrated in Figs. 13 and 14. The way in which these different methods of illumination affect the visibility of a colorless refractive object is illustrated in Figs. 15, 16 and 17.

**Visibility of Microscopic Objects.**—In the use of the microscope it is necessary to pay some attention to the factors upon which visibility depends. An object may be distinguished and perceived by the eye only when the light coming from the object

differs from that coming from its surroundings either in quantity or in quality, and the greater the extent of this difference the more distinctly visible will the object be. Uncolored transparent objects are visible by virtue of their ability to refract light and so to present darker and lighter zones. If the surrounding medium possess the same refractive power as the colorless trans-

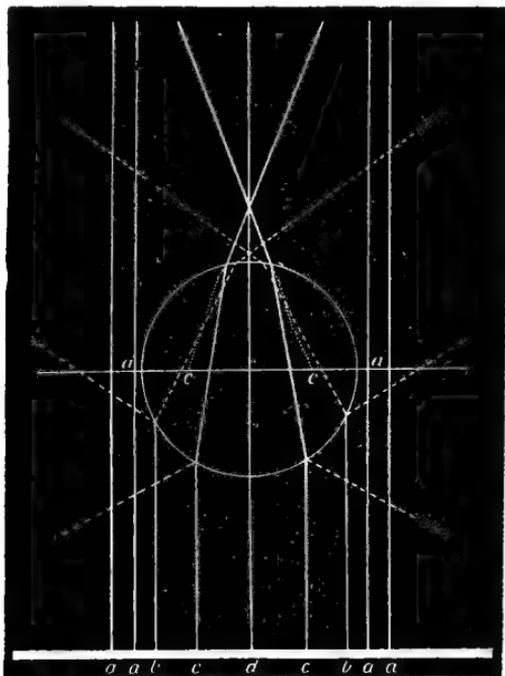


FIG. 15.—Showing the manner in which the "dark outline picture" is produced.  
(After A. E. Wright.)

parent object, the latter is invisible.<sup>1</sup> Microscopic objects may conceivably be invisible or so nearly invisible as to have escaped detection for this very reason. If, however, the object be suspended in a medium of lower refractive index, then it may be defined by light and shade, and it is most clearly defined when illuminated in one of two ways, either by a rather narrow direct

<sup>1</sup> This may be illustrated fairly well by immersing clean, perfectly clear glass beads in oil of cedar wood.

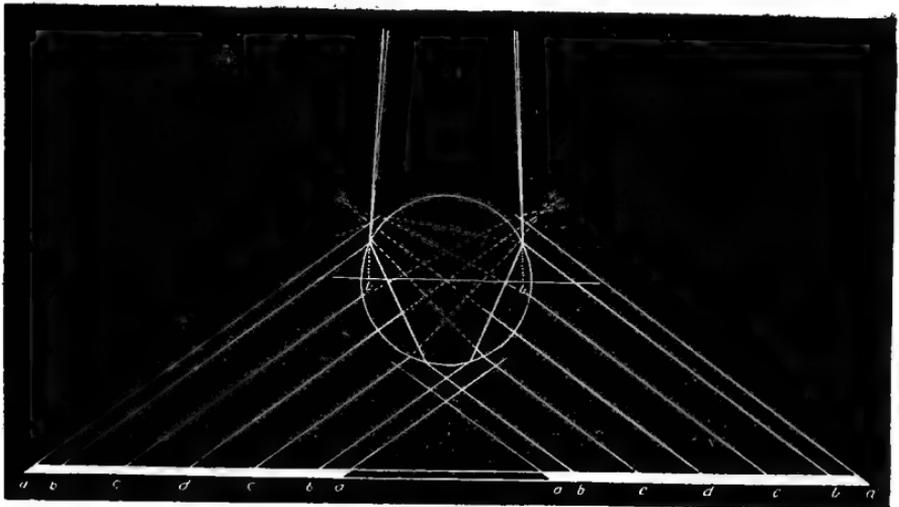


FIG. 16.—Showing the manner in which the "bright outline picture" is produced.  
(After A. E. Wright.)

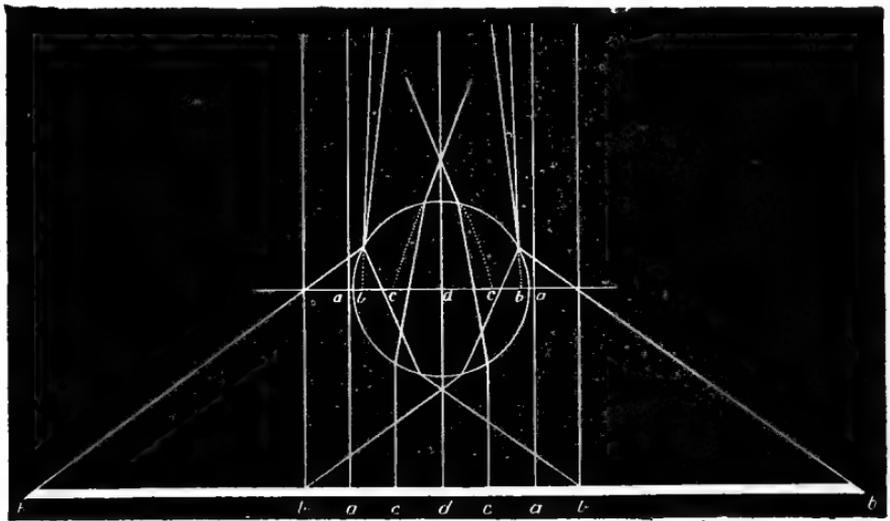


FIG. 17.—Showing the manner in which the outlines are obliterated when an object is illuminated by a homogeneous illuminating field. (After A. E. Wright.)

beam of light passing from behind it directly toward the eye, in which case the object is defined by dark outlines upon a white field; or by oblique beams directed at an angle from the sides, when the object is defined by bright outlines on a dark background. If, however, the object be illuminated from all sides or from behind and from both sides by light of similar intensity, its outlines become less distinct and may even be completely obliterated so that the object becomes invisible. These facts may be crudely illustrated by holding a test-tube full of water, (1) between the eye and a window, (2) between the eye and a dark wall between two windows, and (3) against the center of the window pane. Their importance in microscopy may be readily illustrated by examining a simple preparation of living bacteria, (1) with the iris diaphragm nearly closed, (2) with the dark-field condenser, and (3) with the ordinary condenser with the iris wide open. It will be evident that the third arrangement is fatal to the definition of colorless transparent microscopic objects. It will also be observed that the dark field offers an advantage in the ease with which the objects can be seen, the small luminous outline on the dark background being more distinct than the dark outline on the luminous background. The former might be compared in this respect to a star at night, and the latter to a sun spot in the daytime, which though many times larger may not be readily perceived.

The method of making objects visible by a difference in quality of light (color) usually involves the necessity of staining. Colored preparations have certain very important advantages for microscopic study. If an object can be differentially colored, that is, stained a different color or a different shade of the same color from the material by which it is surrounded, it becomes clearly visible even in the absence of different refractive power. Refraction may be largely eliminated by replacing the fluids of the preparation by other fluids of high refractive index, such as cedar oil or balsam, and this elimination of refraction eliminates the opacity of the preparation, "clears" it, and makes possible the distinct definition of minute objects situated in the deeper optical

planes of the preparation.<sup>1</sup> A proper appreciation of this microscopical principle will at once suggest the importance of differential staining methods in microscopy.

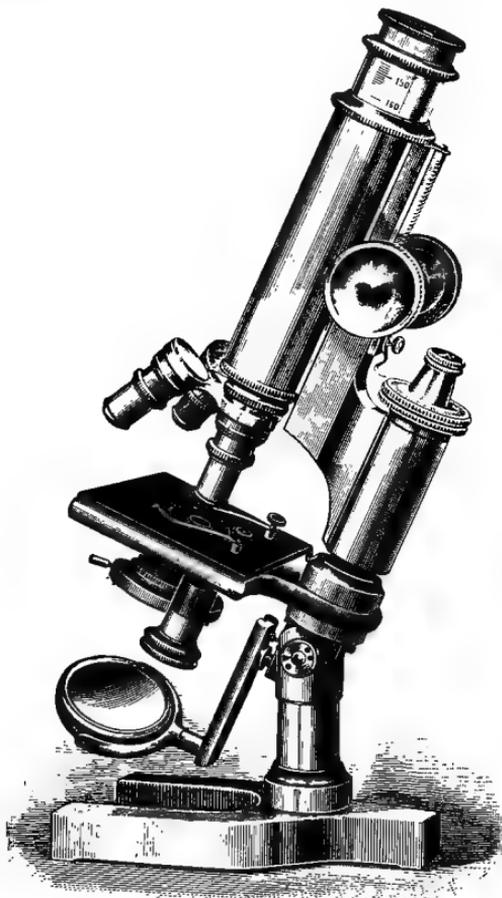


FIG. 18.—Microscope.

**The Bacteriological Microscope.**—The bacteriological microscope consists of a tubular body which carries the optical parts, and

<sup>1</sup>This principle may be crudely but clearly illustrated by covering a colored glass bead with a deep layer of colorless glass beads in a test tube, so as to conceal the former when looked at from above. By replacing the air between the beads with homogeneous cedar oil the layer of colorless beads is rendered perfectly transparent and the colored bead is distinctly seen from above. Filter paper may be rendered transparent in a similar way.

which can be raised or lowered for focusing. The objectives should be three in number, and should be attached to the body by means of a triple nose-piece, which permits any objective to be turned into the optical axis at will. The eye-piece slips into the upper and opposite end of the body or tube. The arrangements for focusing consist of a rack and pinion, which accomplish the coarse adjustment, and a more delicate fine adjustment. The stage, upon which the objects to be examined are placed, has an opening in the middle. In this opening an iris diaphragm and Abbé condenser are inserted. The iris diaphragm enables one to alter the size of the opening as desired. Beneath the stage is a movable mirror, of which one side is plane and the other concave. All of these parts are supported on a short, heavy pillar which is fixed in the horseshoe-shaped base.

The essential parts of the microscope are, of course, the eye-piece (German, *Ocular*), and the objective. Objectives are given

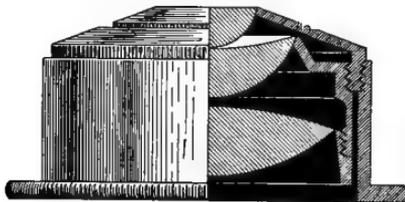


FIG. 19.—Abbé Condenser. On the right side the figure gives a sectional view.

various names by different makers, for instance, A, B, C, etc., or 1, 2, 3, etc.; or they are named according to their focal distances, as  $\frac{2}{3}$  inch,  $\frac{1}{4}$  inch,  $\frac{1}{2}$  inch, etc. In bacteriological work a rather "low power"  $\frac{2}{3}$  or  $\frac{3}{4}$  inch objective, an ordinary "high power"  $\frac{1}{6}$  to  $\frac{1}{8}$  inch dry

objective, and a high power  $\frac{1}{12}$  inch oil-immersion objective are needed. The magnification with the  $\frac{2}{3}$  or  $\frac{3}{4}$  inch objective is about 75 to 100 diameters; with the  $\frac{1}{6}$  to  $\frac{1}{8}$  inch 400 to 700 diameters; with the  $\frac{1}{12}$  immersion 750 to 1,000 diameters. The magnification varies according to the eye-piece used, as well as with the objective. A 1 inch and  $1\frac{1}{2}$  inch eye-piece (Leitz No. 2 and No. 4) serve well for most purposes. The eye-pieces are usually named arbitrarily, like the objectives. The oil-immersion objective is used in the examination of bacteria where a very high power is desired. A layer of thickened oil of cedar-

wood is placed between the lower surface of the objective and the upper surface of the glass covering the object under examination. The oil must be wiped away from the surface of the objective when the examination is finished. For this purpose the soft paper sold by dealers in microscopical apparatus serves admirably. Care must be taken not to scratch the lower surface of this objective. Oil of cedar-wood furnishes a medium having nearly the same refractive index as the glass of the lens and the glass on which the object is mounted, and it obviates the dispersion of light which takes place when a layer of air is interposed between the objective and the object, as happens with the ordinary dry lens.

The microscope should be placed in front of the observer on a firm table. The observer should be able to bring the eye easily over the eye-piece when the tube of the microscope is in vertical position. Daylight should be employed if possible. When artificial illumination is necessary, an ordinary lamp, a Welsbach burner or an incandescent electric light may be used. It is well to modify the artificial light by inserting a sheet of blue glass between the light and the mirror or to employ a special incandescent nitrogen bulb made of "daylight" glass, which can be obtained from dealers in electric lamps.

In order to focus upon any object, having first secured a satisfactory illumination with the mirror, it is best, beginning with the low power and using the coarse adjustment for focusing, to bring the objective quite close to the object, and then, with the eye in position, to raise the tube until the object comes into focus. The exact focusing is done with the fine adjustment. The observer should keep both eyes open when using the microscope, and should be able to use either eye at will.

All measurements of microscopic objects are expressed in terms of a micromillimeter. This is one-thousandth of a millimeter (0.001 mm.), which is about  $\frac{1}{25000}$  of an inch. This unit is designated as a micron, and is denoted by the Greek letter  $\mu$ . For example,  $5\mu = 0.005 \text{ mm.} = \frac{1}{5000} \text{ inch.}$

**The Platinum Wire.**—The substance under examination is placed upon thin slips of glass called cover-glasses or directly upon thicker strips of glass, called slides. The material is spread over the glass by means of a platinum wire which has been fixed in a glass rod about six inches long. Such a platinum wire is used

constantly in doing bacteriological work. The platinum wire must be stiff enough not to bend too easily, and yet it should not be so large that it will not cool rapidly after heating. A good size for most purposes is No. 28, English standard gauge, diameter .014 inch. Instead of the expensive platinum wire, one may use nichrome wire, which is much cheaper and serves as well for all ordinary purposes. The wire may be straight throughout its length, or the tip may be bent to form a loop. It is well to follow, from the beginning, certain rules which make the use of the wire safe and accurate. Every time it is taken into the hand and before using it for any manipulation, heat it in the flame of a Bunsen burner or an alcohol lamp to a red heat; and always, *after using and before putting it down, heat it again to a red heat.* After the needle has become wet by

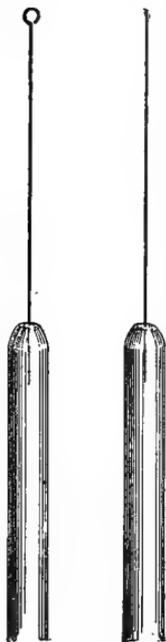


FIG. 20.—Needles used for inoculating media.

dipping it in a fluid and is to be sterilized in the flame, it is necessary to avoid "sputtering" of the fluid by bringing the wet needle gradually to the flame, so as to dry the material adhering to it before burning it or, better, by holding it within the central cooler cone of the Bunsen flame to accomplish the same purpose. This procedure must be done with great care when the wire has been dipped in milk or other substances containing oil. When the needle "sputters," as it is called, from too rapid heating, particles that have not yet been sterilized may be thrown some distance. On no account should the needle touch any object other than that which it is intended it should touch. With such a platinum

wire, which has been properly sterilized, one can easily remove portions from a culture of bacteria, or from a fluid in which bacteria are supposed to be present. The rod in which the wire is fixed should be held between the thumb and forefinger of the right hand like a pen.

**Glass Pipettes.**—Sterile glass tubes drawn out to form slender capillaries, Pasteur pipettes, are very convenient instruments for handling bacteriological materials, and, for many kinds of work, really indispensable. They serve nearly all the purposes of the platinum wire and are capable further of use to transfer large quantities of fluid without contamination. They are also especially useful in collecting material from patients

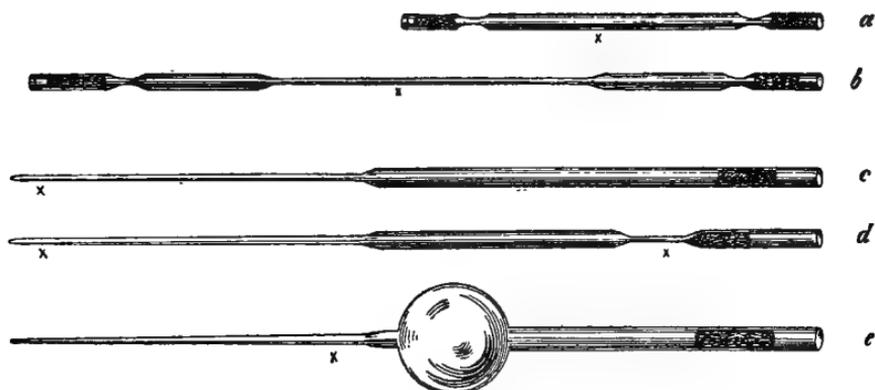


FIG. 21.—Drawn-out tube pipettes of Pasteur. *a*, Plugged, sterile tube as kept in stock; *b*, the same heated at *x* in blast-lamp and drawn out; then sealed at *x*; *c* and *d*, completed pipettes; *e*, the same with bulb. (After Novy.)

and at autopsy. Each pipette is sterilized and discarded after use.

These pipettes are made by cutting glass tubing of a suitable size, diameter 3 mm. to 9 mm., into pieces from .20 to 40 cm. in length. The cut ends are smoothed in the flame. In the tubes of larger caliber it is well to make a constriction about 5 cm. from each end. Each end is plugged with cotton. The tubes are then sterilized by dry heat. By heating the middle of the tube in a blast lamp or over a large Bunsen flame, the glass may be

softened and then drawn out into a capillary of any desired length and caliber. This is melted in the middle and severed by the flame, giving two pipettes. When a large capacity is desired a bulb may be blown in the tube between the capillary and the cotton plug. This requires a little practice. The tip of the pipette is finally broken off with aid of a file, sterilized by the flame and the pipette is ready for use. The various steps in the preparation of pipettes are illustrated in the figures (Fig. 21).

**The Hanging-drop.**—Living bacteria may be studied with the microscope while suspended in some fluid substance. The platinum loop having been heated to a red heat in the flame and having been allowed to cool, a small portion of the culture or other material may be removed with it and deposited in the center of an ordinary cover-glass. The needle should again be sterilized in the flame. When cultures on solid media are to be examined, a small particle may be mixed with a drop of sterilized water or



FIG. 22.

bouillon. The cover-glass should have been carefully cleaned and sterilized over the flame. The cover-glass with the small drop of fluid material held in sterilized forceps is now to be inverted over a sterilized glass slide, which has a concavity ground in the middle of it. Around the concavity, the slide should be smeared with vaseline. In this manner a small air-tight chamber is made. This slide and cover-glass is next put upon the stage of the microscope. A good dry lens, if of sufficiently high power, is more convenient for examining the hanging-drop than an oil-immersion. If the latter be used, having placed a drop of cedar-oil on the center of the cover-glass, and a good light having been secured, the oil immersion objective should be brought down upon this drop of oil. The beginner often experiences difficulty in focusing upon a hanging-drop. It is necessary to shut off most of the light by means of the iris diaphragm, for as has

already been pointed out (page 26), colorless objects may be clearly seen only when illuminated either by a narrow central beam or by oblique illumination (dark-field). Often it is well to secure the focus roughly upon the extreme outer edge of the chamber, or to find the edge of the drop of fluid with the low power and then focus upon this edge with the oil-immersion objective. Above all things guard against breaking the cover-glass by forcing the objective down upon it. The motility of certain bacteria is one of the most striking phenomena to be observed in the hanging-drop. It is not to be confused with the so-called "Brownian movement" which is exhibited by fine particles suspended in a watery fluid. It is well for the beginner to observe the character of the Brownian movement by rubbing up some carmine in a little water, and with the microscope to study the trembling motion exhibited by these particles of carmine. It will be noticed that, although the particles oscillate, no progress in any direction is accomplished unless there are currents in the fluid. Such currents might give rise to the impression that certain bacteria possessed motility when they were, in fact, powerless to move of themselves. In the hanging-drop the multiplication of bacteria can be studied, the formation of spores and the development of spores into fully formed bacteria. The hanging-drop is also used extensively for the demonstration of the agglutination reaction with the bacillus of typhoid fever. Sometimes bacteria must be watched in the hanging-drop for hours, or even days, and it may be necessary to keep it at the temperature of the human body for this length of time. Various complicated kinds of apparatus have been devised for this purpose, but they are needful only for special kinds of work. When the hanging-drop preparation is no longer required, the slide and cover-glass should be dropped into a 5 per cent carbolic acid solution and afterward sterilized by steam.

**The Hanging-block.**—Hanging-block preparations, which were introduced by Hill,<sup>1</sup> make use of a cube of nutrient agar

<sup>1</sup> *Journal of Medical Research*, Vol. VII, March, 1902.

instead of a drop of fluid. Bacteria are distributed on the surface of the agar, which is then applied to a cover-glass, and mounted like a hanging-drop. The bacteria are thus kept in a layer close to the glass, where growth may be studied.

**The Microscopic Preparation for Study by Dark-field Illumination.**—The central portion of a clean glass slide is encircled with a ring of vaseline, and a drop of the fluid to be examined is deposited on the clean surface in the center of the ring by means of a capillary tube. It is then covered with a clean large cover-glass so that the fluid spreads out in a moderately thin layer beneath the cover-glass and is confined on all sides by the vaseline, thus preventing evaporation and resulting currents in the preparation.

Best results with the dark-field microscope are obtained only in a dark or dimly lighted room. An electric arc or a powerful gas-light or, better still, a powerful daylight-glass nitrogen-bulb incandescent electric light, may be employed as the source of light, and it is well to put a flask of water between the light and the microscope to eliminate the heat-rays. The substage condenser of the microscope is replaced with the special dark-field condenser and this is carefully centered. A large drop of immersion oil is placed on the upper surface of the condenser. The slide is carefully placed upon the stage so that the oil fills in completely the space between the condenser and slide and remains free from air-bubbles. The preparation is then ready for examination. Objectives of numerical aperture wider than 1.0 cannot be successfully used with the ordinary dark-ground condensers and therefore it is necessary to stop down the aperture of the oil-immersion objective before using it. A special funnel stop is furnished for this purpose. When this has been attached the preparation may be studied with the oil-immersion objective in the usual way. Skill in this method of studying unstained microbes is quickly acquired, offering, as a rule, less difficulty than the method of central illumination which is employed for the hanging-drop and hanging-block.

**Smear Preparations for Staining.**—The examination of bacteria with the microscope is carried out to a very large extent by means of smears made upon thin slips of glass. Such slips of glass are generally called cover-glasses. It is best to obtain the kind sold by dealers as No. 1,  $\frac{3}{4}$  inch squares.

The cover-glass may be cleaned best by immersion in a mixture of sulphuric acid and bichromate of potassium solution, and afterward washed thoroughly in distilled water, and finally in alcohol. A stock of clean cover-glasses may be kept in a bottle of alcohol, or perhaps preferably in alcohol containing 3 per cent of hydrochloric acid.

#### CLEANING FLUID

Potassium bichromate.....	40 grams.
Water.....	150 c.c.
Dissolve the bichromate of potassium in the water, with heat; allow it to cool; then add slowly and with care sulphuric acid, commercial.....	
	230 c.c.

When they are needed for use they should be wiped clean with a piece of linen cloth. As a rule, cover-glasses cleaned in this way still retain a small amount of oily matter on their surfaces, sufficient to prevent the proper spreading of a drop of water. This difficulty may be overcome by passing each glass several times through the flame. It is better, when time permits, to fill an Esmarch dish with clean cover-glasses and then heat them in the oven at  $200^{\circ}\text{C}$ . for half an hour. Cover-glasses treated in this way will allow the droplet of bacterial suspension or other material to spread perfectly. They must be carefully preserved in a covered dish from which they are to be removed only by clean (flamed) forceps. Carelessness in this matter may necessitate recleaning of the whole lot of cover-glasses.

An ordinary pair of fine forceps may be used to pick up the cover-glass and insert it between the blades of such special forceps as those of Cornet or of Stewart. Perhaps the most convenient style of forceps is that devised by Novy, provided with a clasp.

Bacteria may be placed upon the cover-glass by allowing the glass to fall upon one of the colonies of bacteria, on a gelatin or agar plate (see page 110), which will adhere to it in part, producing an "impression preparation" (German, *Klatschpreparat*). Such a preparation, after drying in the air, is to be fixed by pass-



FIG. 23.—Cornet forceps for cover-glasses.

ing it through the flame three times. (See below). The forceps with which it is handled should be sterilized in the flame.

Generally bacteria contained in fluids, like sputum, or taken from the surface of a culture, are smeared over the cover-glass by means of the platinum wire or loop, which must be heated to

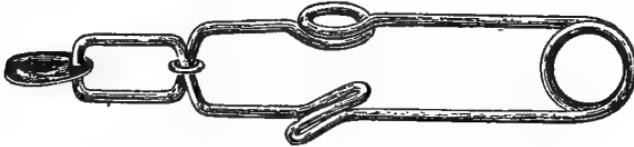


FIG. 24.—Stewart forceps for cover-glass.

a red heat before and after the operation. Such preparations are called smear, cover-glass, cover-slip, or film preparations. When the material to be spread is thick or very viscid, a small drop of distilled water must first be placed in the center of the cover-glass so as to dilute it. Beginners generally take too much



FIG. 25.—Novy's cover-glass forceps with clasp. (After Novy.)

material on the wire. As thin a smear as possible is made. It is allowed to dry in the air; this should occupy a few seconds. The drying may be hastened by holding the forceps with the cover-glass a long distance above the flame, at a point where the

heat would cause no discomfort to the hand. Having dried the preparation, it is to be passed through the flame of a Bunsen burner or alcohol lamp three times, taking about one second for each transit. The heat of the flame serves to dry the bacteria upon the cover-glass and fix them permanently in position; it is not sufficient, however, when applied in this manner, to kill all kinds of bacteria, especially those containing spores. After it has been passed through the flame three times the preparation may be stained with one of the aniline dyes, and after washing in water and drying, may be mounted, face down, in Canada balsam upon a glass slide. It makes a suitable object to be examined with the oil-immersion objective. The slide is a thin slip of glass, 3 inches by 1 inch, with ground edges.

The smear preparation may equally well be made directly upon the glass slide provided this be cleaned and heated to insure

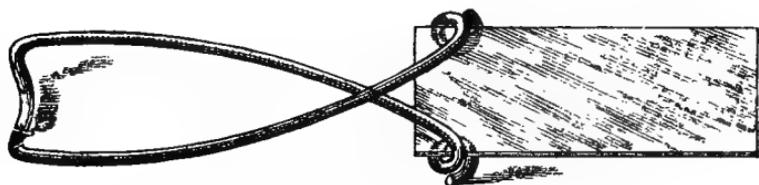


FIG. 26.—Kirkbride forceps for holding slides.

a clean surface free from oily matter. The fixation in the flame must then occupy a longer time than with the small and thin cover-glass. Such preparations have the advantage that several may be made upon one slide, and that after staining them they may be examined in cedar-oil, with the oil-immersion lens, without the use of the cover-glass and Canada balsam. They are also less readily broken in handling. Special care should be exercised to avoid soiling the surface of the objective with the material on the slide, for inattention to this matter may permit the confusion of material from two different specimens, a bit being carried from one smear to another by the lens. The forceps of Kirkbride will be found convenient when staining on the slide. The aluminium

dish devised by Krauss,<sup>1</sup> or some similar dish, will be found useful when the stain has to be heated. Experiments have shown that the ordinary method of fixation in the flame, when applied to bacteria spread upon slides, has little effect on the vitality of many species. The beginner is, therefore, advised to make his preparations on cover-glasses.

When very resistant or dangerous pathogenic bacteria are being handled, after fixation by heat upon the slide or cover-glass, the preparation may, if desired, be immersed in 1-1000 solution of bichloride of mercury long enough to kill the bacteria, without injuring the preparation or its staining properties.

**Staining Solutions.**—The staining of bacteria is done for the most part with the aniline dyes. The object of staining bacteria is to give them artificially some color which makes them distinct and easily visible without imparting this color to the substance or medium in which they are imbedded. The substances known as aniline dyes are derivatives of coal-tar, but not always of aniline. These dyes are of great importance in bacteriological work. Their number is very large, but only a few are in common use.

It is simplest to classify the aniline dyes as acid or basic. Eosin, picric acid and acid fuchsin are acid dyes; they tend to stain tissues diffusely. Fuchsin, gentian-violet and methylene blue are basic dyes; they have an affinity for the nuclei of tissues and for bacteria; they therefore are the dyes used chiefly in bacteriological work. The other varieties may be employed as contrast-stains; another contrast-stain frequently used is Bismarck brown. It is best to keep on hand saturated solutions of the aniline dyes in alcohol, which are permanent, but cannot be employed directly for staining. In order to prepare the simple staining solutions, the alcoholic solution is diluted about ten times, or so as to make a liquid which is just transparent in a layer about 12 mm. in thickness, after filtering. These watery solutions deteriorate after a few weeks.

Fuchsin and gentian-violet stain rapidly and intensely.

<sup>1</sup> Krauss, *Jour. A. M. A.*, Apr. 6, 1912, Vol. LVIII, p. 1013.

Methylene blue works more slowly and feebly; it is to be preferred where the bacteria occur in thick or viscid substances, like pus, mucus, and milk.

*Aniline-water Staining Solutions.*—The intensity with which aniline dyes operate may be increased by adding aniline oil to the solution:

Aniline oil.....	5 c.c.
Water.....	100 c.c.

Mix, shake vigorously, filter through wet filter paper. The fluid after filtration should be perfectly clear. Add—

Alcohol.....	10 c.c.
Alcoholic solution of fuchsin (or gentian violet, or methylene blue).....	1 c.c.

Aniline-water staining solutions do not keep well, and need to be freshly prepared about every two weeks. The applications of the aniline-water stains will be given under separate headings. In general, however, they are employed where a stain of unusual power is required.

**Sterling's Gentian Violet.**—Mix 2 c.c. aniline oil with 10 c.c. of 95 per cent alcohol and add 88 c.c. of distilled water. Grind 5 grams of gentian violet in a mortar, slowly adding the mixed liquid while grinding. Filter. The solution keeps and stains rapidly in Gram's method.

**Carbol-gentian-violet.**—Grind 1 gram gentian-violet in a mortar with 10 c.c. absolute alcohol; then add 2 grams pure crystals of carbolic acid and mix. Add distilled water, 70 c.c., and transfer the whole to a clean bottle. Rinse the mortar with a further 30 c.c. of distilled water and add this to the contents of the bottle. Filter into a clean bottle after 24 hours. This solution keeps well and is used for staining by Gram's method.

**Carbol-fuchsin.**—The intensity of staining may also be increased by the presence of carbolic acid. The most common example of this is carbol-fuchsin.

Saturated alcoholic solution of fuchsin.....	10 c.c.
5 per cent aqueous solution carbolic acid.....	100 c.c.

This solution keeps for some months. It is employed especially where very intense action is required, as in staining spores, flagella, and acid-proof bacteria.

**Löffler's Methylene Blue.**—A very useful solution, which keeps well, is Löffler's alkaline methylene blue:

Saturated alcoholic solution of methylene blue.....	30 c.c.
1-10,000 aqueous solution of potassium hydroxide..	100 c.c.

This solution stains more intensely than simple methylene blue, and also gives rise to useful differential staining in smears and even in sections of tissue.

**Nocht-Romanowsky Stain.**—This requires two solutions, one of ripened alkaline methylene blue, the other of eosin.

*Solution 1.*

Methylene blue.....	1.0 gram.
Sodium carbonate.....	0.5 gram.
Distilled water.....	100.0 grams.

Heat at 60° C. for two days until solution shows a slight purplish color.

*Solution 2.*

Eosin, yellowish, water soluble.....	1.0 gram.
Distilled water.....	100.0 c.c.

In staining, a few drops of each of these solutions are mixed with about 10 c.c. of distilled water in an Esmarch dish, and the smear, which has previously been fixed in absolute methyl alcohol, is floated on this mixture for about ten minutes. Considerable practice is necessary before the best results are obtainable. The method is especially useful in staining blood films, and protozoa in blood, in feces or in culture.

**Leishman's Stain.**—Leishman has utilized the principle of Jenner's stain<sup>1</sup> and has added to it the important additional constituents found in polychrome methylene blue by substituting this for the ordinary methylene blue used by Jenner.

<sup>1</sup> Jenner (*Lancet*, 1899, I, p. 370) first employed the solution of eosin and methylene blue in methyl alcohol as a stain for blood films.

*Solution A.*—To a 1 per cent solution of medicinally pure methylene blue in distilled water add 0.5 per cent sodium carbonate and heat at 65° C. for 12 hours, then allow it to stand 10 days at room temperature.

*Solution B.*—Eosin extra B. A. (Grübler) 0.1 per cent solution in distilled water.

Mix Solutions *A* and *B* in equal amounts and allow to stand six to twelve hours, stirring at intervals. Filter and wash the precipitate thoroughly. Collect, dry and powder it. 0.15 gram is dissolved in 100 c.c. of pure methyl alcohol to form the staining solution. It keeps perfectly for at least five months. To stain, cover the dried but unfixed film of blood with the staining solution. After 30 to 60 seconds add about an equal amount of distilled water. Allow this mixture to act for five minutes. Wash in distilled water for about one minute, examining the specimen mounted in water under the microscope. Blot, dry thoroughly, mount in balsam, or preserve the specimen as an unmounted film.

Numerous imitations or modifications of Leishman's stain have been described.

**Giemsa's Stain.**—This stain contains certain of the essential constituents of polychrome methylene blue and eosin, the whole being dissolved in a mixture of glycerin and methyl alcohol. Giemsa's Azur I is the substance methylene azure and his Azur II is this substance mixed with an equal amount of methylene blue. His Azur II-eosin is the compound precipitated when aqueous solutions of Azur II and eosin are mixed. The Giemsa solution is made according to the following formula:

Azur II-eosin.....	3.0 grams.
Azur II.....	0.8 gram.
Glycerin.....	250.0 grams.
Methyl alcohol.....	250.0 grams.

Dissolve the powdered dyes in the glycerin at 60° C.; then add the methyl alcohol previously heated to the same temperature. After mixing, let it stand 24 hours at room temperature, and

filter. To stain, mix one drop of this solution with 1 c.c. of water and immerse the film, previously fixed, for 15 minutes to 24 hours.

*Direct Preparation of Romanowsky Stains.*—In a study of the essential constituents of the Romanowsky stain, MacNeal<sup>1</sup> found both methylene azure and methylene violet to be present and participating in the nuclear staining. The preparation of solutions directly from the pure dyes, methylene azure, methylene violet, methylene blue and eosin, has been recommended as the best manner of preparing these staining solutions, as the proportion of the various constituents may be varied at will to obtain various kinds of differentiation. As a routine blood stain for study of leukocytes and staining of hematozoa, the following is recommended:

*Solution A.*

Methylene azure.....	0.3
Methylene violet (Bernthsen's, insoluble in water).....	0.2
Methylene blue, medicinal.....	1.0
Methyl alcohol, pure.....	500.0

*Solution B.*

Eosin, yellowish, water soluble.....	1.0
Methyl alcohol, pure.....	500.0

These solutions keep for at least a year. They are mixed in equal parts and filtered to prepare the actual staining solution, which keeps for a few weeks. This final mixture is employed in the same manner as Leishman's stain. It keeps for a few months.

**Method of Staining Cover-glass Preparations.**—(a) A smear preparation of bacteria having been made and fixed in the manner above described, and a watery solution of either fuchsin, gentian violet or methylene blue having been prepared, the cover-glass is to be dropped into a dish containing the dye, or the dye may be dropped upon the cover glass held in the forceps.

(b) Allow the stain to act for about thirty seconds.

(c) Wash in water.

(d) Examine with the microscope in water directly or after drying and mounting in Canadian balsam.

<sup>1</sup> *Journ. Infectious Diseases*, Vol. III, 1906, pp. 412-433.

The rapidity and intensity of staining may be increased by warming the solution slightly. The bacteria will usually appear more distinct if, directly after pouring off the stain, the preparation is rinsed for a few seconds in 1 per cent solution of acetic acid, and then thoroughly washed in water. The acetic acid solution serves to remove in a measure any color which has been imparted to the background, and which is undesirable.

Preparations that are mounted at first in water may be made permanent by moistening the edge of the cover-glass so that it may be easily removed from the slide, then drying and mounting in Canada balsam. Cover-glass preparations which have been stained are examined with the oil-immersion objective, employing the plane mirror, having the iris diaphragm open and the condenser close to the lower surface of the glass slide. The purpose is to obtain the most intense illumination possible over a small field.

**Gram's Method.**—Cover-glass preparations, having been prepared and fixed in the usual manner (see page 38), are stained as follows:

(a) Stain in carbol gentian violet or in aniline-water gentian violet solution, from two to five minutes. The intensity of the stain may be increased by warming slightly.

(b) Gram's solution, one and one-half minutes:

Iodine.....	1 gram.
Potassium iodide.....	2 grams.
Water.....	300 c.c.

In this solution the preparation becomes nearly black.

(c) Wash in alcohol repeatedly; the alcohol becomes stained with clouds of violet coloring matter; the alcohol is used as long as the violet color continues to come away, and until the preparation is decolorized or has only a faint steel-blue color.

(d) When desired, the specimens may be stained, by way of contrast, with a watery solution of Bismarck brown, dilute fuchsin, safranin or eosin.

(e) Wash in water, and examine either in water directly or after drying and mounting in Canada balsam. Gram's method and its modifications should not be regarded as absolute means of distinguishing between Gram-positive and Gram-negative bacteria in every case, as much depends upon the condition of the bacteria, and very much upon the technic of staining. When the Gram stain is used for diagnosis, it is well to put a smear of a known Gram-negative and a smear of a known Gram-positive organism on the same slide or cover-glass along with the unknown, and subject them all to the same technic.

**Some bacteria that are stained by Gram's method :**

Staphylococcus aureus,  
Streptococcus pyogenes,  
Micrococcus lanceolatus (of pneumonia),  
Micrococcus tetragenus,  
Bacillus of diphtheria,  
Bacillus of tuberculosis,  
Bacillus of leprosy,  
Bacillus of anthrax,  
Bacillus of tetanus,  
Bacillus welchii (aërogenes capsulatus),  
Ray fungus of actinomycosis.

Of these the tubercle bacillus and the bacillus of leprosy require a much longer exposure to the stain than other bacteria in the list.

**Some bacteria that are not stained by Gram's method :**

Gonococcus,  
Diplococcus intracellularis (meningitidis),  
Micrococcus melitensis,  
Bacillus of chancroids (Ducrey),  
Bacillus of dysentery (Shiga),  
Bacillus of typhoid fever  
Bacillus coli,  
Bacillus pyocyaneus,  
Bacillus of influenza,

Bacillus of bubonic plague,  
 Bacillus of glanders (*Bacillus mallei*),  
 Bacillus proteus,  
 Spirillum of Asiatic cholera,  
 Spirillum of relapsing fever.

**Staining of Acid-proof Bacteria.**—A very large number of methods have been proposed for staining the tubercle bacillus, all of which depend upon the principle that, after adding to solutions of aniline dyes certain substances, like aniline water, carbolic acid, or solutions of ammonia or soda, the tubercle bacillus is stained with great intensity, and gives up its stain with difficulty. Solutions of acids will remove the stain from all parts of the preparation excepting from the tubercle bacilli, which retain the dye, having once acquired it. The rest of the preparation may now be given a different color—contrast-stain.

Bacilli that resist decolorization by acids are called *acid-proof* or acid-fast.

**Some acid-proof bacteria :**

Bact. tuberculosis,  
 Bact. lepræ,  
 Bact. smegmatis,  
 Grass bacillus of Moeller,  
 Butter bacillus of Rabinowitsch,  
 Certain streptothrices,  
 Certain bacilli common in the feces of cattle,  
 Certain bacteria found in distilled water,  
 Spores of many bacteria.

Occasionally other bacteria, micrococci and horny epithelial cells are imperfectly decolorized, but their forms distinguish them from tubercle bacilli. Minute crystalline needles which have a shape like that of bacilli, are often encountered in sputum, but their nature will be recognized after a little practice.

The stain for acid-proof bacteria is most frequently used for specimens of sputum from cases of suspected pulmonary tuberculosis; it may be applied to other fluids and secretions equally

well. It is not reliable, however, when applied to milk, as the oil present in milk interferes with its operation, and milk and its products quite often contains other acid-proof bacilli. The smegma of the external genitals also frequently contains acid-proof bacilli that are not tubercle bacilli. On this account all fluids and discharges from the genito-urinary tract need to be examined with particular care not to confuse tubercle bacilli with smegma bacilli. Too much reliance should not be placed on the possibility of distinguishing between tubercle and smegma bacilli by decolorizing in alcohol. In doubtful cases an animal should be inoculated.

Patients should be given minute instructions concerning the collection of sputum. The bottle used should be new, wide-mouthed, clean, and kept tightly stoppered with a clean cork. The patient should be cautioned against allowing the expectoration to get on the outside of the bottle. Probably whatever risk is incurred by those who examine sputum comes chiefly from the outside of the bottle having been soiled with sputum containing tubercle bacilli. It is well to disinfect the exterior of the bottle when it is received at the laboratory. Often little white particles may be seen floating in the mucous portions of the sputum. These particles should be selected for the investigation, and may be spread in a thin film on the cover-glass with the platinum wire, which is sterilized in the flame before and after using. The selection of the little white particles will be facilitated if the sputum be poured into a clean glass dish, which may be placed on a black surface. A form of porcelain dish is furnished by dealers, the bottom of which is black, and which is convenient for these manipulations. The smears may be made moderately thick as a larger amount of sputum may thus be examined in a short time. Uniform thickness is difficult to obtain and is not absolutely essential. It is hardly necessary to observe that the operator must be scrupulously careful not to contaminate the material under examination with any kind of extraneous matter. The cover-glasses and slides which are used should be new, and

should have been cleaned with bichromate of potassium and sulphuric acid (see page 37). When the work is completed, the bottle containing the sputum should be sterilized by steam or boiling.

*Method for staining the tubercle bacillus:*

(a) The cover-glass or slide preparation is made, dried, and fixed by passing through the flame three times.

(b) The cover-glass, held in forceps or in a watch-crystal is covered with steaming carbol-fuchsin for five minutes. If a slide is employed it may be conveniently stained in the Krauss staining dish, being turned face downward.

(c) Wash in water.

(d) Wash in alcohol containing 3 per cent of hydrochloric acid one minute, or longer if necessary to remove the red color.

(e) Wash in water.

(f) Stain with methylene-blue solution (see page 42) thirty seconds.

(g) Wash in water.

(h) Examine in water directly, and after drying and mounting in Canada balsam. If the preparation has been made on a slide it may be dried and examined directly in cedar oil with the  $\frac{1}{12}$  in. objective. When the preparation is mounted in water, tubercle bacilli may be obscured by refraction in the thicker portions of the smear. Tubercle bacilli take a brilliant red color; other bacteria and the nuclei of cells are stained blue.

Of the numerous methods of staining tubercle bacilli only a few others can be mentioned. Aniline-water fuchsin, aniline-water gentian violet, or carbol-fuchsin may be used. The intensity of the stain must then be increased by warming the preparation till it steams or boils, then allowing the warm stain to act on the specimens for from three to five minutes; the preparation may also be left in the cold stain over night. Decolorization may be effected with a 25 per cent solution of sulphuric acid used till the red color disappears, or a 30 per cent solution of nitric acid, which operates very rapidly. If the red color persists after washing in water,

dip in the acid again. After either acid the preparation is to be washed in alcohol until the last trace of the stain has been removed. An excellent decolorizing agent is a 3 per cent solution of hydrochloric acid in alcohol, used for about a minute. The contrast stain may be omitted entirely if it is desired. A suitable contrast stain after fuchsin staining is a solution of methylene blue; after gentian-violet staining, safranin.

Those who have had experience in staining tubercle bacilli soon discover that the bacilli exhibit some differences in their resisting power to strong acids. One encounters occasionally bacilli that are perfectly stained side by side with others that are more or less completely decolorized. These facts show the necessity of practice with any method, and of exercising caution and judgment in making a diagnosis where the number of bacilli happens to be scanty. If tubercle bacilli are not found in the first preparation, other preparations should be made. Sometimes a large number of cover-glasses must be examined.

Various expedients have been devised to concentrate tubercle bacilli when only a small number may be present in a sample of sputum. Antiformin (a preparation of chlorinated sodium hydroxide) has been employed for this purpose. The following method is that of Williamson.<sup>1</sup> The sputum is measured and transferred to a clean flask of resistant glass. An equal volume of 50 per cent antiformin is added, mixed with the sputum, and the mixture brought to a boil over the flame. This dissolves the sputum promptly. The material is then cooled and to each 10 c.c. of material in the flask, 1.5 c.c. of a mixture of chloroform, one part, and alcohol, nine parts, is added. The mixture is thoroughly shaken. As a result the tubercle bacilli imbibe some of the chloroform and become heavier. The material is next centrifugalized at high speed for 15 minutes, which separates it into three layers, antiformin above and chloroform below with the layer of sediment between the two. This layer is removed and mixed with egg albumen (egg albumen + 0.5 per cent carbolic acid) on a slide and then

<sup>1</sup> Williamson, *Journ. A. M. A.*, Apr. 6, 1912, Vol. LVIII, p. 1005-07.

spread into a smear between two slides. The smears are then dried and stained in the usual way. Instead of using albumen to fix the sediment to the slide, it is convenient to save some of the original sputum and mix it with the sediment for this purpose.

**Staining of Spores.**—The method is applicable to cover-glass preparations which may be prepared in the usual way from material supposed to contain spores.

(a) After drying the smear on the cover-glass, fix it with heat by passing through the flame three times.

(b) Float the cover-glass face downward on the surface of steaming hot carbol-fuchsin or aniline-water fuchsin for three to five minutes.

(c) Wash in 3 per cent hydrochloric acid alcohol one minute, or less.

(d) Wash in water.

(e) Stain with watery solution of methylene blue half a minute.

(f) Wash.

(g) Dry.

(h) Balsam.

The spores are intensely stained by the fuchsin. The stain is removed from everything except the spores by the acid alcohol. The methylene-blue solution stains the bodies of the bacteria, the spores remaining brilliant red. There are various other methods for staining spores, but this procedure usually gives good results. The principle is the same as in staining the tubercle bacillus, except that more pains are needed to impregnate spores with the dye.

When it fails, the cover-glass preparation may be treated by Moeller's method previous to staining. After fixation, the preparation is immersed in chloroform for 2 minutes, drained and dried in the air. It is then immersed in 5 per cent chromic acid for 2 minutes, washed thoroughly in water, and stained as above described.

**Staining of Capsules.**—The capsules which many bacteria possess, appear to be made of some gelatinous substance, which is difficult to stain.

**Method of Hiss.**—(a) Cover-glass preparations are made in the ordinary way and fixed in the flame as soon as dry.

(b) Use the following stain, heated till it steams:

Saturated alcoholic solution of gentian violet or fuchsin.....	5 c.c.
Distilled water.....	95 c.c.

(c) Wash in 20 per cent solution of cupric sulphate crystals.

(d) Dry and mount in Canada balsam.

The method of Hiss is recommended to be used for bacteria that have been cultivated on serum-agar with 1 per cent of dextrose. This method gives excellent results with preparations of capsulated bacteria in smears of animal tissues.

**Method of Huntoon**<sup>1</sup>.—In this method two solutions are required.

*Solution 1.*—Sift 3 grams nutrose into 100 c.c. distilled water and steam for one hour. Then add 5 c.c. of 2 per cent. aqueous solution of carbolic acid. Allow the sediment to settle and employ the supernatant liquid as the diluent.

*Solution 2.*—Mix the following, 100 c.c. of 2 per cent aqueous carbolic acid; 0.25 to 0.50 c.c. of concentrated lactic acid; 1 c.c. of 1 per cent acetic acid; 1 c.c. of saturated alcoholic fuchsin; and 1 c.c. of old carbol-fuchsin solution.

Solution 1 is placed on the slide and the bacteria are emulsified in it and then spread with the platinum loop. When dry the film is covered at once with solution 2 for thirty to fifty seconds. Wash quickly in water, dry and examine.

**Staining of Flagella.**—Flagella are among the most difficult of all objects to stain. The best-known method is that of Löffler. It is important to use young cultures (4 to 10 hours old), preferably on agar.

(a) A small amount of the growth is gently mixed with a

<sup>1</sup> Huntoon, *Journal of Bacteriology*, May, 1917, Vol. II, p. 241.

large drop of distilled water on a clean slide, so that the water is made very faintly cloudy. From the top of this drop one or two transfers are made to a second drop with a small platinum loop. From this second drop a loopful is transferred to a perfectly clean (flamed) cover-glass, spread with minimum manipulation and dried quickly, high over the flame.

(b) After drying, fixation is effected by passing through the flame three times, holding the cover-slip between the thumb and fore finger to avoid overheating.

(c) The essential point in this method is the use of a mordant as follows:

Tannic acid, 10 per cent solution.....	20 c.c.
Saturated solution of ferrous sulphate.....	4 c.c.
Saturated alcoholic solution of fuchsin.....	1 c.c.

This solution should be freshly prepared from pure substances, and should be filtered at once after mixing. It may deteriorate in a few hours but sometimes keeps for a few days or weeks. A few drops are placed on the cover-glass, or the cover-glass is placed, face down, in a dish containing the mordant; it is then left for one to five minutes, the mordant being heated until it steams.

(d) Wash in water.

(e) Stain with aniline-water fuchsin, or carbol-fuchsin, hot, for one to two minutes.

(f) Wash in water.

(g) Dry.

(h) Mount in Canada balsam.

Another and very valuable method is that of *Van Ermengem*.

(a) Make and fix cover-glass preparations as in the preceding method.

(b) Use the following mordant for one-half hour at room temperature or for five minutes at 50° to 60° C.

Osmic acid 2 per cent solution.....	1
Tannic acid 10 to 25 per cent solution.....	2

(c) Wash carefully in distilled water and then in alcohol.

(d) Place for a few seconds in a 0.25 to 0.50 per cent solution of nitrate of silver—"the sensitizing bath."

(e) Without washing transfer to the "reducing and reinforcing bath":

Gallic acid.....	5 grams.
Tannic acid.....	3 grams.
Fused potassium acetate.....	10 grams.
Distilled water.....	350 c.c.

(f) After a few seconds, replace the preparation in the nitrate of silver solution, in which it is kept constantly moving, till the solution begins to acquire a brown or black color.

(g) Finally wash in distilled water, dry, mount in Canada balsam. It is difficult to avoid the formation of precipitates; otherwise the results of this method are usually good.

**Wet Fixation of Protozoa.**—The fluid containing the protozoa is spread on a cover-glass or slide and immediately dropped upon a solution of the fixing agent, commonly sublimate alcohol heated to 60° C. This is prepared by mixing saturated aqueous solution of mercuric chloride, 100 c.c, with absolute alcohol, 50 c.c., and acetic acid, 5 drops. After a few minutes the preparation is carefully washed in water, and passed through graded alcohols to harden. It may then be stained, dehydrated in graded alcohols, cleared in xylol and mounted in balsam. The preparation should not be allowed to dry at any stage of the process.

**Heidenhain's Iron Hematoxylin.**—The preparation to be stained by this method should be fixed in mercuric chloride or alcohol. The stain is prepared by dissolving hematoxylin crystals, 1 gram, in hot absolute alcohol 10 c.c., and then adding distilled water 90 c.c. This solution is allowed to stand in an open, cotton-plugged bottle for about four weeks, and it is then diluted with an equal volume of water before using. The iron solution is made by dissolving 2.5 grams of ferric ammonium sulphate (lavender-colored crystals) in 100 c.c. of distilled water. The preparation to be stained is first soaked in the iron solution for four to eight hours, then rinsed and immersed in the hematoxylin

for twelve to twenty-four hours. It is again rinsed and now differentiated by immersion in the iron solution until black clouds cease to be given off. When the desired differentiation has been obtained the preparation is washed, dehydrated by passing through graded alcohols, and absolute alcohol, cleared in xylol and mounted in balsam.

**Preparation and Staining of Blood Films.**—Blood films are best made on *clean, flamed* slides. A small drop of fresh blood is received on the surface of one slide near one end. The end of another slide is applied to the first at an acute angle so that the blood spreads laterally in the angle between the two slides. The second slide is then pushed along the surface of the first with the blood *following* it in the angle. The thickness of film may be regulated by varying the size of angle between the two slides as well as by the speed of movement.

For staining blood films, either Leishman's or Giemsa's stain or some modification of them should be used as a general rule. After fixation in absolute alcohol, blood films may be stained with Löffler's methylene-blue or by Gram's method.

**Staining Bacteria in Tissues.**—Pieces of organs about 1 cm. in thickness may be taken. Alcohol is the best agent for preserving them. The hardening will be completed in a few days. It is best to change the alcohol. The amount of the alcohol must be twenty times the bulk of the tissue to be preserved.

Ten parts of the standard 40 per cent solution of formaldehyde, with 90 parts water make a good mixture for fixation; after twenty-four hours change to alcohol.

**Imbedding in Collodion or Celloidin.**—From absolute alcohol the pieces of tissue are placed in equal parts of alcohol and ether twenty-four hours; thin collodion ( $1\frac{1}{2}$  per cent.), twenty-four hours; thick collodion of a syrupy consistency (6 per cent) twenty-four hours. The specimen is laid upon a block of wood and surrounded by thick collodion, and then inverted in 70 per cent alcohol. The collodion makes a firm mass, surrounding and permeating the tissue, and permits very thin sections to be cut.

The soluble cotton sold by dealers in photographer's supplies serves as well as the expensive preparation known as celloidin. To make collodion, dissolve it in equal parts of alcohol and ether. Soluble cotton is also called pyroxylin, and is a kind of gun-cotton.

**Imbedding in Paraffin.**—(a) Pieces of tissue 2 to 3 mm. thick which have already been fixed in alcohol or formaldehyde are to be placed in absolute alcohol for twenty-four hours.

(b) In pure xylol one to three hours.

(c) In a saturated solution of paraffin in xylol one to three hours.

(d) In melted paraffin having a melting-point of 50° C., which requires the use of a water-bath or oven, one to three hours. The xylol must be entirely driven off, and the tissue thoroughly infiltrated.

(e) Change to fresh paraffin for one hour.

(f) Finally, place the tissue in a small dish or paper box and pour the melted paraffin about it. Harden as quickly as possible with running water. It is important to fix the piece of tissue in a suitable position, if the position is of importance, before pouring in the melted paraffin. Sections of exquisite thinness may now be cut. The knife need not be wet. Paraffin imbedding is especially desirable when serial sections are to be made.

In order to mount the sections, proceed as follows:

(a) Place the sections on water in a porcelain capsule.

Warm slightly, when the sections will flatten. Smear the surface of a slide with a very thin layer of Mayer's glycerin-albumen mixture. Dip the slide under the sections; lift them; and then drain off the water, leaving the sections in their proper positions. Let them dry for some hours in the incubator, and they will be firmly fastened to the slide.

#### GLYCERIN-ALBUMEN MIXTURE (MAYER)

Equal parts of white of egg and glycerin are thoroughly mixed, and then filtered. Add a little gum-camphor to preserve.

(b) Dissolve out the paraffin in one of the numerous solvents (xylol, a few minutes).

(c) The xylol should be washed off with absolute alcohol, and then 70 per cent alcohol and finally distilled water.

(d) The section is stained.

(e) Dehydrate in absolute alcohol.

(f) Clear in xylol.

(g) Mount in balsam.

**Section Cutting.**—Cutting is best done with an instrument called a microtome. The tissues may be imbedded in collodion or paraffin; or when they have been hardened with formaldehyde

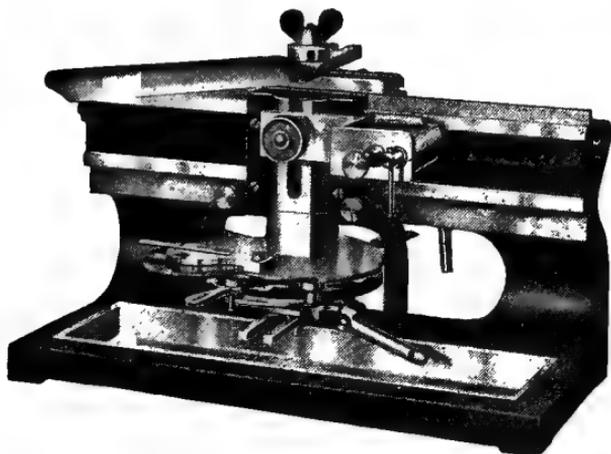


FIG. 27.—Automatic laboratory microtome.

they may be cut after freezing. Bacteria stain admirably in frozen sections. For routine work collodion imbedding will be found as convenient a process as any. Paraffin imbedding gives the thinnest sections.

A microtome consists of a heavy, sliding knife-carrier, which moves with great precision on a level, and of a device for elevating the object which is to be cut, any desired distance after each excursion of the knife. The thickness of the section will be the distance which the object is elevated. The knife is kept wet with alcohol during the cutting of collodion sections, otherwise it is left dry. The microtome is usually provided with a special form of knife. A razor will serve nearly as well, after having

had the lower side ground flat. If a razor is used, a special form of razor-holder must be attached to the microtome to receive the razor. Above all, it is necessary that the knives should be kept in good condition. Only occasionally will they need honing, using a fine water-stone or Belgian hone. The movement in honing should be from heel to toe, always placing the back of the knife next the hone when turning. The knife should be stropped frequently. The leather of the strop should be glued to a strip of wood to make a flat surface. The movement in stropping should be from toe to heel. Sections should be cut to a thickness of not more than  $25\ \mu$ . Thinner sections ( $5$  to  $10\ \mu$ ) are to be desired.

**Staining of Section.**—A watery solution of one of the aniline dyes is used—fuchsin, gentian violet or methylene blue—made by adding a few drops of the alcoholic solution to a dish filled with water. Löffler's solution of methylene blue serves very well.

By this process most bacteria are stained; also the nuclei of cells; frequently, also certain granules contained within some cells which may easily be mistaken for bacteria by the inexperienced (basophilic granules).

(a) Place the section in the staining solution from two to five minutes.

(b) Wash in water.

Place in a watery solution of acetic acid, 1 per cent, for one minute.

(d) Alcohol, one to two minutes; change to absolute alcohol. Touch the sections to blotting-paper to remove the superfluous alcohol.

(e) Xylol until clear; xylol is to be preferred to other clearing agents, like oil of cloves, most of which slowly remove aniline colors. It has the disadvantage of not clearing when more than a trace of water is present; dehydration in alcohol must, therefore, be complete. The section should be removed from the xylol as soon as it is cleared; otherwise wrinkling occurs.

(f) The section is placed upon a glass slide; a drop Canada

balsam is placed upon it and then a cover-glass. The Canada balsam should be dissolved in xylol.

The section is to be manipulated with straight or bent needles. The removal from xylol to the glass slide is managed best with a spatula or section-lifter.

The above statements apply to frozen sections or to sections imbedded in celloidin. Paraffin sections are preferably attached to the slide with glycerin-albumen. The different steps in the process follow in the same order. The stain may be poured on the slide, or the slide may be placed in a large dish full of staining fluid. (See page 44.) Celloidin sections may also be stained on the slide. If the section be well spread and flattened thoroughly with blotting paper, it will usually adhere to the slide, and is less likely to wrinkle. It must not be allowed to dry.

**Gram's Method** may be applied to the staining of sections of tissues as well as to smears upon cover-glasses.

(a) Place the section in aniline-water gentian violet, one to five minutes.

(b) Rinse briefly in water.

(c) Iodine solution (see page 45), one and one-half minutes.

(d) Alcohol, until decolorized to a faint blue-gray.

(e) Xylol.

(f) Mount on a slide in balsam.

**Weigert's Modification of Gram's Method, or Weigert's Stain for Fibrin.**—(a) Place the section in aniline-water gentian violet solution, five minutes or more.

(b) Wash briefly in water.

(c) Place the section upon a slide by means of a section lifter; having straightened it carefully, absorb the water with blotting-paper.

(d) Gram's solution (see page 45) one to two minutes.

(e) Absorb the iodine solution with blotting-paper.

(f) Add aniline oil, removing it from time to time with blotting-paper, and adding fresh aniline oil until the color ceases to come away. (Aniline oil serves in this connection both to decolorize

and to dehydrate. It absorbs the water rapidly and efficiently. However, on account of its decolorizing tendency, it must be removed before the specimens can be mounted permanently.)

(g) Add xylol; remove it with blotting-paper; and add fresh xylol several times, in order to extract the last trace of aniline oil.

(h) Mount in Canada balsam.

This method is more convenient for the staining of sections than the Gram method. The results, however, are essentially the same as far as the bacteria are concerned; fibrin and hyaline material are stained blue, bacteria violet. It is often impossible to decolorize the nuclei completely without decolorizing the bacteria also. The parts of the nuclei which remain stained often present pictures that resemble bacteria, and which may lead to error if not recognized. Basophilic granules also retain the stain, as do the horny cells of the epidermis. These remarks apply also to Gram's method, except as regards fibrin. Very beautiful preparations can be obtained according to this or the Gram method when the sections have previously been stained in carmine; the nuclei will then be colored red, bacteria violet.

**Tubercle bacilli** may be stained in sections as follows:

(a) Use carbol-fuchsin, or aniline-water gentian violet for one-half to two hours with very gentle warming, or over night without warming.

(b) Wash in water.

(c) Decolorize with some one of the decolorizing agents mentioned in connection with the staining of tubercle bacilli in cover-glass preparations, preferably 3 per cent hydrochloric-acid alcohol. Decolorization must be continued until the red color has disappeared, which requires one-half to several minutes.

(d) Wash in alcohol.

(e) Wash in water.

(f) Use hematoxylin as a contrast-stain for fuchsin preparations, and carmine for gentian violet preparations. (It is better to stain with carmine first of all and before staining the bacilli. The carmine is not affected by the subsequent treatment.)

- (g) Wash in water.
- (h) Alcohol.
- (i) Xylol.
- (j) Balsam.

**Nuclear stains**, which may be used as contrast-stains for sections:

DELAFIELD'S HEMATOXYLIN

Hematoxylin crystals.....	4 grams.
Alcohol.....	25 c.c.
Ammonia alum.....	50 grams.
Water.....	400 c.c.
Glycerin.....	100 c.c.
Methyl alcohol.....	100 c.c.

Dissolve the hematoxylin in the alcohol, and the ammonia alum in the water. Mix the two solutions. Let the mixture stand four or five days uncovered; it should have become a deep purple. Filter and add the glycerin and the methyl alcohol. After it has become dark enough, filter again. Keep it a month or longer before using; the solution improves with age. At the time of using, filter and dilute with water as desired.

LITHIUM-CARMINE (ORTH)

Carmine.....	2.5 grams.
Saturated watery solution of lithium carbonate....	100.0 c.c.

Add a few crystals of thymol. The carmine dissolves readily in the lithium carbonate solution. Filter the stain at the time of using. Sections are to be left in the stain five to twenty minutes.

Sections stained in carmine are placed directly in acid alcohol (1 part hydrochloric acid, 100 parts 70 per cent alcohol) for five to ten minutes. They acquire a brilliant scarlet color. When used as a contrast-strain for tissues containing bacteria, it is best to use it before staining the bacteria, which might be decolorized by the acid alcohol.

## CHAPTER II

### STERILIZATION—DISINFECTION—ANTISEPSIS— FOOD PRESERVATION

**Definitions.**—By sterilization is meant the killing or the removal of all micro-organisms in or on a body or substance. Disinfection has a somewhat analogous signification, but denotes the destruction or removal of infectious microbes, and this may or may not be accomplished without complete sterilization, according to the nature of the particular case in hand. Antiseptics means the inhibition of growth of micro-organisms without ordinarily killing or removing them, and is especially applied to the checking of microbic activity in wounds and the effects produced thereby (sepsis). Food preservation involves similar principles, depending upon the prevention of microbic activity in dead organic matter either by sterilization or by the presence of inhibitive substances, similar to antiseptics, but in this instance called preservatives.

In connection with sterilization we shall consider those agents which remove or destroy a part of the microbic flora without producing complete sterility, as well as the methods which insure complete sterilization. A few examples of each general class will be considered.

**Physical Sterilization.**—Among the physical means by which sterilization may be accomplished, those which are merely *mechanical* may be mentioned first. The removal of microbes from an infected surface by washing them away is a method of wide application. Complete sterility may sometimes be attained in this way. In ordinary disinfection of woodwork, walls and floors, or of the hands, mechanical cleaning is of primary impor-

tance, even though it does not insure complete sterilization. The process removes not only many of the bacteria, but also much other material which serves to protect them and even to furnish food for their development. Another mechanical method is that of comminution, actual crushing of the bacterial cells. It is of very narrow application and not to be relied upon. High pressures have been employed to destroy bacteria, but hydrostatic pressure of even 1000 atmospheres does not produce complete sterilization. Sedimentation is a method of primary importance, especially in the removal of suspended bacteria from the atmosphere. It also operates to remove a large proportion of the bacteria from drinking water when stored in suitable reservoirs. Filtration of fluids is an important means of sterilizing them. Air may be sterilized by drawing it slowly through a sufficient layer of cotton. Water becomes bacteria-free as it filters through the soil, so that waters from the depths of the earth are sterile. Liquids are commonly sterilized in the laboratory by forcing them through a layer of unglazed porcelain (Pasteur-Chamberland filter) or through a compact wall of diatomaceous earth (Berkefeld filter). Liquids rich in bacteria, such for example as cultures in broth, may be rendered bacteria-free in this way. These filters have also been employed for the sterilization of drinking water, but their use for this purpose requires intelligence and care, and when carelessly employed they are worse than useless.

*Desiccation* is destructive to many microbes, especially those which do not form spores. The germs of Asiatic cholera are dead in a few hours after complete drying. The spores of the anthrax bacillus on the other hand remain alive for at least ten years after drying. Most bacteria resist drying long enough so that they may be transferred by air currents as dust and still be capable of growth.

*Light* is injurious to bacteria and direct sunlight is rapidly fatal to them, even in spore form. Light seems to act by producing powerful chemical germicides, probably organic peroxides, in the medium surrounding the bacteria. Such substances are

known to be produced under these circumstances. They rapidly decompose.

*Cold* appears to be fatal to some pathogenic forms, and a considerable percentage of the bacteria in a culture are usually killed by freezing. Cultures cannot be completely sterilized even by exposure to the temperature of liquid air. Cold is therefore not to be regarded as an efficient germicide, although it may completely check the growth of bacteria.

*Heat* is the most important of the physical means and doubtless the most important of all means of destroying bacteria. Its value as a purifying agent was recognized among the ancients. Heat is applied under conditions insuring the presence of liquid water, so-called moist heat, and in the absence of water, so-called dry heat or hot-air sterilization. The most reliable methods of sterilization by dry heat are those which accomplish the combustion or destructive distillation of organic matter in general. Actual combustion of clothing and bedding, and even of houses has been resorted to in the past as a method of disinfection. Heating to redness in the naked flame is the routine method of sterilizing our platinum wire, and glass articles, such as capillary pipettes, cover-glasses and slides are commonly sterilized in the flame. Flaming may even be employed for sterilization of surgical instruments in an emergency, although such treatment quickly destroys steel instruments. Sterilization of large objects and of combustible material by dry heat is generally accomplished in an oven or hot-air sterilizer. The common laboratory sterilizers are boxes of sheet iron with double walls, with air space between to allow the hot gases from the flame completely to surround the inner compartment. The door, which occupies one full side, is usually double. A tubulation through the top allows a thermometer to be inserted into the interior so that the temperature may be read off at any time. Even the best hot-air sterilizers fail to give an even temperature throughout the interior, so that the thermometer bulb at one corner cannot be implicitly relied upon to record the temperature of other parts. Ordinarily a temperature of  $150^{\circ}$  C.

for one hour,  $170^{\circ}$  C. for 30 minutes, or  $200^{\circ}$  C. for one minute will kill all bacteria. Such exposure browns cotton of good grade only slightly. One fallacy in hot-air sterilization needs to be guarded against. Glassware and other apparatus must be dry before it is put into the oven to sterilize. A tube containing water may be left in the oven until the thermometer records a temperature of  $200^{\circ}$  C. in the upper corner of the sterilizer, and subsequently the

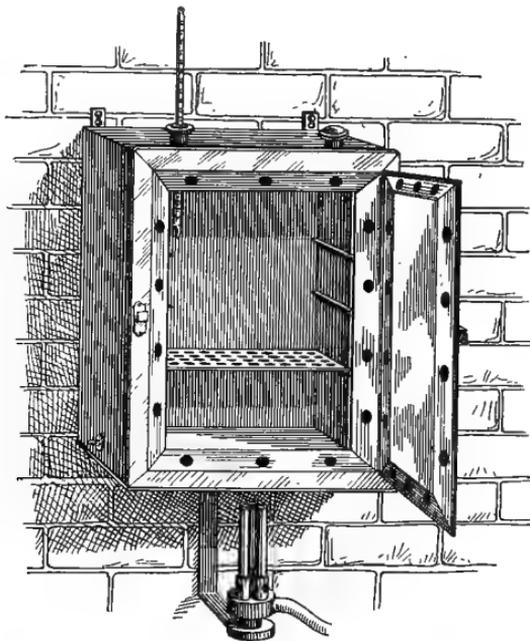


FIG. 28.—Hot-air sterilizer.

tube may be removed from the oven with the most of the water still in it. Hot-air sterilization is employed for glassware, tubes with cotton plugs, granite-ware, stone-ware, and for metals not injured by heat.

*Moist heat* or heat in the presence of liquid water must be used whenever drying is to be avoided, especially in the sterilization of culture media and various solutions. It is employed as continuous sterilization at a single exposure and as discontinuous sterilization, heating for a short time on several consecutive days.

The temperature employed varies according to the effect desired. A temperature of  $60^{\circ}$  C., maintained throughout a water liquid for twenty minutes will kill most vegetative bacteria, and practically all pathogenic bacteria which do not form spores. Such partial sterilization is called Pasteurization. Boiling water,  $100^{\circ}$  C., kills vegetative bacteria in a very short time, less than two minutes for most bacteria, and the spores of many species are destroyed

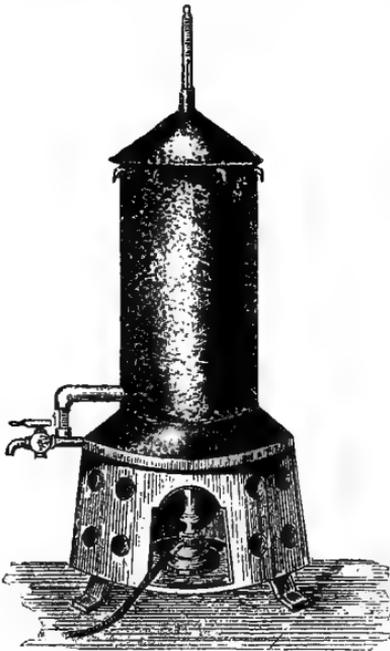


FIG. 29.—Koch's steam sterilizer.

by boiling for 5 to 30 minutes. Some spores, however, for example those of some varieties of *B. vulgaris*, may survive a boiling temperature for several hours. Boiling is one of the most useful practical methods of disinfection. Nearly all pathogenic bacteria are quickly killed in boiling water. Surgical instruments are commonly boiled in water to which sodium carbonate, 1 to 2 per cent, has been added. Rusting and corrosion may also be prevented by adding 10 per cent of borax to the water in which metal instruments are boiled. Sterilization of bacteriological media is usually done by means of streaming steam, rather than by immersion in boiling water. The Koch steam sterilizer is a comparatively simple device for this kind of sterilization. It is a tall, cylindrical, tin vessel covered with asbestos or felt. The lower portion is filled with water; on the side is a water-gauge indicating the height of the water, in order that one may observe when there is danger of the sterilizer boiling dry. Over the top there is a tight-fitting cover. The steam is generated by a Bunsen burner standing underneath. A perforated shelf placed some distance

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above the surface of the water is for the reception of the tubes and flasks that are to be sterilized. The Arnold steam sterilizer is somewhat more complicated but is very convenient and efficient. It consists of a cylinder of tin or copper with a cover, which is enclosed in a movable cylindrical outer cover or hood. The inner cylinder has an opening in the bottom through which steam may enter, the steam coming from a small chamber underneath with a copper bottom to which the flame is applied. The peculiarity of this form of sterilizer consists in the fact that the steam which escapes from the sterilizing chamber condenses beneath the outer cover or hood and falls back upon the pan over the chamber in which the steam is generated. The bottom of this pan is perforated with three small holes, which allow the water of condensation to return into the chamber where the steam is generated. The sterilizer, therefore, to a certain extent, supplies itself with water, although not by any means perfectly. It is, however, less likely to boil dry than other forms of sterilizers, and it has the ad-

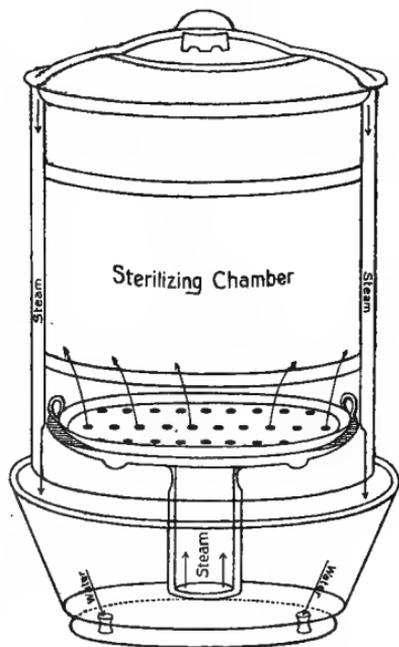


FIG. 30.—Diagram of the Arnold steam sterilizer.

vantage of being reasonably cheap and quite effective. The space inclosed by the hood also serves as a steam-jacket and helps to prevent fluctuations in temperature. A great improvement upon the ordinary Arnold sterilizer is the modification of it devised by the Massachusetts Board of Health.

In the use of this, or any form of steam sterilizer, the time is noted from the period when boiling is brisk and it is evident that the sterilizing chamber is filled with hot steam; or, what is better,

when the thermometer registers  $100^{\circ}$  C., if the sterilizer be provided with a thermometer. With a large Arnold sterilizer a temperature of  $100^{\circ}$  C. may not be reached until it has been heated with a rose-burner for twenty to thirty-five minutes. When bulky articles or large amounts of material are to be sterilized, allowance must be made for the time necessary to bring the temperature in the middle of the mass to  $100^{\circ}$  C.

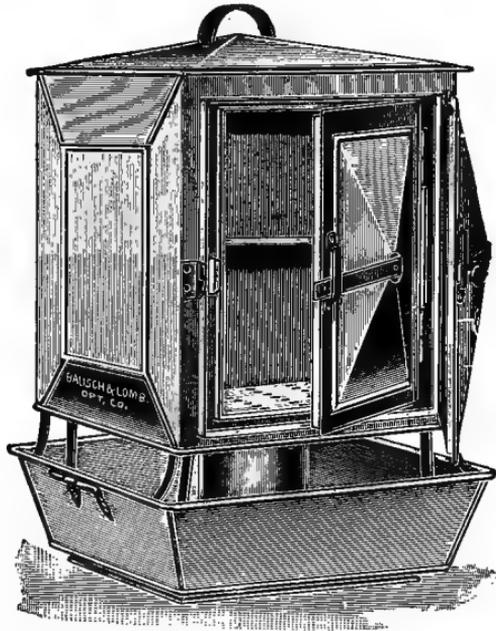


FIG. 31.—Steam sterilizer, Massachusetts Board of Health.

*Autoclave Sterilization.*—Sterilization in the presence of moisture and at temperature above  $100^{\circ}$  C., requires a pressure greater than that of the atmosphere and the apparatus used for this purpose is known as the autoclave. All bacteria and their spores are killed by heating at  $110^{\circ}$  C., in the presence of water, for fifteen minutes, and in about five minutes at  $120^{\circ}$  C. The steam pressures corresponding to these temperatures are approximately  $7\frac{1}{2}$  pounds and 15 pounds per square inch or  $\frac{1}{2}$  kilogram and 1 kilogram per square centimeter, respectively. The autoclave

consists of a metal cylinder with a movable top, which is fastened down tightly during sterilization. It is furnished with a pressure gauge, a stop-cock, and a safety-valve which is set to allow the steam to escape when the desired pressure is attained and thus prevents it from running too high. Heat is furnished by a gas-burner underneath. The lower part of the cylinder contains water. The objects to be sterilized are supported above this water on a perforated bottom or shelf.

It is necessary to observe certain precautions in the use of the autoclave. Allusion has already been made to the necessity for having the steam saturated with moisture. This is effected by allowing the air to escape after the heat is applied, and in order to be sure that all the air has really been expelled, the stop-cock, with which all autoclaves are provided, is left open until the steam escapes freely. The stop-cock is then closed, and the pressure begins to rise. After leaving the articles to be sterilized in the autoclave for the length of time desired, the apparatus must not be opened while the steam contained within it is still under

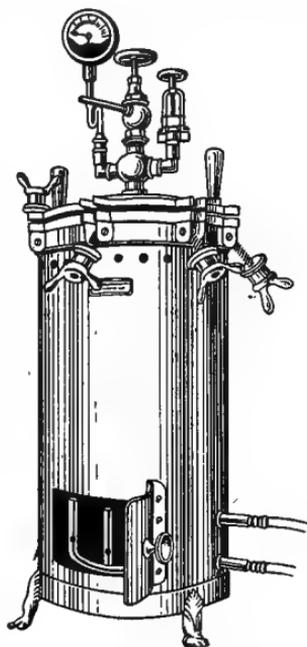


FIG. 32.—Autoclave.

pressure, as there may be a sudden evolution of steam upon the removal of the pressure which may blow the media out of their tubes and flasks. After the pressure has fallen to zero it is well to open the stop-cock only a little way so that air may not be drawn in too rapidly to replace the condensing steam. The autoclave may be opened as soon as the internal and external pressure become equal.

The length of exposure necessary to accomplish sterilization in the autoclave depends upon the protection which the article to be sterilized affords the bacteria. In sterilizing agar, a con-

siderable interval elapses before the agar becomes liquified, especially if it be in large flasks, and it is well to allow 30 to 35 minutes at  $110^{\circ}$  C., for its sterilization. Closely packed surgical dressings serve to protect the interior, and considerable time may be required for penetration of a sterilizing temperature into such packages. In such instances it is unwise to rely upon the gauge as an indicator of the temperature throughout the materials being sterilized. It is well to test the efficiency of the sterilization from time to time by enclosing test objects in the center of several packages. A convenient test object for surgical autoclaves may be made by spreading spores of *B. subtilis* or *B. vulgatus* on a sterile cover-glass and placing it in a sterile test-tube plugged with cotton, and then drying the preparation thoroughly in the incubator for 24 hours. A number of these may be prepared and subsequently kept in the refrigerator until used. After the test object has been exposed in the autoclave, sterile broth is added to the tube by means of a capillary pipette. The development of culture from the spores indicates lack of efficiency in the process of sterilization.

*Discontinuous or fractional sterilization* by moist heat is employed to sterilize certain kinds of culture media, more especially blood serum and gelatin, which are likely to be injured by heating above  $100^{\circ}$  C., or by prolonged heating. In this method the medium is exposed to a temperature deemed sufficient to kill the vegetative forms of bacteria but not the spores. An interval is then allowed for the generation of these spores, whereupon the heat is again applied. This sequence is repeated until, according to past experience, sterilization may be regarded as almost certainly accomplished. In the case of gelatin, steaming ( $100^{\circ}$  C.) for 15 to 20 minutes on three consecutive days is the usual practice; with inspissated serum, exposure for 1 hour at  $60^{\circ}$  to  $70^{\circ}$  C. on six successive days is usually sufficient. These methods are applicable only to media in which spores may germinate and they may fail to sterilize even in case of such materials, especially in the presence of rapidly growing spore-producing bacteria

and when there are spores of anaerobic bacteria in the material to be sterilized. On this account, materials sterilized in this way should not be injected into patients.

*Electricity* has little or no direct demonstrable germicidal action. An electric current may generate sufficient heat to kill bacteria, or it may produce powerful germicides by electrolysis, such for example as acids and alkalies.

**Chemical Agents.**—Sterilization by means of chemicals is not employed in the preparation of culture media because of the difficulty of removing the added substance after the desired effect has been obtained. It is necessary in every case to consider the other effects which the use of chemical germicides entails, and their usefulness is therefore somewhat more limited than that of the physical agents for sterilization. Their efficiency is also subject to great variation according to the nature of the materials with which they come in contact. Nevertheless they have a very important place in practical sterilization and disinfection.

The common *soaps*, and more particularly green soap, have a slight germicidal value, and this in conjunction with their solvent action upon fats and protein, and the mechanical cleansing which accompanies their use, justifies assigning them an important place among the chemical disinfectants.

*Acids*, especially those which are strongly dissociated, are powerful germicides. Hydrochloric acid apparently owes its power entirely to its acidity, and in fairly weak solution, 0.2 to 1.0 per cent, it kills vegetative bacteria in a short time. Strong sulphuric acid actually carbonizes organic matter, while nitric acid oxidizes and also forms special combinations with protein, the reactions resulting in death of living protoplasm. Sulphurous acid (sulphur dioxide) also possesses marked germicidal properties, probably due to oxidation effects.

*Sulphur dioxide* gas has been employed extensively in the fumigation of rooms, and is usually prepared by burning sulphur. Much difference of opinion exists regarding the value of it as a disinfectant. The spores of anthrax are not killed by several

days' exposure to the liquefied gas. Anthrax and other vegetative bacilli are destroyed in thirty minutes when exposed on moist threads in an atmosphere containing one volume per centum of the gas. An exposure of twenty-four hours in an atmosphere containing four volumes per centum of the gas will destroy the organisms of typhoid fever, diphtheria, cholera and tuberculosis. The presence of moisture greatly enhances the activity of the disinfectant, owing to the formation of the more energetic sulphurous acid.

For the destruction of insects, such as mosquitoes, this agent is superior to formaldehyde. Its application for this purpose is important in preventing the spread of yellow fever and malaria.

In practice, at least 3 pounds of sulphur per 1000 cubic feet should be used, and moisture must be present. This latter requirement can be fulfilled by evaporating several quarts of water within the tightly closed room just prior to generating the gas. In using powdered or flowers of sulphur, the necessary amount is placed on a bed of sand or ashes in an iron pot, which should rest on a couple of bricks in a pan or other vessel containing an inch or two of water. The sulphur is ignited by means of some glowing coals, or by moistening with alcohol and applying a match. Difficulty is often experienced in keeping the sulphur burning, and for this reason it is surer and more convenient to use the so-called sulphur candles. In operating with these, a sufficient number are placed on bricks in a pan of water and the wicks lighted. Liquefied sulphur dioxide may be used, and can be obtained in convenient tin receptacles containing a sufficient quantity for the disinfection of an ordinary room. The can is opened by cutting through a soft metal tube projecting from the top. The fluid vaporizes at the room temperature, and it is simply necessary to place the can in a convenient porcelain dish and allow the fluid to evaporate.

Sulphur dioxide is objectionable on account of its lack of germicidal power when dry, and on account of its corrosive action on metal and its bleaching effect on hangings and draperies in the

presence of moisture; it is, therefore, preferable to use formaldehyde for room disinfection when possible.

*Alkalies*, especially the caustics, sodium hydroxide and potassium hydroxide, are powerful germicides. Commercial lye is also valuable as a disinfectant. Perhaps the most important of the alkalies is calcium hydroxide,  $\text{Ca}(\text{OH})_2$  which, because of its low cost, is extensively used for the disinfection of excreta.

*Lime*.—The addition of 0.1 per cent of unslaked lime to fluid cultures of the typhoid bacillus and cholera spirillum will render them sterile in four or five hours. Typhoid dejecta are sterilized in six hours when thoroughly mixed with 3 per cent of slaked lime; the addition of 6 per cent will accomplish the same result in two hours. A convenient form for practical use is an aqueous mixture containing 20 per cent of lime—so-called milk of lime. Typhoid and cholera dejecta are sterilized in one hour after mixing with 20 per cent of this mixture. In practice it is safer to use a considerable excess of lime. From the foregoing facts it would seem probable that lime or whitewash as ordinarily applied would possess disinfectant properties. Experimental work has demonstrated this to be a fact. The organisms of anthrax, glanders and the pus cocci are destroyed within twenty-four hours by one application. For spore-forming organisms and the bacillus of tuberculosis the power is not so great, the latter organism not being destroyed by three applications of the whitewash. Practically, whitewashing is an effective means of disinfecting woodwork, perhaps because those microbes which are not killed at once are caught in the whitewash and their further distribution prevented.

*Oxidizing agents* are usually germicidal. Chlorine, bromine and iodine, ozone, nitric acid, potassium permanganate, chlorinated lime, organic peroxides and peracids, and hydrogen peroxide, belong to this class. Chlorine, employed as chlorinated lime, is a valuable disinfectant for excreta. In the form of bleaching powder it has been extensively used in the disinfection of drinking water and of swimming pools. Liquid chlorine is also employed

for the same purpose. Bromine and iodine have long been employed in surgery, and solutions of iodine are often applied to the skin before surgical incision. Iodine probably acts to some extent as a germicide in this instance, but also as an antiseptic, remaining in the skin for some time after its application. Hydrogen peroxide is a germicide, as it quickly decomposes to form water and oxygen. It is placed on the market in solutions varying in strength from 10 to 30 volumes, the mode of expression indicating that corresponding solutions will liberate ten to thirty times their volume of oxygen when appropriately treated. It decomposes rapidly when in contact with purulent secretions, setting free abundant oxygen, and on this account is used for cleansing infected wounds. It deteriorates in strength so rapidly that only fresh solutions of known strength should be used.

*Potassium Permanganate.*—Koch asserts that a 3 per cent solution will destroy anthrax spores in twenty-four hours, but that a 1 per cent solution cannot be depended upon to kill pathogenic organisms. Its disinfectant value in practice is very low on account of its ready decomposition by inert material. In the dilute solutions usually used for medicinal injections and irrigations no disinfectant action occurs.

*Iodoform.*—This substance possesses little if any disinfectant power. It is mildly antiseptic in moist wounds, due to the gradual liberation of small quantities of iodine.

*Inorganic Salts.*—Mercuric chloride,  $HgCl_2$ , is probably more commonly used than any other one germicide. But Geppert, whose work in this direction has been abundantly corroborated by others, found that the potency of corrosive sublimate as a germicide had been greatly overrated. The inhibitory action of corrosive sublimate, on the other hand, is very great, and the veriest trace of it left adhering to the bacteria is sufficient to prevent them from growing. Corrosive sublimate is difficult to remove by ordinary washing and traces of it remain even after very thorough washing. But if the last traces are removed by treatment with ammonium sulphide or other reagents which pre-

precipitate the mercury salt without themselves injuring the bacteria, growth takes place even where the corrosive sublimate solutions have been used which are apparently efficacious. Thus anthrax spores will not grow in culture media when they are exposed for even a few minutes on silk threads to the action of corrosive sublimate solution of the strength of  $\frac{1}{10}$  per cent and then washed thoroughly in water and rinsed in alcohol; but Geppert showed that the spores so treated were only apparently killed, for it took twenty hours' exposure to corrosive sublimate solution of this strength where the spores were not dried on silk threads, but suspended in water, and where the last trace of corrosive sublimate was removed by treatment with ammonium sulphide. It is claimed that its affinity for albuminous bodies and the readiness with which it combines with such substances detract from its value for some purposes. On the other hand, many observers claim that the albuminous combinations formed under such circumstances are soluble in an excess of albuminous fluid, and that its value as a germicide is not affected thereby. To obviate this possible difficulty it is customary in practice to combine the bichloride of mercury with some substance that will prevent the precipitation of the mercury salt by albumin. For this purpose 5 parts of any one of the following substances to 1 part of bichloride of mercury may be used—hydrochloric acid, tartaric acid, sodium chloride, potassium chloride, or ammonium chloride. A very practical stock solution for laboratory purposes has the following composition:

Hydrochloric acid.....	100 c.c.
Bichloride of mercury.....	20 grams.

Five c.c. in a liter of water makes a solution of about 1-1000 strength.

*Mercuric Iodide.*—An extremely high antiseptic value has been placed on this substance by Miquel, who claims that the most resistant spores are prevented from developing in a culture medium containing 1-40,000. In combination, as potassium-mercuric iodide, it has been used in soaps (McClintock) with very

favorable results. The substance is not extensively employed and further investigation is necessary to determine its true value.

*Silver Nitrate.*—This salt probably occupies the position next to the bichloride of mercury in germicidal power. Behring claims it to be superior to bichloride of mercury in albuminous fluids. The anthrax bacillus is killed by a solution of 1–20,000 after two hours' exposure. At least forty-eight hours' exposure to a 1–10,000 solution is required to kill the spores of anthrax. It is very irritating, and possesses strong affinities for chlorides, forming with them insoluble chloride of silver, a salt without germicidal value. For these reasons the use of silver nitrate is limited. In the solutions usually employed for douching the cavities of the body the available silver nitrate is immediately converted into the insoluble chloride, and little if any germicidal action takes place. To this fact may be ascribed the varying clinical results reported.

Many proprietary silver compounds are on the market, introduced to replace the nitrate and its objectionable features. The most important are protargol and argyrol, organic silver combinations. They do not combine with chlorides, are less irritating than the nitrate and, not coagulating albumin, they possess greater penetrating power.

**Organic Poisons.**—Carbolic acid is one of the most important and most widely used disinfectants. It is usually employed in strengths of from 1 to 5 per cent. A 3 per cent solution will sometimes kill the spores of anthrax after two days' exposure. In the absence of spores, the anthrax bacillus is destroyed by a 1 per cent solution in one hour. The less resistant pus cocci are destroyed rapidly by a 2 per cent solution. Combination with an equal proportion of hydrochloric acid enhances the efficacy of carbolic acid to a marked extent. This is due to the prevention of albuminous combinations, thus allowing greater penetration of the disinfectant.

Many other substances closely related to carbolic acid are used and possess marked germicidal properties. Among them

may be mentioned creolin, cresol and lysol. They are all slightly superior to carbolic acid in actual germicidal value.

*Formalin* is a 40 per cent aqueous solution of formaldehyde,  $H_2CO$ . Numerous investigations have shown it to possess, both in the liquid and gaseous forms, remarkable disinfecting power under certain conditions. In solutions of 1-1000 an exposure of twenty-four hours is necessary to destroy the staphylococcus pyogenes aureus, while 1-5000 is sufficient to restrain its growth (Slater and Rideal). Its use in a gaseous form as a house disinfectant is by far the most important application at the present time.

From 250 to 500 c.c. of formalin together with 500 to 1000 c.c. of water should be vaporized for each 1000 cubic feet of air space in the room, and the room should remain tightly closed for at least four hours, preferably over night. Many methods of vaporizing formaldehyde have been devised. Some form of tank, provided with heating apparatus and with an outlet tube which passes through the keyhole into the room, is perhaps the most convenient, where much disinfection has to be done. If apparatus of this sort is not at hand, good results may be obtained by putting the formalin and the water previously heated to boiling, in a large pail in the center of the room, and then adding rapidly crystalline potassium permanganate, about 200 grams to each 500 c.c. of formalin used. The permanganate oxidizes some of the formaldehyde and produces heat to evaporate the rest of it. From 25 to 50 per cent more formalin should therefore be used for a given air space. It is well also to add about 10 per cent of glycerin to the water so as to raise the boiling-point somewhat and insure more complete vaporization of the formaldehyde.

Formaldehyde penetrates very slightly beneath exposed surfaces so that everything to be disinfected should be completely exposed. Openings about windows and doors should be carefully plugged up and sealed with strips of paper. Mechanical cleansing supplemented by application of 1-1000 solution of mercuric chloride to floors and walls should follow the fumigation. The

persistent odor of formalin may be removed by fumes of ammonia.

*Aniline Dyes.*—Many of the aniline dyes, notably pyoktanin (methyl-violet), possess germicidal properties. Malachite green is said to possess even greater germicidal value than pyoktanin. Methylene blue also possesses considerable germicidal power.

*Alcohol* is a germicide of moderate power. It has little effect upon spores but in concentrations of from 50 to 95 per cent it destroys vegetative bacteria in a few minutes.

Germicides destroy bacteria, as a general rule, because they are general protoplasmic poisons, destructive to all living matter. There is, nevertheless, some selective action. Thus, formaldehyde kills bacteria but has little poisonous effect upon insects, such as mosquitoes, bedbugs, roaches or fleas. Mercuric chloride is rapidly fatal to bacteria when it comes into contact with them, but it has no very immediate destructive effect upon fly larvæ (maggots). Some of the oxidizing agents, such as hydrogen peroxide and acetozone are not poisonous to man because they are decomposed into relatively harmless substances before they can be absorbed. Attempts to discover or to produce chemicals which would exhibit a selective destructive effect upon microbes in the interior of the body have not met with much success. Quinine is perhaps the best known example, as it may circulate in the blood in sufficient concentration to poison the malarial parasites without at the same time killing the host. The effects produced by mercury and by salvarsan in syphilis are perhaps analogous, but they evidently depend to a large extent upon a special susceptibility of the microbe, a susceptibility not yet apparent in most parasites. The specific immune substances may perhaps be classed in the same category. These will be considered in more detail in a later chapter.

**Antiseptics.**—Antiseptic and preservative agents prevent or delay the development of bacteria, without killing them. Very much the same agents are applied to prevent the growth of microbes in living tissues and consequent poisoning of the body

(antiseptis) as in preventing microbic development in dead organic matter (food preservation).

Of the physical antiseptics, desiccation and cold are perhaps of greatest importance. These agencies find application to the living body as well as in preservation of dead material. Substances which increase osmotic pressure, sodium chloride and sugar, are also employed to prevent microbic growth in foods.

The chemical antiseptics are very numerous. In general a germicide in higher dilution exhibits antiseptic effect. Small quantities of the inorganic acids, hydrochloric, nitric, sulphuric or sulphurous acid, prevent bacterial growth. Even boric acid, which has little or no germicidal effect, will delay or inhibit microbic development. Many organic acids possess inhibitive properties toward bacterial action. Acetic and lactic acids probably act merely by virtue of their acidity. Benzoic and salicylic acids seem to be more antiseptic, probably by virtue of other structural features in their molecules. Other organic substances, such as phenol (carbolic acid) and formaldehyde in high dilutions prevent or delay bacterial growth, and weaker germicides such as alcohol, chloroform or ether, are fairly effective preservatives. Oxidizing agents often decompose too rapidly to be of much value as antiseptics. Iodine, however, is one member of this group having considerable antiseptic value.

Of the inorganic salts, mercuric chloride is most important. Small quantities of this agent inhibit the multiplication of bacteria. It is extensively employed in antiseptic treatment of wounds. The borates, nitrates and salicylates, the latter compounds of an organic acid, also inhibit bacterial action to some extent.

In using these substances as antiseptic applications to wounds, the possible poisonous effects upon the body as a whole from absorption of the antiseptic must be kept in mind. Moreover, such substances ought not to be used as food preservatives without due regard to the changes they may induce in the food and the possible effects they may exert upon the consumer.

## TESTING ANTISEPTICS AND DISINFECTANTS

The determination of the antiseptic value of a material is a comparatively simple matter. A virulent culture of the organism used as a test is inoculated into sterile bouillon containing a known quantity of the antiseptic. The process is repeated with varying strengths of the material until the smallest quantity of it capable of preventing growth is determined. This dilution may be considered the antiseptic value of the material in question for the organism used, under the conditions of the test.

Determination of the disinfectant power of a substance is a problem of much greater magnitude, and the method used must be altered more or less to suit the properties of the substance tested. It is obvious that some of the substance tested remains in contact with the organisms in the method given for determining the antiseptic value, and that we do not know whether the bacteria are alive and merely inhibited in growth, or actually killed.

The chemical composition of the medium in which the bacteria are tested may have a marked influence upon the action of germicides. If components of the medium enter into chemical union with the germicide there may be an inert compound formed. There may also be formed dense, flocculent precipitates which envelop the bacteria and protect them from the action of the germicide. It is therefore apparent that the potency of a germicide may appear very different when acting upon the bacteria in water or in physiological salt solution or on bacteria dried on glass rods or on silk threads, on the one hand, and upon the same bacteria in beef broth or in feces or in urine, on the other. For these reasons it is not always possible to draw conclusions from the results of laboratory experiments as to the value of a germicidal agent for practical disinfecting purposes.

*Method.*—To 15 c.c. of sterile water in a 60 c.c. Erlenmeyer flask add 2 c.c. of a virulent culture of the test-organism. Then add a solution of the substance under investigation in the pro-

portion necessary to give the dilution wished. Mix thoroughly, and allow this "action-flask" to stand as long as it is desired to have the germicide in contact with the test-organism (action-period). Transfer 0.5 c.c. from the action-flask to a flask containing 200 c.c. of a solution of some chemical capable of decomposing the substance being tested with the formation of inert or insoluble compounds. In this "inhibition-flask" the strength of the solution should be such that molecular proportions of the chemical are present in sufficient quantity to combine with all the germicide carried over. The inhibition-flask is shaken for 30 seconds, and 1 c.c. transferred from it to 100 c.c. of sterile water in another, the "dilution-flask." After two minutes, three agar tubes are inoculated with 1 c.c. each from the dilution-flask, plated, and growth watched for.

Control-experiments should be performed to determine that the dilution of the test-culture is not too great when carried through the three flasks. It likewise should be determined that the inhibiting chemical has no effect on the bacteria.

What the inhibiting chemical shall be must be determined for each individual case. For salts of the heavy metals ammonium sulphide answers well; for mercury salts, stannous chloride may be used; for formaldehyde, ammonium hydrate; for carbolic acid, sodium sulphate.

The testing of gaseous disinfectants, such as sulphur dioxide and formaldehyde, must be conducted under conditions as nearly parallel to actual practice as possible. The test-organisms may be exposed on threads or cover-glasses, and acted upon by a known volume strength of disinfectant for a known length of time. Subsequent treatment of the organisms with a suitable inhibitor is necessary when possible, and, should growth occur in the cultures following, the test-organism should be recognized in order that possible contamination by extraneous organisms may be excluded.

In determining the value of germicides for sterilizing ligatures, the students can apply methods based on the foregoing principles.

Great care and ingenuity are necessary to arrive at correct conclusions, particularly in the case of animal tendons. In many instances quite stable compounds are formed between tendon and germicide, and living organisms may be so imbedded in such a substance that subsequent growth in a test-culture is impossible. The use of a suitable inhibitor, and, prior to final culture-tests, a prolonged soaking in sterile water, will promote the accuracy of the results.

## CHAPTER III

### CULTURE MEDIA

Culture media are substances in which microbes are artificially cultivated. The variety of such substances is very large, different materials being suited to different purposes. Particular kinds of media have been devised in order to bring to development or especially to favor the development of certain kinds of microbes. Various media are also used to demonstrate the physiological properties of bacteria, especially the physical arrangement of the bacterial cells as they grow under various conditions, and the chemical changes induced in the various constituents of the media by the microbic growth.

**Glassware.**—Micro-organisms are usually grown in glass test-tubes or sometimes in glass flasks. The tubes and flasks should be of more durable glass than those ordinarily used in chemical work, but heavy tubes of glass of poor quality are not to be recommended. For ordinary purposes, test-tubes  $125 \times 15$  mm. are convenient. Larger tubes,  $150 \times 20$  mm., are used to store media to be used in making plate cultures and for roll-tube cultures. New glassware should be thoroughly washed before using, and for critical work it should be boiled in dilute sodium carbonate, rinsed, washed in dilute hydrochloric acid, rinsed repeatedly in running water, finally in distilled water, and then inverted to drain in a warm place, such as the incubator, until perfectly dry. Used glassware should be sterilized in the autoclave at  $120^{\circ}$  C. for half an hour, emptied, cleaned with a swab and hot water, rinsed in distilled water and drained. In case of special difficulty the glassware may, after emptying and washing in water, be cleaned by soaking in a special cleaning fluid, and all organic matter may be readily removed by using this

fluid hot. It should not come into contact with the hands or with any large quantity of organic matter.

#### CLEANING FLUID

Potassium or sodium bichromate.....	40 grams.
Water.....	150 c.c.
Dissolve the bichromate in water, with heat; allow it to cool; then add, carefully, con- centrated commercial sulphuric acid.....	
	230 c.c.

Exact proportions are not necessary in making this fluid. Glassware cleaned in it must be repeatedly rinsed subsequently.

**Plugs.**—The clean dry tubes or flasks are plugged with raw cotton of a good grade which does not char too readily upon heating. The cotton plugs may be carefully made by rolling an oblong rectangular strip, of even thickness, into a firm cylinder of proper size, *rolled plugs*, or more hastily made by stuffing the cotton into the open end of the flask or tube, *stuffed plugs*. The latter kind of plug serves very well for tubes in which media are to be stored temporarily but is not so satisfactory for other purposes.

**Sterilization.**—After plugging, the tubes are placed in a wire basket and sterilized in the hot-air sterilizer or, sometimes, to avoid charring, in the autoclave. This not only renders the glassware free from bacteria but also gives more permanent form to the plugs.

#### THE COMMON CULTURE MEDIA

**Broth.**—Broth, bouillon or beef-tea, is best made from fresh meat, either beef, veal or chicken. Finely chopped lean meat, 450 to 500 grams, is mixed with 1000 c.c. of distilled water and either allowed to stand over night in the refrigerator or else digested for half an hour at temperature of 50 to 55° C. It is then strained through muslin, yielding a filtrate of deep red color. Any excessive amount of fat should be skimmed off. To the filtrate, which should measure 1000 c.c., are added:

Peptone <sup>1</sup> .....	10 grams.
Sodium chloride (common salt).....	5 grams.

These should be dissolved by stirring at a temperature below 60° C. The mixture is then boiled for half an hour over the direct flame, cooled slightly, and filtered through paper previously wet with warm water. The filtrate should be clear and light yellow in color, and should be diluted to 1000 c.c. with distilled water. Its reaction is acid, a reaction unfavorable to the growth of many bacteria, especially to many pathogenic forms.

The amount of alkali to be added is ascertained by titration. For this purpose exactly 5 c.c. of the broth is placed in each of three test-tubes. Five-tenths cubic centimeters of a 5 per cent solution of purified litmus (Merck's highest purity) is added to each tube. An accurately prepared  $\frac{N}{20}$  solution of sodium hydroxide<sup>2</sup> is then run in drop by drop from a graduated burette, the reading of which has been recorded, into one of the tubes until the red color just changes to blue. The burette reading is taken and recorded. The alkali is then run into the second tube rather rapidly until the endpoint ascertained by the first test is nearly reached. By comparing the color of this tube with that of the first one and with the third to which no alkali has yet been added, the exact point at which the color is changing from red to blue may be accurately judged. When this point is reached, the burette reading is again recorded and the amount of alkali necessary to neutralize the 5 c.c. of broth ascertained. The third tube should then be titrated to confirm the previous result. The titration of the broth should now be repeated, using phenolphthalein as an indicator. For this purpose, 5 c.c. of the medium is transferred to a small porcelain dish, diluted by the addition

<sup>1</sup> Commercial peptones are mixtures of albumoses and a small amount of peptone.

<sup>2</sup> A normal solution of sodium hydroxide contains one gram-molecule of anhydrous NaOH, or 40 grams, in a liter. The  $\frac{N}{20}$  solution contains  $\frac{1}{20}$  of this amount or 2 grams in a liter.

of approximately 45 c.c. of distilled water, and boiled for a minute. 1 c.c. of a 0.5 per cent solution of phenolphthalein in 50 per cent alcohol is now added and  $\frac{N}{20}$  solution of sodium hydroxide run in from the burette until the color changes to a faint but distinct and permanent pink color. The burette reading is recorded and the amount of alkali\* necessary to neutralize the 5 c.c. of medium in respect to phenolphthalein thus ascertained. This titration may well be repeated, especially by beginners. As a result of these titrations we shall have ascertained the amount of alkali necessary to neutralize the remaining broth to either indicator. For example suppose that 5 c.c. of the broth titrated as follows:

0.5 c.c. of  $\frac{N}{20}$  alkali with litmus as indicator.

2.0 c.c. of  $\frac{N}{20}$  alkali with phenolphthalein as indicator

In order to neutralize the remaining 980 c.c. of broth to litmus would require  $\frac{980 \times 0.5}{5}$  or 98 c.c. of  $\frac{N}{20}$  alkali. A solution of alkali twenty times as strong as this, namely normal sodium hydroxide, is employed for this purpose, and only  $\frac{98}{20}$  or 4.9 c.c.

of this are necessary to neutralize the 980 c.c. of broth to litmus. The reaction generally required for pathogenic bacteria is slightly alkaline to litmus and for this reason an excess of 10 c.c. of normal alkali per liter is added to the broth, 9.8 c.c. for the 980 c.c., making altogether 14.7 c.c. to be added. Calculation from the result obtained with phenolphthalein in the same way shows that 19.6 c.c. of normal alkali would be required to neutralize the medium to this indicator. The desired final reaction of the medium in respect to phenolphthalein is acid, usually that of 5 to 15 c.c. of normal acid per liter, or 0.5 to 1.5 per 100 c.c., or 0.5 to 1.5 per cent, as it is commonly expressed after Fuller.<sup>1</sup> In this instance,

<sup>1</sup> Fuller. *Journal of Amer. Public Health Assoc.*, 1905.

therefore, 5 to 15 c.c. per liter, or 4.9 to 14.7 c.c. less than the 19.6 for the 980 c.c., would be added, namely 14.7 to 4.9 c.c., according to the purpose for which the broth is to be used.

The amount of normal alkali finally decided upon is added to the broth, which is then weighed in its pan. It is then cooked by boiling over the direct flame for half an hour or by heating in the autoclave at 110° C. for 15 to 20 minutes. It is now cooled to about 50° C., filtered through paper, filled into tubes and sterilized, either in the autoclave at 110° C. for 15 minutes or by fractional sterilization in streaming steam at 100° C. for 15 minutes on three consecutive days.

Broth may be prepared from meat extract instead of meat. Meat extract 3 grams, peptone 10 grams and salt 5 grams are dissolved in 1000 c.c. of water, boiled, filtered and titrated against  $\frac{N}{20}$  sodium hydroxide. The subsequent steps are the same as in preparation of broth from fresh meat.

*Remarks upon Titration.*—The titration of bacteriological media made from meat or meat extract is an important step in their preparation. There is some confusion on this point because of the use of different indicators in ascertaining the reaction. The neutral point indicated<sup>1</sup> by litmus is very nearly the actual neutral point in respect to acidity and alkalinity, and this point is not appreciably displaced in either direction by the addition of a neutral mixture of a feebly dissociated acid and its salts to the solution. The end reaction indicated by phenolphthalein when it turns pink is actually a point at which there is a slight excess of alkali. This is so nearly the actual neutral point in inorganic solutions, when electrolytic dissociation is marked, that the error is not appreciable. In solutions of organic substances, especially when considerable amounts of feebly dissociated substances, such as are contained in peptone or gelatin, are present,

<sup>1</sup> Washburn, E. W., The significance of the term alkalinity in water analysis and the determination of alkalinity by means of indicators. Report Illinois Waterworks Association, 1911.

this error becomes very appreciable. The discrepancy between the end point for litmus and for phenolphthalein will vary for different lots of media. Another source of error and misunderstanding arises from the fact that the reaction of a medium changes somewhat after its neutralization, especially during sterilization, but also upon standing afterward at ordinary temperature. This change is toward decreased alkalinity and increased acidity and its extent is not the same for different media, being most marked, perhaps, in those rich in glucose. Where particular importance is attached to the titre of a medium, it is well, therefore, to determine this upon a sample of the medium taken from the lot at the time it is used, rather than to quote figures obtained before sterilization. The optimum reaction for most microbes is very close to the neutral point for litmus and for most pathogenic bacteria slightly alkaline to this indicator.

A clear understanding of electrolytic dissociation and the measurement of acidity in terms of hydrogen ion concentration, requires a knowledge of the elementary principles of physical chemistry, which the beginning student of bacteriology may not possess. Inasmuch as it is customary to use such terms as  $P_H = 7.0$  to indicate that a medium possesses neutral reaction,  $P_H = 6.0$  for a definite slight acidity, and  $P_H = 8.0$  for a definite slight alkalinity, the student should be acquainted with them.

When a crystalline salt, such as NaCl, is mixed with a quantity of a solvent insufficient to dissolve all of it, the salt exists in three states, (1) undissolved crystalline residue, (2) undissociated salt in solution as NaCl and (3) electrolytically dissociated salt in solution as the dissociated electrically charged ions,  $\text{Na}^+$  and  $\text{Cl}^-$ . If all of the solid substance is dissolved then it exists in the states indicated as (2) and (3). The speed of chemical reaction into which such a solution will enter depends upon the concentration of the ionized portion. In the same way an acid, such as HCl, in solution in water is more or less completely ionized into  $\text{H}^+$  and  $\text{Cl}^-$  and the reactivity or chemical strength of such a solution is measured by the concentration of the ions and its

acidity by the concentration of the hydrogen ion. Hydrogen ion concentration, therefore, is the physical measure of acidity.

Pure water is in part a solution of dissociated  $H^+$  and  $OH^-$  in undissociated  $H_2O$ . Roughly the actual amount of dissociated  $H^+$  in a liter of water is 0.000,000, 1 gram and the dissociated  $OH^-$  is 0.000,001, 7 gram, in each instance representing a concentration of  $\frac{1}{10,000,000}$  Normal or Normal  $\times 10^{-7}$ , which is Normal  $\log - 7.0$ .

Sørensen and others after him have employed the symbol  $P_H$  to signify the logarithm of the reciprocal of the hydrogen ion concentration, thus omitting the minus sign, so that in pure water  $P_H = 7.0$ . In a watery solution the concentration of hydrogen ions  $\times$  the concentration of hydroxyl ions = a constant which is

$\frac{1}{100,000,000,000,000}$  Normal or Normal  $\times 10^{-14}$ . As hydrogen ion

concentration increases, hydroxyl ion concentration diminishes. Thus a Deci-normal NaOH has an  $H^+$  concentration of approximately Normal  $\times 10^{-13}$ . This is expressed on Sørensen's scale as  $P_H = 13$ . Conversely Deci-normal HCl has an  $H^+$  concentration of about Normal  $\times 10^{-1}$  or  $P_H = 1$ .

In the presence of feebly dissociated compounds, phosphates, salts of organic acids and especially proteins, a large fraction of the hydrogen ions, introduced by the addition of a mineral acid, are quickly combined in the undissociated state. The same fate is met by hydroxyl ions introduced. The relatively lesser degree of dissociation in such solutions therefore requires the addition of greater amounts of acid or of alkali in order to bring about a definite alteration in chemical reaction or measured alteration in hydrogen ion concentration. This property of such solutions is spoken of as a buffer effect. Obviously bacteriological media are such as to exhibit this property in a conspicuous manner and to a different degree, according to their composition.

Clark and Lubs<sup>1</sup> recommend a series of indicators which change color at various hydrogen ion concentrations and their chart has

<sup>1</sup> Clark, W. M. and Lubs, H. A.: Journ. Bact., 1917, II, 1, 109, 191.

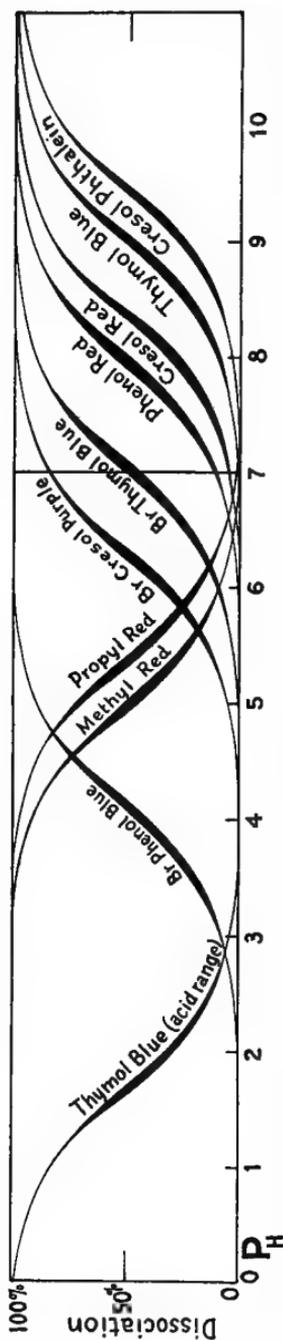


FIG. 33.—Dissociation curves of indicators considered as simple mono-basic acids, showing percentage of color change with  $P_H$ . Shaded portions of curves indicate the useful ranges. (After Clark and Lubbs.)

been used by the Committee<sup>1</sup> on the Descriptive Chart of the Society of American Bacteriologists. To the left of  $P_H = 7$  is the acid range and to the right of 7 the alkaline range. The useful range of each indicator is shown by the heavily shaded portion of the respective curve.

**Gelatin.**—Finely chopped meat, 450 to 500 grams, is mixed with a liter of distilled water and digested on the water bath for half an hour at  $50-55^\circ$ , with stirring. It is then strained through muslin, yielding a filtrate of deep red color, which should be made to equal 1000 c.c. This filtrate is placed in the inner compartment of a double boiler (rice cooker) and to it are added 10 grams peptone, 5 grams sodium chloride and 100 to 150 grams of sheet gelatin of the best quality ("gold label" gelatin). The larger amount of gelatin should be used during warm weather if no low temperature incubator is at hand. These constituents are dissolved by stirring at a temperature below  $55^\circ\text{C}$ . After complete solution, the reaction is titrated as has been described for the titration of broth. From 30 to 50 c.c. of normal alkali are usually required to give the proper reaction

<sup>1</sup> Conn, H. J. and others: Journ. Bact., 1916, IV, 6167.

to a liter of the medium. After this has been ascertained, and the amount added, the medium is thoroughly mixed and then left covered and undisturbed while the water in the outer compartment of the cooker is boiled for an hour. It is well to have boiling water at hand in another receptacle so that the supply in the cooker may be replenished if it gets low, without chilling the medium. The gelatin is now filtered through paper wet with hot water, and should be kept warm during filtration by means of a funnel-heater, or by a steam bath, although these are not indispensable. If it gets cold it may be poured out of the funnel and warmed again in the pan. A portion of the filtrate should be boiled in a test tube over the flame for a minute or two. It should then remain (1) perfectly clear, (2) alkaline to litmus paper, and (3) should solidify on cooling in tap water. After filtration the medium is filled into tubes and sterilized in streaming steam by the fractional method, 20 minutes at  $100^{\circ}\text{C}$ . for 3 consecutive days. Gelatin may be sterilized in the autoclave at  $110^{\circ}\text{C}$ . for 10 minutes, but it should be chilled in cold water at once after removal, and even then its gelatinizing property may be seriously impaired.

In filling gelatin into tubes it is important that the medium should not be spilled on the mouth of the tube or on the cotton plug, as this accident causes the latter to be glued in position. The filling apparatus indicated in Fig. 34 will be found convenient for filling any sort of liquid medium into tubes, and with proper care one may fill tubes rapidly without soiling the mouths of tubes and their cotton plugs.

Gelatin may be made from beef extract. The extract, peptone, salt and gelatin are dissolved at a temperature below  $60^{\circ}\text{C}$ . or

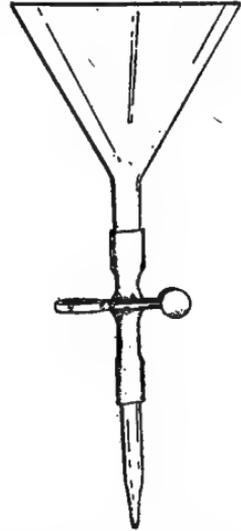


FIG. 34.—Apparatus for filling media into tubes. It is held in a ring-stand support.

the medium is cooled to this temperature after solution has been accomplished. It is titrated and the proper amount of alkali added. An egg is beaten up in water and then stirred into the medium. It is then boiled on the water bath for an hour, filtered, tested, tubed and sterilized.

**Nutrient Agar.**—To a liter of nutrient broth, prepared as above described (page 84) add 15 grams of finely cut agar shreds. Weigh the pan with its contents. Boil the material over the direct flame for one to two hours, with constant stirring to avoid burning, adding hot distilled water from time to time to compensate for the loss by evaporation. Instead of boiling it is convenient to cook the medium in the autoclave at  $110^{\circ}\text{C}$ . for 45 minutes to an hour. In either case, the agar should be very completely dissolved. The medium is then cooled to  $60^{\circ}\text{C}$ . and an egg previously beaten up in water is added and thoroughly mixed with the agar. It is then boiled again for 10 minutes over the free flame, with constant stirring at the bottom, or for 45 minutes on the water bath, or for 15 minutes in the autoclave at  $110^{\circ}\text{C}$ . Distilled water is added to restore the original weight, and the medium is then filtered, usually through a layer of cotton wet with hot water, although filter paper may be used. Filtration is favored by keeping the funnel hot, either with the hot-water funnel heater or in a steam bath, and it may be hastened by the use of suction. The filtrate need not be perfectly clear, and it usually clouds on cooling unless it is acid in reaction. The reaction should be alkaline to litmus. After filling into tubes or flasks, agar should be sterilized in the autoclave at  $110^{\circ}\text{C}$ . for 30 to 35 minutes.

Time may be saved by using the more expensive powdered agar in place of the agar shreds and when a very clear medium is desired the liquid agar may be allowed to sediment in a water bath at  $60^{\circ}\text{C}$ . for some hours before filtration.

**Modifications of the Common Media.**—Broth is made nearly free from sugar by fermenting the meat infusion over night at  $37^{\circ}\text{C}$ . after inoculating it with *B. coli*, and then proceeding with

the filtrate in the usual way. This medium is designated as sugar-free broth. Various sugars or other substances are added to such broth in order to test the ability of bacteria to ferment them. Acetic acid, 0.5 per cent, is added to broth to make a selective medium for acid-resisting bacteria. Glycerin, 5 to 7 per cent, is added to broth for the cultivation of the tubercle bacillus. Naturally sterile ascitic fluid or blood is added to broth to promote the growth of certain types of microbes, and to encourage anaërobes. Bits of naturally sterile tissue are added to broth for similar purposes.

Gelatin is modified by the addition of various sugars, especially dextrose and lactose, often with the further addition of litmus. The production of acid by fermentation of the sugar is at once evidenced by the reddening of the litmus. Glucose litmus gelatin is also a useful medium for anaërobes. It is best to sterilize the litmus separately and add it from a sterile pipette at the time the medium is used.

Agar is modified by the addition of 5 to 7 per cent of glycerin, and such glycerin-agar is used extensively for cultivation of the tubercle bacillus and several other pathogenic bacteria. Various sugars, supplemented by the addition of litmus, are dissolved in agar to test the fermentation properties of bacteria. Glucose agar is extensively employed as such for the cultivation of anaërobes. Agar also forms the gelatinizing base for a number of more or less complex special media.

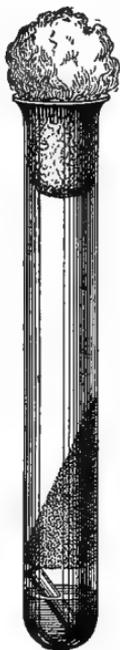


FIG. 35.—  
Potato in  
culture tube.

#### STERILIZABLE SPECIAL MEDIA

**Potato.**—Potatoes were perhaps the first solid medium employed in the cultivation of micro-organisms. Boiled or steamed potatoes kept in a moist place, such as a large covered glass dish, may well be employed as an illustration of primitive technic, and excellent cultures of the common chromogenic bacteria may be

obtained in this way. For most purposes it is better to put pieces of potato in test-tubes where they are more perfectly protected from contamination, as suggested by Bolton.<sup>1</sup> The potato is carefully washed, a slice removed from each end, and a cylinder is cut out with a cork-borer or with a test tube cut off near its bottom. This cylinder is divided diagonally into two pieces. The pieces are washed in running water for twelve to eighteen hours.

They are placed in test-tubes containing a little water to keep the potato moist, and are supported from the bottom on a piece of glass tubing about 1 to 2 cm. in length (or in cotton, or in a specially devised form of tube with a constriction at the bottom). The tubes are plugged, and sterilized in the autoclave at 110° C. for 30 minutes. Potato is best when freshly prepared; it is likely to become dry and discolored with keeping.

**Milk.**—Milk fresh as possible is placed in a covered jar, steamed for fifteen minutes, and then kept on ice for twenty-four hours. At the end of that time the middle portion is removed by means of a siphon. The upper and lower layers must not be taken; the upper part contains cream, and the lower part particles of dirt, both of which are to be avoided. About 7 to 10 c.c. are to be run into each test tube. The tube is plugged with cotton, and sterilized in the autoclave at 110° C. for 30 minutes.

The coagulation of milk, which is accomplished by certain bacteria, is a very valuable differential point. A little litmus tincture may be added to the tubes of milk before sterilizing, until they acquire a blue color, to indicate whether or not acids are formed by the bacteria which are afterward cultivated in the milk. Better results are obtained by sterilizing the litmus solution separately and adding it to the sterilized milk with aseptic technic.

### Dunham's Peptone Solution.

Peptone.....	10 grams.
Sodium chloride.....	5 grams.
Water.....	1 liter.

Boil, filter, sterilize in the usual manner.

<sup>1</sup> Bolton, *The Medical News*, Vol. 1, 1887, p. 318.

Dunham's solution is valuable to test the development of indol by bacteria (see Part II., Chapter VIII.). The development of acids may be detected after the addition of 2 per cent of rosolic acid solution (0.5 per cent solution in alcohol); alkaline solutions give a clear rose-color which disappears in the presence of acids.

**Nitrate Broth.**—Dissolve 1 gram of peptone in 1000 c.c. of distilled water, and add 2 grams of nitrite-free potassium nitrate. Fill into test-tubes, 10 c.c. in each, and sterilize in the autoclave at 110°C. for 15 minutes.

**Blood-serum.**—The blood of the ox or cow may be obtained easily at the abattoir. It should be collected in a clean jar. When it has coagulated, the clot should be separated from the sides of the jar with a glass rod. It may be left on the ice for from twenty-four to forty-eight hours. At the end of that time the serum will have separated from the clot and may be drawn off with a siphon into tubes. These tubes are sterilized for the first time in a slanting position, as the first sterilization coagulates the serum. The coagulation may be done advantageously, as advised by Councilman and Mallory, in the hot-air sterilizer at a temperature below the boiling-point. After coagulation, sterilize in the autoclave at 110° C. for 20 minutes. This serum makes an opaque medium of a cream color. Blood-serum may be more conveniently sterilized in the Koch serum inspissator (Fig. 36). A clear blood-serum is to be obtained by sterilization at a temperature of 58° C. for one hour, on each of six days, if a fluid medium is desired, or of 75° C. on each of four days if the serum is to be solidified. In the latter case the tubes are to be placed in an inclined position. Opaque, coagulated blood-serum has most of the advantages of the clear medium. Blood-serum may be secured from small animals by collecting blood directly from the vessels, and with proper technic may be obtained in a sterile condition; and the serum may be separated and stored in a fluid state. Human blood-serum is sometimes obtained from the placental blood. The preservation of blood-serum is sometimes

accomplished with chloroform, of which 1 per cent is to be added to the medium; in this manner the serum may be preserved for a long time. It may be filled into tubes, solidified and sterilized as required; the chloroform will be driven off by the heat, owing to its volatility. Blood-serum media which are sterilized at low temperatures should be tested for twenty-four hours in the incubator to prove that sterilization has been effective; if it has not, development of the contaminating bacteria will take place and be visible to the eye.

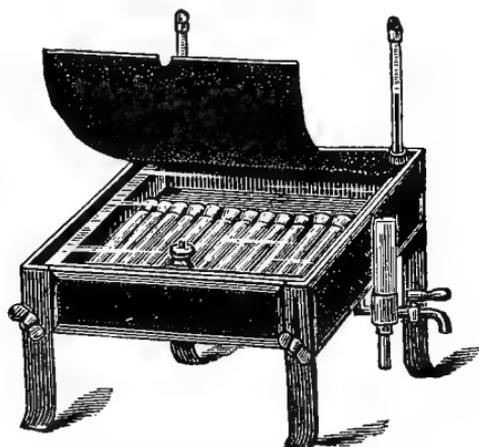


FIG. 36.—Koch's serum sterilizer.

**Löffler's blood-serum** consists of one part of bouillon containing 1 per cent of glucose, mixed with three parts of blood-serum. It is sterilized like ordinary blood-serum. It is used largely for the cultivation of the bacillus of diphtheria.

**Fresh eggs** in their shells may be used without other preparation than washing the surface thoroughly with bichloride of mercury solution; or after sterilization by steam, which of course coagulates the albumen. The egg is easily inoculated through a small opening made with a heated needle, which may be closed afterward with collodion. Hueppe recommended eggs closed in this manner for the cultivation of anaërobic bacteria.

**Dorset's Egg Medium.**<sup>1</sup>—Perfectly fresh eggs are washed and the shells sterilized with bichloride solution. The eggs are then carefully broken and the yolks and whites mixed in a sterile dish. The mixed material is poured into sterile tubes and solidified in the slanting position by heating at 70—75° C. for two hours. Contamination with bacteria should be carefully avoided throughout the preparation of the medium. The tubes should be sealed with rubber caps or with wax and incubated for a week before use. It is well to moisten the surface with a few drops of sterile water from a pipette before inoculating. This medium is used for growing the tubercle bacillus.

**Bread-paste.**—Dry or toasted bread is broken into small crumbs, filled into tubes or flasks, moistened with water and sterilized in the autoclave. This medium is used for cultivation of molds.

#### MEDIA CONTAINING UNCOOKED PROTEIN

Culture media containing naturally sterile uncooked protein have made possible the cultivation of microbic forms not cultivable on other media. Many microbes which may also grow on cooked media do much better on those containing uncooked protein. It would seem that media of this kind are to play an important part in the further development of our knowledge of pathogenic micro-organisms.

**Collection of Sterile Blood.**—A few drops of blood may be obtained from the ear lobe. The skin is cleansed with soap and alcohol and then dried perfectly with sterile cotton. It is punctured with a sterilized lancet and the blood quickly transferred to the surface of an agar slant by means of a platinum loop or a sterile capillary pipette. It should be incubated before use to insure sterility.

Larger quantities of sterile human-blood may be obtained with far less danger of contamination from the median basilic vein or other large vein at the elbow. The skin is washed, disinfected

<sup>1</sup> Dorset: *American Medicine*, April 5, 1902.

with alcohol and bichloride and dried. An elastic bandage is applied about the arm to distend the veins. A sterile needle attached to a special sterilized blood pipette is thrust into the

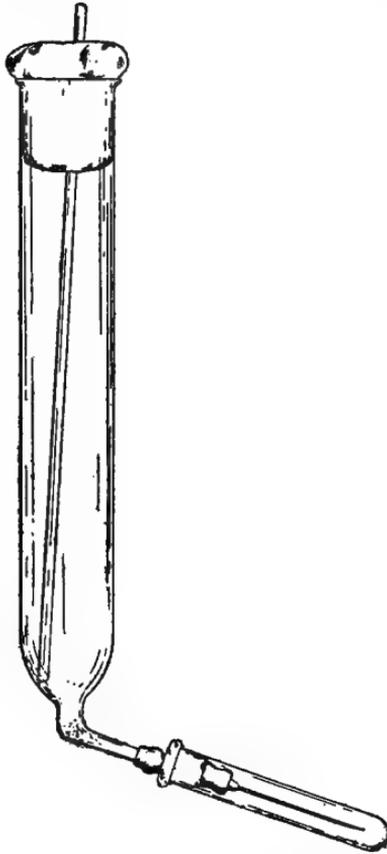


FIG. 37.—Pipette with needle attached for drawing human blood from a vein for use in culture media. The glass rod inside is used to defibrinate the blood.

vein and the desired amount of blood collected (see Fig. 37). It may be allowed to clot if sterile serum is desired, or it may be defibrinated by stirring with the glass rod if a mixture of corpuscles and serum is desired, or it may be kept in the fluid state by the addition of sterile 10 per cent solution of sodium citrate so that the final mixture may contain 1 per cent of citrate. The bandage is removed from the arm before the needle is withdrawn. Pressure over the wound with cotton wet in alcohol for five minutes prevents subcutaneous hemorrhage. No dressing is required. The inlet to the blood pipette is closed by kinking the rubber tube. The blood or the serum is subsequently handled by means of sterilized pipettes, and most conveniently by means of the Pasteur bulb pipettes. (See page 33.)

Blood from small laboratory animals serves as well as human blood for most purposes. It may be drawn from the carotid artery by aseptic technic into a special blood pipette, the lower end of which is drawn out into a capillary, which is inserted directly into the artery (see Fig. 38). This blood may be defibrinated, citrated or allowed to clot.

Small amounts of sterile blood may be obtained from laboratory animals without killing them by means of heart puncture. The needle of a Luer glass syringe is inserted through the chest wall, after anesthetizing the animal and shaving and disinfecting the skin, so as to enter the cavity of the right ventricle. A quantity of blood not greater than  $\frac{1}{50}$  the weight of the animal may be removed. The needle is withdrawn and the blood quickly forced out into a sterile tube where it may be defibrinated or mixed with citrate solution, or allowed to clot, as may be desired.

Very large amounts of sterile blood are best obtained from the juglar vein of the horse or the superficial abdominal veins of the cow. The skin is shaved, washed and cauterized with 95 per cent carbolic acid. When this has dried the vein is punctured with the needle, which is attached to a suitable glass receptacle by means of rubber tubing.

#### Collection of Sterile Ascitic Fluid.—

For this purpose a large trochar and canula provided with a lateral outlet, and made so that the trochar can be drawn back beyond this outlet without being completely removed, is most convenient. The instrument is oiled with liquid paraffin. A rubber tube about 40 cm. in length is attached to the outlet and the whole is wrapped in a cloth and sterilized in the autoclave. The site selected

for puncture should be cleansed and painted with tincture of iodine and the skin may be frozen with ethyl chloride if desired. One man inserts the trochar and canula, taking care not to contaminate it after it is removed from the cover. Another manipulates the attached rubber tube, carefully guarding it from contamination

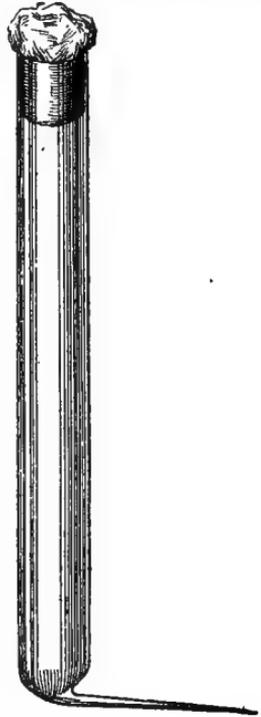


FIG. 38.—Pipette with capillary tip for drawing blood from carotid artery of an animal. (After *Novy*.)

and allowing the fluid to flow into sterilized flasks of 1000 c.c. capacity which are handled by an assistant. The mouth of each flask should be flamed after removing the cotton plug and again before it is inserted after filling the flask. With proper technic the ascitic fluid will as a rule be found bacteria-free. It should be stored in a cool place, and is most conveniently handled by means of large Pasteur bulb pipettes.

In collecting hydrocele fluid or other fluids to be used for culture media, similar aseptic technic should be employed.

**Sterilization of Contaminated Fluids.**—Any of the clear fluids may be sterilized, when this is necessary, by filtration through the Berkefeld filter. The filtrate will usually prove less valuable as a medium than the corresponding unfiltered naturally sterile material.

**Collection of Sterile Tissue.**—For this purpose, a healthy animal is first bled to death as described above (page 98) for the collection of sterile blood. The skin is then thoroughly wet with water or with bichloride solution. With sterile instruments, an incision is made in the median line and the skin carefully stripped back. It is then well to sear the abdominal wall with a hot iron along the median line and also crosswise and cut along these lines with sterile scissors, opening the abdominal cavity. The organs desired are quickly removed with sterile instruments and placed in covered sterile glass dishes. The liver, kidneys and testes are the organs most frequently employed in culture media. They are divided into pieces of suitable size with sterile scissors. Brain tissue may be readily obtained from the rabbit. The top of the head is skinned and an opening made by cutting away the skull between the orbits with the bone forceps. An area of the anterior portion of the brain is exposed. This is thoroughly seared with a hot iron, as well as the adjacent structures. A Pasteur bulb with a large capillary (internal diameter at least 5 mm.) is convenient for drawing out the brain tissue. This large capillary is inserted through the seared area and the brain is

broken up by moving it about in the cranial cavity, while the tissue is drawn into the bulb by suction.

**Pfeiffer's Blood-streaked Agar.**—A large loopful of naturally sterile human blood, freshly taken from the ear, is spread over the surface of an agar slant, and incubated to insure sterility. This medium is employed for cultivation of the influenza bacillus.

**Novy's Blood-agar.**—The agar is melted and cooled to 50° C. The naturally sterile defibrinated blood, usually rabbit's blood, is warmed to about 40° C. The blood is mixed with the agar in various proportions, and the mixture is allowed to solidify in the inclined position. The medium should be fairly firm in consistency and some fluid should collect at the bottom of the slant. The medium is useful for cultivation of the gonococcus, the influenza bacillus, streptococcus, pneumococcus and meningococcus, but more especially for cultivation of the flagellated hematozoa such as trypanosomes and related organisms, including the various species of *Leishmania*.

**Smith's Broth Containing Sterile Tissue.**—Pieces of naturally sterile organs, usually liver or kidney, are placed in broth, more particularly in fermentation tubes of broth. The bits of tissue are conveniently handled by touching with a hot platinum wire or glass capillary, to which they will adhere. The medium is especially useful for the culture of anaërobic bacteria. Naturally sterile blood added to the broth also serves for this purpose.

**Ascitic-fluid-agar.**—This is made in the same way as the Novy's blood-agar except that naturally sterile human ascitic fluid is employed instead of blood. The medium is beautifully transparent, and may be employed for plating as well as for tube cultures. It is especially valuable for cultivation of the gonococcus and also for the streptococcus, pneumococcus and meningococcus.

**Noguchi's<sup>1</sup> Ascitic Fluid with Sterile Tissue.**—Naturally sterile tissue is placed in a tall tube. A deep layer of ascitic fluid is added, and for some purposes this is covered with a layer of

<sup>1</sup>Noguchi: *Journ. Exp. Med.*, Jan. 1, 1912, Vol. XV, pp. 90-100.

sterile paraffin oil. The medium is used more especially for the cultivation of the blood spirochetes which cause relapsing fever.

*Special Media* of very special and limited usefulness will be discussed in the chapters dealing with the particular organisms for the growth of which they are employed.

## CHAPTER IV

### COLLECTION OF MATERIAL FOR BACTERIOLOGICAL STUDY

Bacteria under natural conditions are usually associated as mixtures of several species living together. Only under rather exceptional circumstances will a single kind of bacteria be found growing alone. This does occur in disease, however, where the living host may be able to keep out all but the one kind of microbe. But even diseased tissues or exudates originally harboring only one kind of bacteria may quickly acquire others in abundance after removal from the living body. It is well therefore to regard any material presented for bacteriological examination as potentially, and in all probability actually, harboring several kinds or species of bacteria. The direct planting of such material on a culture medium will, therefore, in most instances give rise to a mixed culture, in which those forms least prominent in the original material may easily appear as most important. If the material be allowed to stand, especially if it be a favorable medium for bacterial growth, the relationships present may become seriously confused. It should, therefore, be examined as fresh as possible. When immediate examination is impossible the material should be kept on ice.

*Samples of water, milk or other fluid* should be collected in sterilized tubes or bottles. Samples of solid food should be seared or charred all over the surface and divided with a sterilized knife. A small piece of the interior is then removed to a sterilized glass dish and covered.

*Material removed from the human or from the animal body* during life or at autopsy may be bacteria-free, or it may contain one or more species of microbes. It is important that the picture be not confused by the addition of bacteria from the surface of

the body, from instruments or from the air during the collection and transportation to the laboratory. Unfortunately the laboratory study of such material is too often rendered difficult, untrustworthy or even worthless through lack of attention to this point.

When merely microscopic examination is to be undertaken, contamination may not be serious, and an antiseptic, such as two per cent of carbolic acid; may be added to the material, if fluid, and if solid it may be immersed in ten per cent formalin. The bottles used should be new and clean. Such material may also be spread on microscopic slides or cover-glasses in a thin layer, dried, fixed in the flame, and transported to the laboratory. This method is not always free from danger when the material passes through several hands. Special precautions for collecting material for microscopic examination will be considered in discussing the specific pathogenic microbes.

*Specimens of sputum* should be raised from the trachea, bronchi and lungs after previously cleansing the mouth. Sputum should be received into a sterile wide-mouthed bottle, and stoppered with a sterilized cork. The exterior of the bottle should then be carefully washed with 5 per cent carbolic acid.

*Urine* should be collected by catheter with careful aseptic technic, and should be received in a clean sterilized bottle. When the passage of a catheter is deemed unwise, the urine should be received directly into a sterile bottle after surgical cleansing of the urinary meatus and must be examined in the laboratory without delay, contamination being assumed to have taken place.

*Blood and transudates* are collected by the technic previously described (page 97). Blood is drawn from the vein by means of the Luer syringe and is quickly ejected into several flasks of broth (150 to 250 c.c.) and into Petri dishes where it is mixed with melted agar (cooled to 50° C.) before clotting takes place.

A most convenient tube for collecting blood has been described by Taylor.<sup>1</sup> The side arm (Fig. 39) is accurately ground at the

<sup>1</sup> Taylor, R. M.: Proc. N. Y. Path. Soc., 1914, 14, p. 37.

tip to fit the ordinary Luer needle. The tube is sterilized by hot air and the needles are sterilized by boiling in liquid paraffin (albolene). By putting 1 c.c. of 10 per cent sodium citrate into the tube, the blood specimen may be kept fluid and transported a considerable distance to the laboratory before inoculating the culture media.

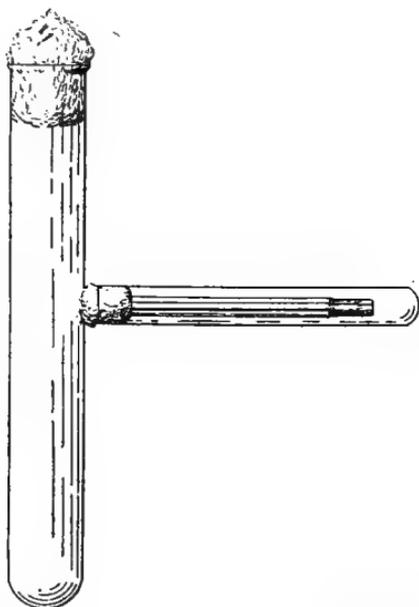


FIG. 39.—Taylor's tube for vein punctures.

*Cerebro-spinal fluid* is obtained by inserting a sterilized needle (4 cm. long for children, 8–10 cm. long for adults, and with lumen 1 mm.) in the median line in the back, so that it enters the spinal canal between the second and third, or between the third and fourth, lumbar vertebræ. Aseptic technic is essential. The fluid coming from the needle is received in a sterile tube.

*Feces* from infants and young children are best collected by means of a heavy glass tube closed and rounded off at the end, and provided with a lateral opening near the closed end. This is enclosed in a larger tube and sterilized. It is inserted well into the rectum with aseptic technic and the entrance of fecal material

through the lateral opening is favored by gently moving the tube. It is then withdrawn and replaced in its original container to be transported to the laboratory. From adults the feces are passed directly into a sterilized covered agateware basin without other special apparatus.

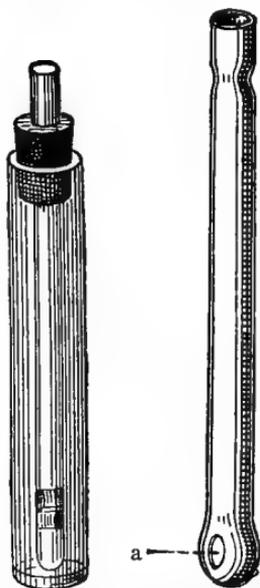


FIG. 40.—Two types of instrument for obtaining feces from infants for bacteriological examination. (After Schmidt and Strasburger.)

*Intestinal juice* from the duodenum may be obtained in infants<sup>1</sup> by inserting a sterile rubber catheter, closed below with a sterilized gelatin capsule, through the esophagus and stomach into the duodenum. The capsule is then blown off by pressure from a sterile syringe attached at the other end of the catheter and the fluid contents of the duodenum aspirated. In adults<sup>2</sup> the Einhorn duodenal tube is employed. The tube is sterilized by boiling and the lower opening sealed with a sterilized gelatin capsule and by finally coating with shellac. The tube is inserted through the esophagus and is carried through the pylorus by peristalsis. Ordinarily it is inserted in the evening. On the following morning the seal at the lower end is broken by pressure of a sterile syringe attached to the free end of the tube and the sample of juice aspirated. Intestinal

juice may be obtained from various levels in the jejunum also by regulating the length of tube inserted.

*Pus and other exudates* are best collected in sterile glass capillary pipettes (see page 33). A sterilized cotton swab, made by winding a pledget of absorbent cotton around the end of a stiff wire, enclosing it in a test-tube and sterilizing it, is also useful, especially when it is impossible or undesirable to employ the glass tube.

<sup>1</sup> Hess: *Journ. Infectious Diseases*, July, 1912, Vol. XI, pp. 71-76.

<sup>2</sup> MacNeal and Chace: *Arch. Int. Med.*, Aug., 1913, Vol. XII, pp. 178-197.

*At autopsies* on human subjects, the same principles for collection of material apply. Fluids are best collected in sterile glass pipettes and even solid organs may be seared and punctured with a strong glass capillary into which some of the pulp is drawn by suction. The tubes may be sealed in the flame and transported considerable distances to the laboratory. This is usually more satisfactory than the inoculation of culture media in the autopsy room, especially if the facilities for bacteriological work there are somewhat limited. Smears on slides or cover-glasses should also be made for microscopic examination, and pieces of the various organs fixed in alcohol or formalin and preserved for sectioning.

## CHAPTER V

### THE CULTIVATION OF MICRO-ORGANISMS

**Avoidance of Contamination.**—Micro-organisms are so numerous on the body of man and in his environment that they are likely to be present on all articles about us unless special precautions are taken to remove or destroy them. The dust blown about in the air contains bacteria and spores of molds. The primary essential in all bacteriological culture work is the exclusion of these extraneous micro-organisms. The unskilled or careless worker may quickly add some of these chance organisms to the material which he is attempting to study, introducing an element of almost hopeless confusion unless it is recognized. Another essential of great importance, especially when working with pathogenic microbes, is the complete destruction of all living bacteria before they are allowed to pass beyond strict and absolute control. The unskilled or careless worker in the laboratory, who allows micro-organisms to escape from him while he is attempting to study them, is a serious menace not only to himself but to all others in the laboratory. These two primary essentials must be mastered by practice in handling harmless forms.

Every instrument with which bacteria are handled should be sterilized before it is used, and again after use. In the case of the commonly used platinum wire, this sterilization is accomplished in the flame. The wire is heated to a glow and allowed to cool before handling bacteria, and immediately after its use, *before it leaves the hand*, it is brought close to the flame so as to dry the material on it and then again heated to redness. Careful drying in this way avoids sputtering and consequent scattering of bacteria, which is almost certain to occur if moist material, especially fat or protein, is carelessly thrust into the flame.

In using the Bunsen flame for sterilization, the innermost cone near the base of the flame may be utilized for drying material on the end of the wire. This inner cone is not burning and is comparatively cool, and after a little practice the end of the wire is easily brought into it and dried without sputtering. Slowly elevating the wire brings it gradually into hotter zones of the flame until it glows.

Bacteria do not of themselves leave a moist surface. They are not even removed by moderate currents of air unless they have been previously dried. Their distribution about the laboratory, therefore, results from relatively gross accidents or gross carelessness. When material containing bacteria is accidentally spilled, it should be covered at once with disinfectant solution such as 1-1000 mercuric-chloride solution. As a routine procedure it is well to wash the work table daily with bichloride solution and, when working with pathogenic bacteria, to wash the hands at the end of the day's work, first with the bichloride solution and then with soap and water.

**Isolation of Bacteria.**—In order to study any kind of bacteria it is necessary to have the particular species separated from other sorts with which it may be mixed. The earlier bacteriologists endeavored to separate bacteria of different sorts by successive transplantations through a series of tubes of fluid media, one kind of bacteria outgrowing the rest. Isolation was also accomplished by diluting the material very highly and then inoculating one drop into each of a large number of tubes of broth. Some tubes would thus receive no bacteria, others would receive several, and occasionally one would receive only a single germ and would give rise to a pure culture. Another early method of separating a pathogenic species was by inoculation of animals. The ability of the animal to prevent the development of all but one species contained in the inoculated material was utilized to obtain the first pure cultures of anthrax bacilli and tubercle bacilli. These methods are successfully employed only for relatively few bacterial species.

Methods of isolating bacteria, which are of more general application, were introduced by Koch. The essential characteristic of these methods is the dilution of the bacteria in a fluid medium which quickly becomes solid so that each germ develops in a definite fixed position in the medium.

It is impossible in most cases to distinguish between bacteria of different varieties by microscopical examination alone. Bacteria of widely different species and quite unlike one another in their properties may present similar appearances under the microscope. The differences which they exhibit are usually apparent when they are grown in culture-media. The growth, called a *colony*, which results from the multiplication of a single bacterium, is in many cases very characteristic for the species. By the plate-method, the individual bacteria in a mixture are separated from one another by dilution. They are fixed in place by the use of a solid medium. They are allowed to grow, and from each individual there arises a colony. It is usually possible to distinguish between *colonies* arising from different species when it is not possible to distinguish between *the individual bacteria* of these species. A convenient comparison has been suggested by Abbott. A number of seeds of different sorts may appear very much alike, and considerable difficulty may be found in distinguishing one from another with the eye. Let them be sown, however, and let plants develop from them, and these plants will easily be distinguished from one another.<sup>1</sup>

**Method of Making Plate-cultures.**—Melt *three* tubes of gelatin or agar. (There is some difficulty in keeping agar in a fluid state while dilutions are being made. It is convenient to have some form of water-bath with a thermometer for the purpose.) Let the liquefied agar cool to 45° C. Gelatin may be used at a temperature anywhere between 28° and 40° C. Take a small

<sup>1</sup> It must be understood that *no close comparison can be drawn between higher plants*, which simply complete the development of parts potentially present in the seed, *and colonies of bacteria*, which are aggregates of individuals, the progeny of one individual of the same kind.

portion of the material to be examined—pus, for example—and introduce it with a sterilized platinum wire or loop into one of the tubes. The plug of the test-tube is to be withdrawn, twisting it slightly, taking it between the third and fourth fingers of the left hand, with the part that projects into the tube pointing toward the back of the hand. It must not be allowed to touch any object while the inoculation is going on. Pass the neck of the tube through the flame. If any of the cotton adheres to the neck of the tube, pull the cotton away with sterilized forceps, while the neck of the tube touches the flame, so that the threads of cotton may be burned and not fly into the air of the room.

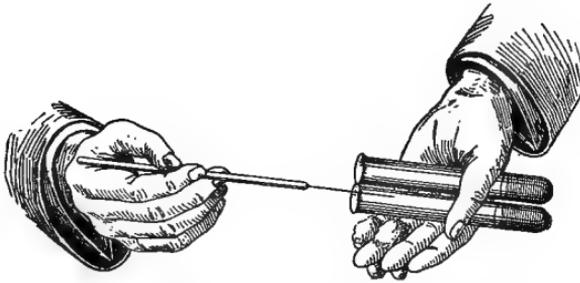


FIG. 41.—Method of inoculating culture media.

The tube is held as nearly horizontal as possible, in the left hand between the thumb and forefinger, resting upon the palm, and the neck of the tube pointing upward and to the right. Mix the material introduced thoroughly with the liquefied culture-medium, taking care not to wet the plug. Now remove the plug again, and, having sterilized the platinum wire, insert it into the liquefied medium. Carry three loopfuls in succession from this tube, which is No. 1, into tube No. 2. When two tubes are being used at the same time, they are placed side by side between the thumb and forefinger of the left hand. The two plugs are held between the second and third and the third and fourth fingers of the left hand, respectively. The wire may now be passed into the first tube, which we will suppose to hold some material containing bacteria, and a little of this material may be removed on the tip

of the wire from the first tube to the second. When the needle is introduced into or removed from either tube it should not touch the side of the tube at any point, and should only come in contact with the region desired. After inoculation of the second tube has been effected, the wire is heated to redness in the flame, the necks of the tubes are passed through the flame, and the plugs are returned to their respective tubes. In the same manner transfer three loopfuls from tube No. 2 into tube No. 3. The original material will obviously be diluted in tube No. 1, more in tube No. 2, and still more in tube No. 3. The most convenient form of plate is that known as a Petri dish, a small glass dish about 10 cm. in diameter and 1.5 cm. in height, provided with a cover which is a little larger but of the same form. This dish should be cleaned,

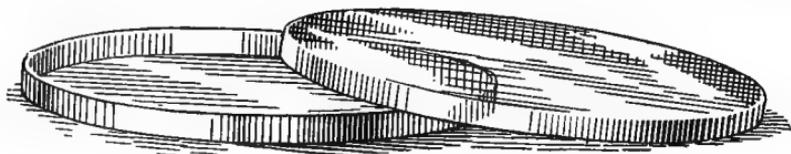


FIG. 42.—Petri dish.

dried and sterilized for an hour in a hot-air sterilizer at 150° C. or higher. When it is cool it may be used.

Such dishes having previously been prepared, the contents of tube No. 1 are poured into one dish, and those of tube No. 2 into another, and those of tube No. 3 into a third. They are labeled Nos. 1, 2, and 3.<sup>1</sup> In pouring proceed as follows: remove the plug of tube No. 1; heat the neck of the tube in the flame; allow it to cool, holding it in a nearly horizontal position. When the tube has cooled, lift the cover of the Petri dish a little, holding it over the dish; pour the contents of tube No. 1 into the dish, and replace the cover. The interior of the dish should be exposed as little and as short a time as possible. Tubes Nos. 2 and 3 are to be treated in the same manner. Burn the plugs, and

<sup>1</sup> The labels should be moistened with the finger, which has been dipped in water. They should not be licked with the tongue. While working in the bacteriological laboratory it is best to make it a rule that no object is to be put in the mouth.

immerse the empty tubes in 5 per cent solution of carbolic acid. Where much culture work is being done, it will be found convenient to sterilize the mouth of each tube by thorough heating in the flame after pouring out its contents, and then to replace the plug. The tube may then be placed in a special receptacle which is sterilized with its contents in the autoclave at  $120^{\circ}$  C. for 20 minutes, at the end of the day's work.



FIG. 43.—Colonies in gelatin plate showing how they may be separated and the organisms isolated.

The culture-medium in the Petri dish will soon solidify. Petri dishes of agar should be inverted after the medium is firmly set; otherwise the water, which evaporates from the surface and condenses on the inside of the lid, may overflow the surface of the agar, confusing the result. Agar plates are usually developed in the incubator. Gelatin plates must be developed at a temperature below the melting-point of the medium, which is usually between  $22^{\circ}$  and  $28^{\circ}$  C. Colonies usually appear in from one to two days. In plate No. 1 they will be very numerous, in plate No. 2 less numerous, and in plate No. 3 still less numerous. Where the number is small the colonies will be widely separated

and can readily be studied. They may be examined with a hand-lens, or the entire dish may be placed on the stage of the microscope and the colonies be inspected with the low power. The iris diaphragm should be nearly closed and the plane mirror should be used. Dilution-cultures prepared as described in the next paragraph, where the principle is the same, are shown in Fig. 45. In tube No. 1 the colonies are so numerous as to look like fine white dust. In tubes 2 and 3 they are less numerous and larger.

**Esmarch's Roll-tubes.**—Use liquefied gelatin or agar. The dilutions in tubes 1, 2 and 3 are made as above. Tubes containing a rather small amount of the culture-medium are more con-

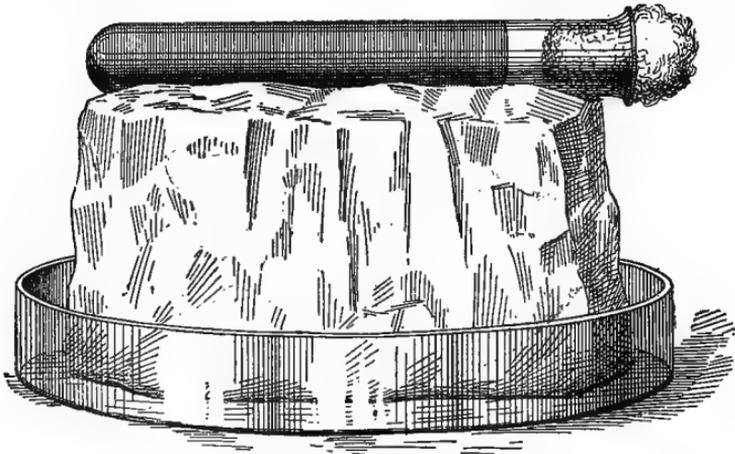


FIG. 44.—Manner of making Esmarch roll-tube.

venient. A block of ice should be at hand, and, with a tube filled with hot water and lying horizontally, a hollow of the size of the test-tube should be melted on the upper surface of the ice. In this hollow, place the tube of liquefied gelatin or agar; roll it rapidly with the hand, taking care that the culture-medium does not run toward the neck as far as the cotton plug. The medium is spread in a uniform manner around the inside of the tube, where it becomes solidified. Gelatin roll-tubes must be kept in a place so cool that there is no danger of their melting; in handling them they are to be held near the neck, so that the warmth of the hand

may not melt the gelatin. Agar roll-tubes should be kept in a position a little inclined from the horizontal, with the neck up,

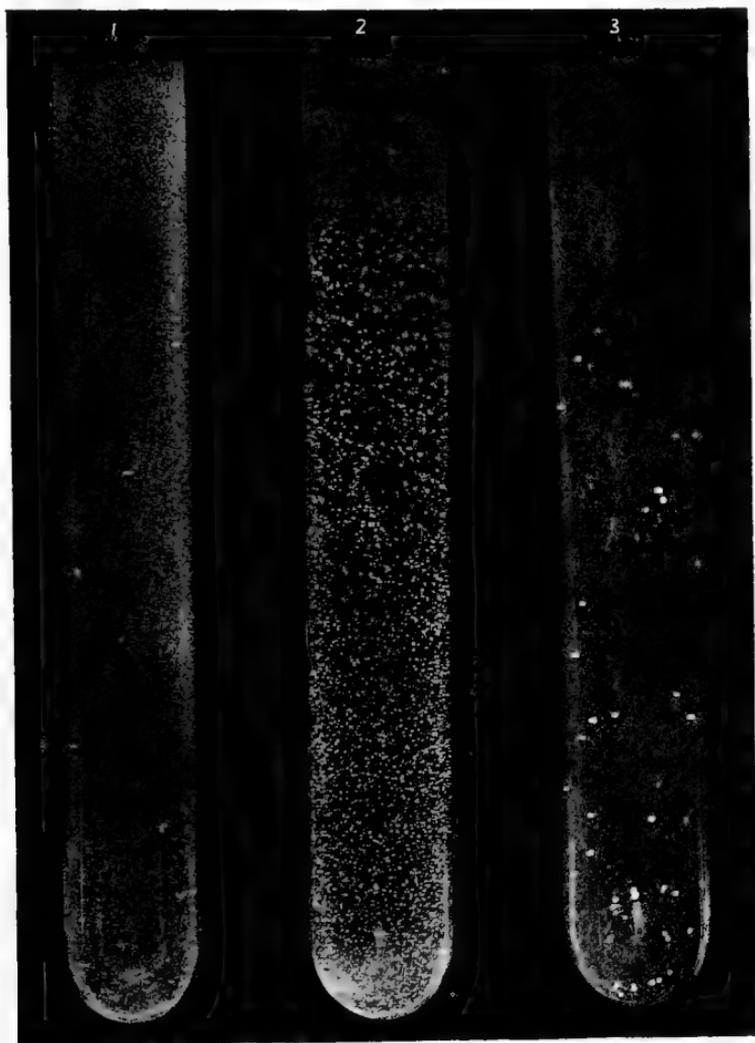


FIG. 45.—Dilution-cultures in Esmarch roll-tubes. In tube 1 the colonies are very close together; in tube 2 they are somewhat separate; in tube 3 they are well isolated.

for twenty-four hours, so that the agar may adhere to the wall of the tube.

In the plate-method as originally devised by Koch, instead of using Petri dishes, the gelatin was poured upon a sterile plate of glass. This plate of glass was laid on another larger plate of glass, which formed a cover for a dish of ice-water, the whole being provided with a leveling apparatus. The plate was kept perfectly level until it had solidified, which took place rapidly on the cold surface. The glass plates were placed on little benches enclosed within a sterile chamber. The more convenient Petri dish has now displaced the original glass plate.

**Streak Method of Isolating Bacteria.**—The isolation of bacteria may sometimes be effected by drawing a platinum wire containing material to be examined rapidly over the surface of a Petri dish containing solid gelatin or agar; or over the surface of the slanted culture-medium in a test-tube; or by drawing it over the surface of the medium in one test-tube, then, without sterilizing, over the surface of another, perhaps over several in succession. This method is ordinarily less reliable than the regular plating method.

**Veillon's Tall-tube Method.**—Three to six tubes of glucose agar, the agar being at least 6 cm. deep, are liquefied and cooled to 45° C. in a water-bath. A small amount of the material to be examined is placed in the first tube by means of the platinum loop, and carefully mixed. From this dilutions are made in series to tubes 2, 3, 4, 5 and 6, each being carefully mixed without introducing air bubbles. The tubes are quickly solidified by immersion in cold water, and are incubated at 37° C. These culture tubes offer the contained bacteria a wide range of oxygen supply. This is abundant at and near the top, and gradually diminishes lower in the tube until near the bottom almost perfect anaërobic conditions obtain. The method is very useful in isolating *B. bifidus* from feces of infants, *B. acne* from acne pustules, and in studying the oxygen requirements of other bacteria and has been most extensively employed for study of the bacteria in war wounds, where it has proved to be of fundamental importance. Colonies are picked out with sterile glass capillaries, and deeper colonies are reached by breaking the tube. The successful use of the method requires some practice and particular care, both in the preparation of

the agar and the cultivation of bacteria, is essential to success in studying toxic edema and gaseous gangrene of war wounds.

**Appearance of the Colonies.**—The colonies obtained in the Petri dishes or in tubes (Fig. 45) may be studied with a hand-lens or with a low power microscope. In the latter case, use the plane mirror with the iris diaphragm nearly closed. The colonies present various appearances. Some of them are white, some colored; some are quite transparent and others are opaque; some are round, some are irregular in outline; some have a smooth surface, others appear granular, and others present a radial striation. Surface colonies often present different appearances from those occurring more deeply. Surface colonies are likely to be broad, flat and spreading. If the colony consists of bacteria which have the property of liquefying gelatin, a little funnel-shaped pit or depression forms at the site of the colony. The appearance of colonies may be of great assistance in determining the character of doubtful species. The appearance in gelatin plates of the colonies of the spirillum of Asiatic cholera, for instance, is one of the most characteristic features of this organism.

**Pure Cultures.**—From these colonies pure cultures may be obtained by the process called "fishing." Select a colony from which cultures are to be made, touch it lightly with the tip of a sterilized platinum wire, taking great care not to touch the medium at any other point. Introduce the wire into a tube of gelatin after removing the plug and flaming the mouth of the tube. Sterilize the wire and plug the tube. In a similar manner, and from the same colony, inoculate tubes of agar, bouillon, milk, potato and blood-serum. Gelatin tube cultures are usually inoculated by introducing the platinum needle into the medium vertically, making a "stab-culture." Inclined surfaces such as those of agar, potato or blood-serum are inoculated by drawing the wire lightly over the surface of the medium, making a "smear-culture" or "streak-culture" (Figs. 46 and 47). Liquid media are inoculated by simple introduction of a small mass of bacteria and mixing them with the medium. At the same time it is well to

make a smear preparation from the colony and to stain with one of the aniline dyes so as to determine the morphology of the bacteria. The growths which take place in the tubes should contain one and the same kind of bacteria. As seen under the microscope these bacteria should have the same general form and appearance as those seen in the colony from which they were

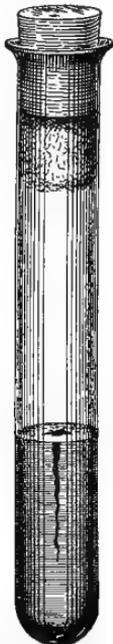


FIG. 46.—Stab-culture.  
A rubber stopper may  
\*be used to prevent drying,  
see page 119.



FIG. 47.—Smear-culture.  
This tube shows the  
rubber cap used to prevent  
drying.

derived. This will be the case, provided the colony has resulted from the development of a single bacterium.

*A pure culture is a culture which contains only the descendants of a single cell.*

**Stock Cultures.**—To maintain their vitality bacteria need to be transplanted from one tube to another occasionally; the time varies greatly with different species. Many bacteria grow on culture-media with difficulty at the first inoculation, but having

become accustomed to their artificial surroundings, as it were, they may be propagated easily afterward; this is especially true of the tubercle bacillus. After they are developed, stock cultures are best kept in a refrigerator, and it is well to seal them so as to prevent drying. Rubber caps or rubber stoppers are useful for this purpose (Figs. 46 and 47).

Some bacteria flourish better on one culture-medium than another. The tubercle bacillus grows best on blood-serum and glycerin-agar; the bacillus of diphtheria grows best on Löffler's blood-serum; the gonococcus on human serum-agar or ascitic-fluid-agar.

The virulence of most pathogenic bacteria becomes diminished after prolonged cultivation upon media. In some forms the virulence is lost very quickly, for example, the *streptococcus* and *pneumococcus*.

#### REGULATION OF TEMPERATURE

**High-temperature Incubator.**—Many bacteria flourish best at a temperature about that of the human body, 37° C. Some species will grow only at this temperature. The pathogenic bacteria in particular, for the most part, thrive best at a point near the body temperature.

The ordinary incubator is a box made of copper, having double walls, the space between the two being filled with water. The outer surface is covered with some non-conductor of heat, such as felt or asbestos. At one side is a door, which is also double. The inner door is of glass, the outer door is of copper covered with asbestos. At one side is a gauge which indicates the level at which the water stands in the water-jacket. The roof is perforated with several holes, some of which permit the circulation of the air in the air-chamber inside the box; some of them enter the water-jacket. A thermometer passes through one of these holes into the interior of the air-chamber, and often another into the water standing in the water-jacket. A gas-regulator passes through another hole, and is immersed in the water standing in

the water-jacket. There are various forms of gas-regulators more or less complicated. The simplest and least expensive thermo-regulators for gas are made of glass and filled with mercury or with mercury and some lighter liquid, the heavy mercury

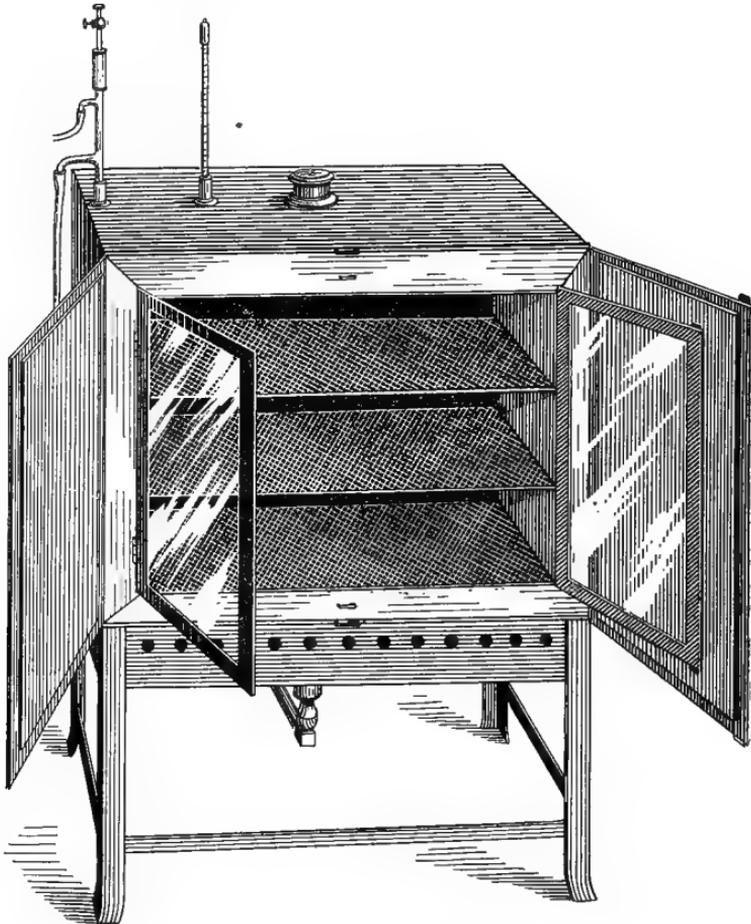


FIG. 48.—Incubator.

servicing to close the chief source of gas supply when the desired temperature has been attained, while a minute opening at another point remains open to furnish sufficient gas to keep the flame alight, but not sufficient to maintain the temperature. Upon

cooling the mercury falls and allows gas to flow again through the larger opening. In this way the supply of gas is made large whenever the temperature is a little below the desired temperature and very small whenever the temperature rises above that point, and the temperature varies within a slight range. The Reichert regulator is designed to operate according to these principles, and various modifications of this regulator are on the market. In many of these instruments the larger supply is only imperfectly shut off at the desired temperature, and, where the weight of the mercury is relied upon to stop this opening, the gas may often bubble out through it unless special precautions are taken to regulate the pressure of the gas supply.

A modification of this type of regulator devised by Mac Neal<sup>1</sup> overcomes this difficulty (see Fig. 50). The inlet tube A leads through the wall of the chamber D, to which it is fused, into an inner upright tube, BC. Near the upper end of this upright is a small opening, O, which allows the minimum supply of gas to pass to the burner to avoid extinction of the flame. The lower end of this upright tube fits quite closely the bottom of the chamber D, around the opening leading into the capillary tube, EF. This end is adjusted so close to the bottom that mercury will not pass through between inner and outer tube at less than twenty millimeters mercury pressure, yet not so close but that an abundant supply of gas may pass. The proper adjustment of this part must be thoroughly tested before the instrument leaves the factory. The upper end of the upright, BC, is closed by a ground glass stopper, which also closes the top of the outer chamber, D. In the ground surface of this stopper a gamma-shaped ( $\Gamma$ ) groove is cut, the vertical limb extending from the

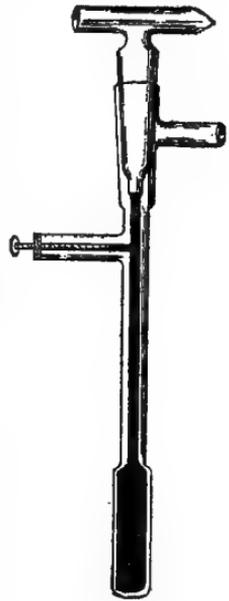


FIG. 49.—Reichert's gas-regulator.

<sup>1</sup> *The Anatomical Record*, August, 1908, Vol. II, No. 5.

lower tip of the stopper to the level of the opening, O. The horizontal limb is deep where it joins the vertical, but gradually becomes shallow and ends about one-quarter the way around the stopper. This groove serves for passage of the gas from the inner

tube BC, to the opening O, and thus to the outer chamber D, and by rotating the stopper, the amount of gas flowing through this passage may be reduced to any desired point. The outlet tube, H, leads from the chamber D to the burner connection.

The capillary, EF, leads to a bulb of sufficient size; the larger the more sensitive the instrument. Either the large bulb with inside capillary, J, to be filled with mercury and toluol, or the smaller simple bulb for mercury alone may be used. A side arm is attached to one side of the capillary EF, for conveniently controlling the height of the mercury column. Either the curved capillary tube with stopcock and a cup on the end, or the simple tube with metal screw cemented in, may be used here, according to the purpose which the regulator is to serve. These parts are similar to those of Novy's modification of the Reichert regulator.

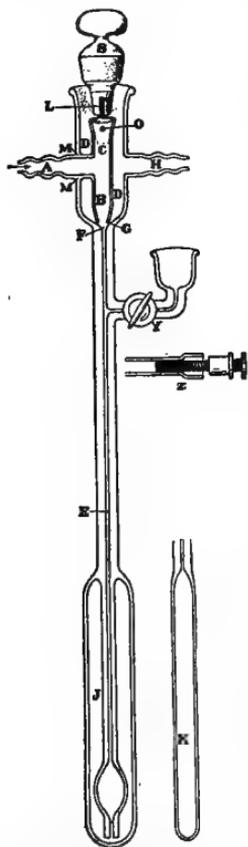


FIG. 50.—Mac Neal gas-regulator.

To fill the instrument, the air is partly driven out by heating the bulb and then the desired liquid is drawn in by cooling, repeating the heating and cooling until the instrument is full of the liquid. For the small bulb, mercury is always used alone. The large bulb, on the other hand, is filled first with either ether, alcohol or toluol, and then part of this liquid is forced out by heat and replaced with mercury so that the capillary EF, the bulb at its lower end, and a small part of the large bulb J, are occupied by the mercury. Ether may be used

when the regulator is not to be heated above  $35^{\circ}$  C., alcohol when it is not to be heated above  $75^{\circ}$  C., and toluol for temperatures between  $75^{\circ}$  and  $100^{\circ}$  C.

A very satisfactory regulator is that of Roux. It is constructed entirely of metal, and its operation is due to the unequal expansion and contraction of two metals which are riveted together. Fig. 51 shows this regulator. The gas passes in at *e* and passes out at *d*. The amount of gas passing through is regulated by a piston on the end of the set screw inside the tube from which the outlet tube branches off. This piston

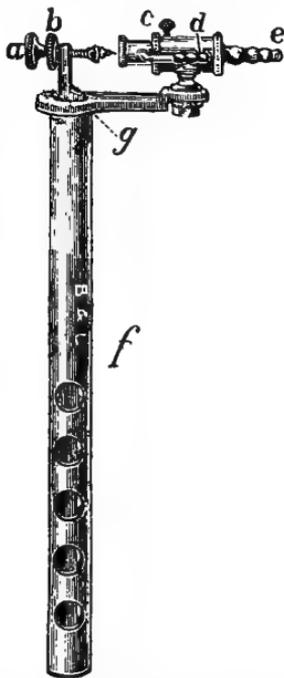


FIG. 51.—Roux bimetallic gas-regulator. *a*, Set screw; *b*, Screw collar; *c*, Clamp; *d*, Outlet for gas; *e*, Inlet for gas.

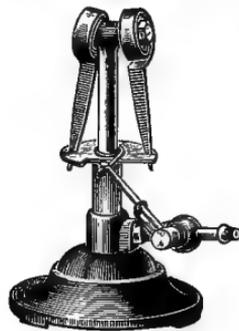


FIG. 52.—Koch automatic gas-burner.

moves in or out according to the changes of temperature of the water jacket of the incubator into which the stem (*f*) of the regulator is inserted. This stem is fenestrated and has the riveted metallic strips running down in it. These strips are pivoted at the collar, *g*.

The gas coming from the gas-regulator passes to a Bunsen burner, which stands underneath the incubator. This burner should have some kind of automatic device for cutting off the flow of gas in case it becomes accidentally extinguished by a sudden

draught of air or from any other cause. The automatic burner invented by Koch is an ingenious, simple and effective device (Fig. 52). The coils of metal seen on each side at the top of the burner are so arranged that when they expand they turn the disk below so as to support the arm coming from the stop-cock; when they cool they turn the disk in the opposite direction, and allow the arm to fall and cut off the gas. Some inconvenience will at times arise from irregularities in the flow of gas from the main supply-pipe. A properly constructed regulator should, however, compensate perfectly for all ordinary variations in pressure of artificial gas. Natural gas is commonly furnished at much higher pressure and it is necessary to install apparatus to reduce the pressure, a gas-pressure regulator, between the gas main and the thermoregulator. Fluctuations of the temperature within the incubator depend very largely upon the external temperature, especially if its outer walls are not well insulated. The incubator should, therefore, be kept in a place free from draughts of air, where the temperature is fairly constant.

In large modern laboratories, the incubators are built in as special insulated rooms, heated by a gas stove. A regulator of large size is installed to control the supply of gas to the stove. These incubator rooms are very satisfactory and provide quite a range of constant temperature according to the height of shelves from the floor.

Culture-tubes which are being kept in the incubator are likely to become dry if their stay is prolonged. In such cases they should be covered with rubber caps, tin-foil, sealing-wax, paraffin, or some other device to prevent evaporation. If rubber caps are used, they should be left in 1-1000 bichloride of mercury solution for an hour, and the cotton plugs should be singed in the flame, before putting them on (Fig. 47). Some bacteriologists prefer rubber stoppers, which may be boiled and stored in bichloride of mercury solution. Cut the cotton plug even with the edge of the tube; singe it in the flame; push it into the tube about 1 cm., and insert the rubber stopper (Fig. 46).

**Low-temperature Incubator.**—An incubator regulated for so-called “room temperature” is very desirable for the cultivation of bacteria upon gelatin and for the bacteriological analysis of water. In our climate the temperature of the rooms of the laboratory often reaches a point at which gelatin melts, and for this reason in a low-temperature incubator provision has to be made for cooling when the room temperature is too high as well as for heating when it is too low.

A form of incubator devised by Rogers<sup>1</sup> for this purpose consists of a refrigerator or of a specially constructed chamber heated by electricity and controlled by an electric thermoregulator. Below is given a description of an incubator constructed according to Rogers' plans. This incubator has been in use for some time and has given entire satisfaction since the precautions noted below were followed. There would appear no reason why this incubator should not be employed for high temperatures as well as for low, but so far it has been run at 22° C. The temperature has kept very constant. The incubator consists of a refrigerator, 30 inches high, 24 inches wide, 18 inches from front to back, all outside measurements. Instead of the ordinary drip pipe, there is a coil of 1-inch galvanized iron pipe run down the back of the cooling chamber attached water-tight to the ice tank. From the bottom of the cooling chamber the coil runs up perpendicularly nearly to the bottom of the ice compartment, and then runs horizontally through the wall of the refrigerator. A bracket on the outside supports a drip-pan. A thermometer encased in a fenestrated metal jacket is inserted about half way up on one side. A lump of ice, about 50 pounds, placed in the ice compartment serves to keep the temperature sufficiently cool. In summer doubtless more ice will be required.

For heating, an ordinary 16-candle-power electric bulb is used, and the electricity is obtained from the public supply. The wire is run through one of the walls, and a part of the current

<sup>1</sup>L. A. Rogers. On electrically controlled low temperature incubators. *Centralblatt für Bakteriologie*, etc., Bd. XV, Abt. II, pp. 236-239, Sept. 23, 1905.

is made to operate a horse-shoe magnet, and another part is conducted through the lamp used for heating.

The accompanying diagram (Fig. 53), will serve to show the arrangement.

A telegraph key is used to supply the horse-shoe magnet which is inserted in the heating circuit in such a way that when the armature is attracted toward the magnet the circuit is completed and the lamp is consequently lighted. The part of the current, *a*, supplying the magnet first passes through a small

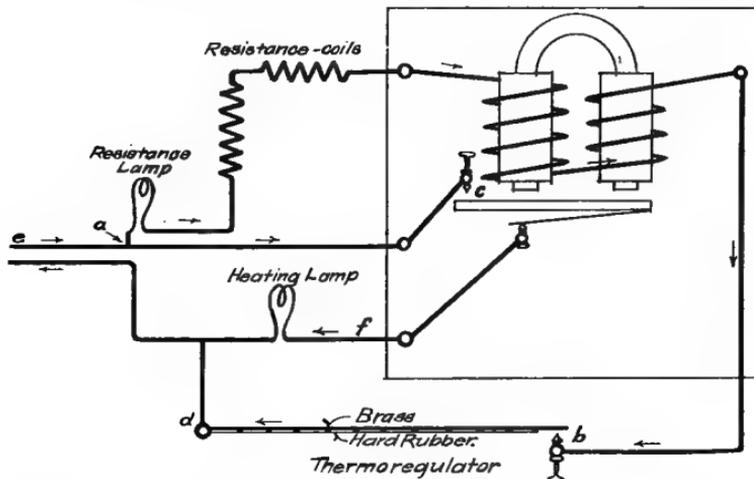


FIG. 53.—Diagram of electric regulator for low-temperature incubator.

lamp and through two resistance coils so as to reduce the current. It then passes through the magnet, and is continued on to the set-screw, *b*, which is so placed that when the thermoregulator comes in contact with it the circuit is complete. The regulator consists of a strip of hard rubber and a strip of brass riveted together. One end is fixed, while the other is free, and when it is heated it tends to bend toward the metal side, when it cools it bends toward the rubber. The brass strip is 15 inches long,  $\frac{1}{2}$  inch thick, and  $\frac{1}{2}$  inch wide; the rubber strip is  $\frac{1}{4}$  inch thick,  $\frac{1}{2}$  inch wide, and a little less than 15 inches long. In the diagram the end is fixed at *d* and is free at *b*. When it is heated, the free

end travels away from the set-screw at *b*; when it cools, it moves toward the set-screw. Rogers also recommends a regulator made of invar and brass instead of hard rubber and brass. Where invar is used instead of the hard rubber the dimensions for the two metals are the same as those given for the brass strip in the hard-rubber-brass regulator just described. As is evident from the description, the circuit controlling the magnet is closed whenever the free end of the regulator comes in contact with the set screw at *b*. When this circuit is closed the magnet attracts the armature, and the heating circuit is closed by the contact formed at *c* between the armature and the set-screw. In the diagram this point of contact is put to one side for the sake of clearness, but as a matter of fact in the instrument in use, the set-screw is above and between the ends of the horse-shoe magnet, and comes in contact with the armature which is extended upward in the shape of a tongue. From the description just given it will be noted that the thermoregulator does not control the heating directly, but indirectly through the electro-magnet.

Certain precautions have been found necessary in practice in order to obtain satisfactory results with this incubator. The set-screw against which the armature strikes at *c* should be so set that the armature does not come in contact with the magnet. In the apparatus described above there is a space of about  $\frac{1}{8}$  inch between the armature and the magnet when contact takes place between the set-screw and the armature. If the set-screw does not project far enough to prevent the armature from coming in contact with the magnet, the armature may adhere to the magnet even after the current is broken at *b*, and when this is the case of course the lamp remains lighted, and the temperature may run up too high. This sticking of the armature to the magnet is said to be due to the residual magnetism left in the core of the magnet. When the current passing through the magnet is broken by the excursion of the end of the thermoregulator away from the set-screw at *b*, the armature is pulled away from the magnet by a coiled spring. Another important precaution is

that the points at which contact is made and broken, *b* and *c*, should be tipped with platinum. A small piece of platinum wire inserted into the ends of the set-screws and a few square centimeters of platinum foil riveted to the opposite point of contact, meet the requirements. If platinum is not used at these points oxidation takes place and prevents contact. The set-screw at *b* is set by experiment for the temperature desired. The further the point of the set-screw projects toward the free arm of the regulator, the higher the temperature maintained.

Electrically heated and electrically regulated incubators for any desired temperature are now to be found on the market. Their initial cost is rather high and, as a rule, they are adapted for use with only one kind of electric current. The exact current available should be stated in ordering.

In many places electric current is not constantly available and in the field one often has to work without gas. Highly satisfactory oil heated, water-jacketed incubators came into very general use in field laboratories in England and France during the war.

#### CULTIVATION OF ANAEROBIC BACTERIA

**Deep Stab Culture.**—Bacteria which cannot grow in the presence of atmospheric oxygen may be successfully cultivated by methods in which the oxygen is excluded or its concentration diminished. The simplest procedure, first practised by Liborius, is to make deep stab cultures into freshly solidified alkaline glucose agar. The agar quickly closes over the needle track and any traces of oxygen introduced into the depths of the agar are absorbed and reduced by the glucose in the presence of the alkali. The bacteria thus find at various points along the puncture all variations in partial pressure of oxygen from almost complete absence up to the concentration existing in the atmosphere at the surface of the medium. Obligate anaërobes begin to grow near the bottom and, as the gases produced replace the air above, the growth extends upward, often even entirely to the surface.

**Veillon Tube Cultures.**—Isolated colonies of anaërobic bacteria may be obtained by a modification of this tube method of Liborius, which seems to have been used first by Veillon. The principle of the method has been given on page 116.

**Fermentation Tube.**—Anaërobic bacteria grow excellently in the Smith fermentation tube filled with glucose broth, especially if a small piece of naturally sterile liver or kidney from a small animal, or a few cubic centimeters of naturally sterile defibrinated blood be added to the medium in the tube. Glucose gelatin to which litmus has been added also furnishes a medium in which anaërobes will grow abundantly without any special precautions to protect them from oxygen or from the air.

**Removal of Oxygen.**—Anaërobic conditions may be furnished by pumping out the air from a container in which the cultures have been placed, a method employed by Pasteur. The oxygen may be absorbed from the air by a mixture of pyrogallic acid and alkali. Buchner's method is carried out as follows: Into a bottle or jar, which can be tightly stoppered, pour 10 c.c. of a 6 per cent solution of sodium or potassium hydroxide, for each 100 c.c. of air contained in the jar. Add one gram of pyrogallic acid for each 10 c.c. of solution. The culture-tube is placed inside of the larger bottle or jar, supported above the bottom, and the stopper, smeared with paraffin, is inserted. The mixture of pyrogallic acid and potassium hydroxide possesses the property of absorbing oxygen.

*Wright's Modification of Buchner's method:* The tube of culture-medium is plugged with *absorbent cotton*, using a plug of large size. The culture-medium is inoculated in the usual way. The plug is cut off close to the neck of the tube, and is then pushed into the tube about 1 centimeter. Now allow a watery solution of pyrogallic acid to run into the plug, and then a watery solution of sodium or potassium hydroxide. Close quickly and tightly with a rubber stopper. Wright recommends that the first solution be freshly made and consist of about equal volumes of pyrogallic acid and water, and that the second solution contain 1

part of sodium hydroxide and 2 parts of water. With 6 inch test-tubes,  $\frac{3}{4}$  inch diameter, the amounts advised are  $\frac{1}{2}$  c.c. solution of pyrogallic acid and 1 c.c. solution of sodium hydroxide.

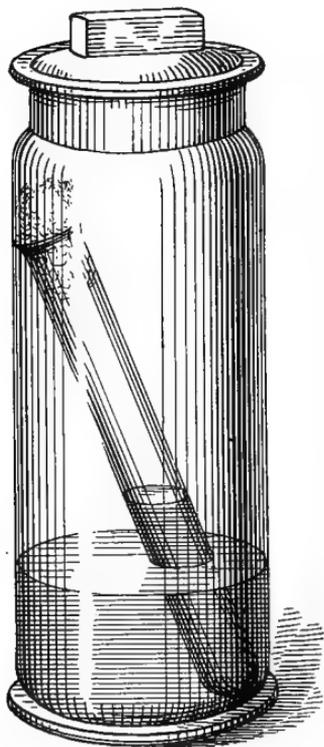


FIG. 54.—Arrangement of tubes for cultivation of anaërobes by Buchner's method.

*Hydrogen Atmosphere.*—The most perfect anaërobic conditions are obtained by replacing the air with hydrogen in a perfectly air-tight container. The method of hermetically sealing such containers full of hydrogen by melting the glass in a flame is really too dangerous to be recommended. The apparatus devised by Novy is most convenient and has practically superseded all other devices for cultivation of anaërobes in hydrogen. The Novy jar is especially valuable for plate cultures. In using this jar, all ground-glass surfaces should be thoroughly coated with a fairly stiff mixture of bees wax and olive oil so as to make all joints air-tight. Rubber gascots or packing should never be employed between the ground-glass surfaces, regardless of the fact that many dealers furnish them for this purpose. After the plate cultures or tubes have been

put into the lower section of the jar, the cover is put on so that the flanges fit together perfectly. A heavy rubber band may then be passed around the circumference of the flanges to cover the circle of contact. Finally two or three clamps, the jaws of which are cushioned with cork or with rubber, are fastened on the flanges, pressing them firmly together. The jar is now attached to a source of pure hydrogen so that the gas enters at the top of the jar. The other opening is connected with a wash bottle containing water which serves as a valve. Hydrogen is

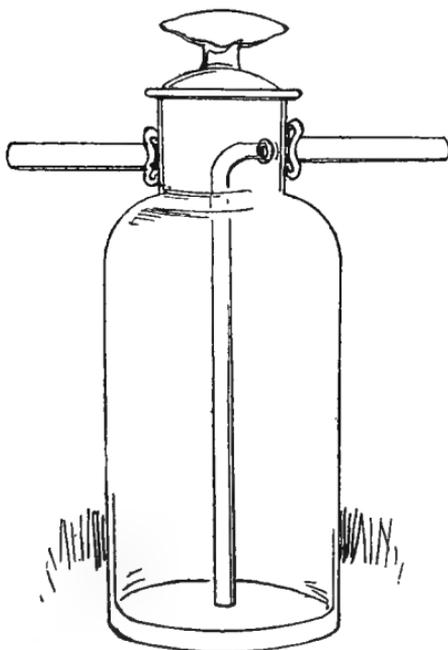


FIG. 55.—Bottle for tube cultures. (*After Novy.*)

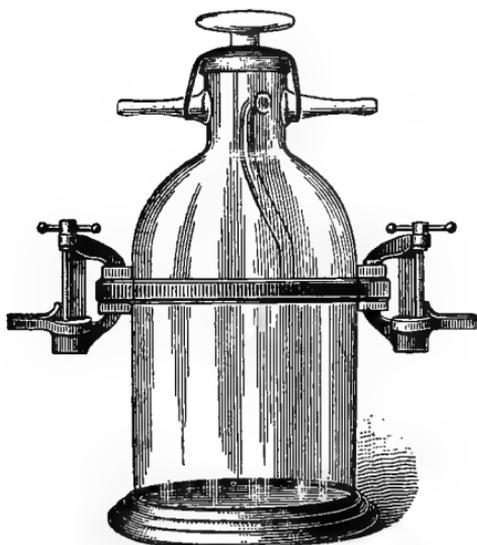


FIG. 56.—Apparatus for Petri dishes or tubes—  
gas or pyrogallate method. (*After Novy.*)

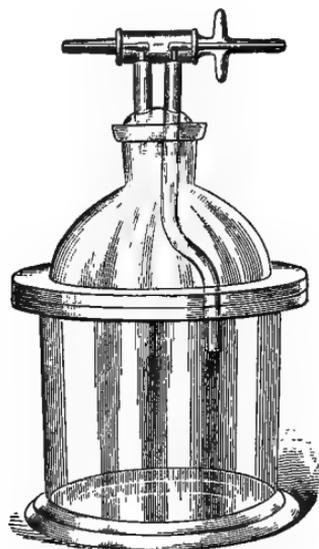


FIG. 57.—Apparatus for plates  
or tubes—gas, pyrogallate or vac-  
uum method. (*After Novy.*)

passed through the jar for two hours or more. It is well to keep all flames away from the apparatus as a precaution against explosion of the hydrogen when mixed with air.

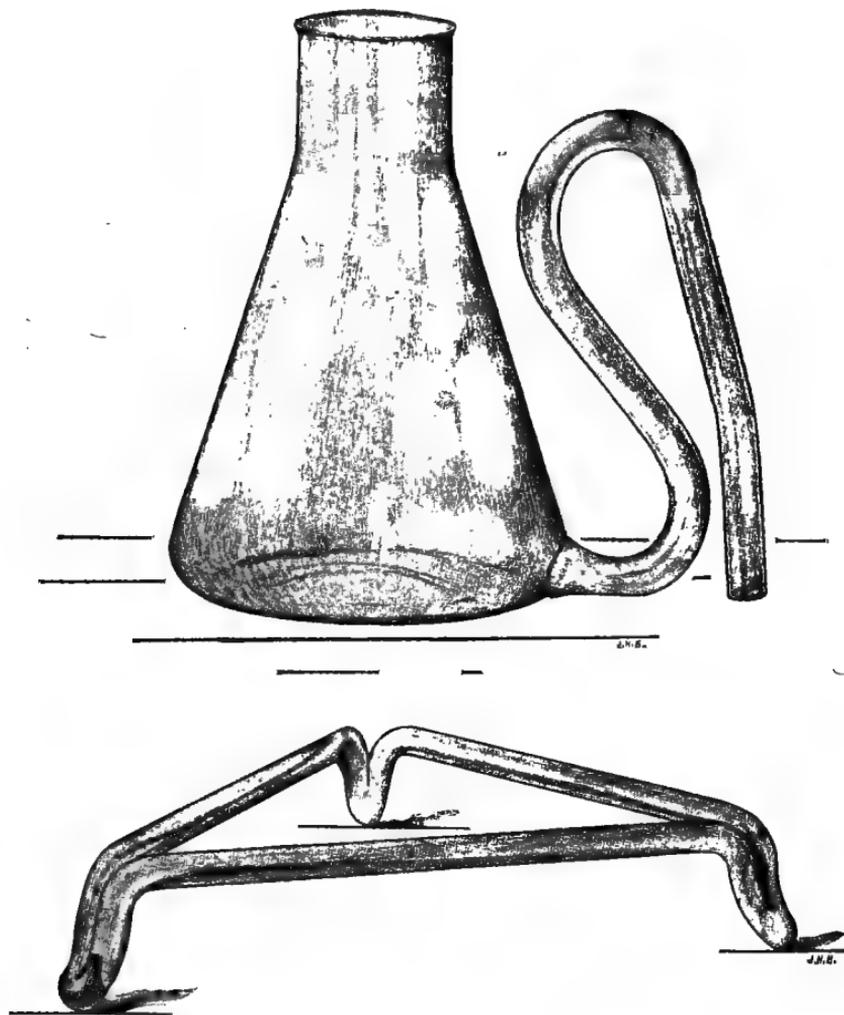


FIG. 58.—Tripod and siphon flask for anaërobic culture by combined hydrogen and pyrogallate method.

The hydrogen is generated by the action of 25 per cent sulphuric acid on granulated zinc. It should be purified by passing through a wash bottle of alkaline lead acetate solution, a second

one containing a solution of potassium permanganate and a third of silver nitrate. In diluting sulphuric acid, the acid must be poured slowly into the water, and the mixture cooled in a bath of cold water, or under the tap. Carelessness in diluting this acid may allow violent boiling to occur, sometimes with serious consequences.



FIG. 59.—An aerobic organism (potato bacillus) that will not grow under a cover-glass.

For critical work in anaerobic culture it is well to combine the pyrogallate and hydrogen methods. This is readily accomplished by placing the Petri dishes on a low glass tripod with a small amount (2 grams) of pyrogallic acid beneath them on the bottom of the Novy jar.<sup>1</sup> On top of the stack of Petri dishes is placed a small flask containing strong solution of sodium hydroxide, and provided with a siphon spout (see Fig. 58). A rubber tube is attached to this spout and leads down to the floor of the jar. After hydrogen has been passed through the jar and it has been finally closed, a slight tipping to one side starts the flow of the alkali through the siphon and so makes the pyrogallic acid available to absorb the last traces of oxygen.

<sup>1</sup> MacNeal, Latzer and Kerr, *Journ. Infect. Diseases*, 1909, Vol. VI, p. 557.

**Further Anaërobic Methods.**—Numerous other expedients have been employed for the cultivation of anaërobes. Koch covered part of the surface of a gelatin plate with a bit of sterilized mica or a cover-glass. Such a method suffices to prevent the growth of strictly aërobic forms but rarely suffices for the successful culture of strict anaërobes. Covering the surface of the medium with sterile liquid paraffin is a more perfect means of excluding air.

In all anaërobic culture methods, the presence of one or more reducing substances in the culture medium is of great importance. Those commonly employed are glucose, litmus and native protein.

## CHAPTER VI

### METHODS OF ANIMAL EXPERIMENTATION

**Value of Animal Experimentation.**—The importance of experimentation upon animals in the development of our knowledge concerning disease-producing micro-organisms can hardly be over-estimated, and animals must be used in considerable numbers in any adequate presentation of the subject to a laboratory class in pathogenic bacteriology. Only in this way has it been possible to discover the causal relation of bacteria to disease and the way in which diseases are transmitted, and it is only by the use of animals that this information can be presented first-hand to students. The inoculation of animals also provides accurately controlled material for studying the course and termination of the disease as well as the gross or microscopic lesions produced by it.

**Care of Animals.**—Laboratory animals should be housed in a light, well-ventilated room which should be heated in winter to about 60° F. If possible a run-way in the open air should be provided. The fixed cages may be constructed with wood or steel frames, but at least the front and preferably both front and back should be made of strong wire netting to provide ample ventilation. For rats and mice it is well to provide an enclosed perfectly dark space inside the cage into which these animals may retire. Smaller movable cages must also be provided for animals acutely sick and those infected with dangerously communicable diseases. These must be sterilizable, and wood should not be used in their construction. Glass jars with weighted covers of wire netting are useful for mice and rats, and for larger animals such as guinea-pigs, rabbits and cats, cages of galvanized iron and wire netting are used. Pigeons may also be kept in such

cages. Very large animals, such as monkeys and dogs, require specially constructed cages. Laboratory animals should receive very careful attention. They should be supplied with new food at least once daily and with clean water twice a day. If food remains at the end of the day, it should be removed and a smaller amount given for the next day. The cages should be completely emptied and cleaned at least once a week, the refuse being incinerated. The animal house should be screened, and insects of all kinds given careful attention. It will be found practically impossible to control the lice and fleas, but winged insects, especially biting varieties, may be kept out; and bedbugs, which sometimes gain entrance on new lots of guinea-pigs or rats, should not be allowed to remain uncontrolled. These possible carriers of infection require serious consideration as sources of confusion where experimental investigations are being carried out, not to mention the element of danger to the human individuals in the neighborhood.

**Holding for Operation.**—Animals to be inoculated or operated upon must be held in a fixed position. Many special mechanical holders have been devised for the various animals, but these are not necessary or especially useful. A pair of long-handled hemostatic forceps with lock, or a pair of placental forceps with lock, will be found most serviceable in handling mice or rats, the loose skin of the animal's neck being caught in the forceps. Guinea pigs are best held by an assistant, the thumb and forefinger of one hand encircling the thorax just behind the fore legs and the other hand holding the hind legs stretched out. Rabbits are held by the ears and hind legs with the body stretched over the knee. Monkeys are to be handled with thick gloves and should be caught around the neck from behind with one hand and by the pelvis or hind legs with the other. A second assistant is required to hold the fore legs. For all work which would cause any considerable pain the animal must be anesthetized, either by putting it into a closed compartment with the anesthetic or by use of a cone. Anesthesia is also necessary when delicate

manipulations are to be carried out. For operations requiring some time the animal is fastened to a board with stout cords, or is held by means of a specially constructed animal holder.

**Inoculation.**—Infectious material may be introduced into the animal body in various ways. The most common methods are injection under the skin and injection into the peritoneal cavity. The hair should be removed from the site selected. A sterilized hypodermic syringe is used, and it is again sterilized by boiling after use. Subcutaneous injection is usually made in the thoracic region as one easily avoids penetrating the chest cavity. For intraperitoneal injection the needle is quickly thrust through the abdominal wall.

*Inoculation into the cranial cavity* is practised especially in studying rabies. The animal, rabbit or guinea-pig, is anesthetized and the scalp is shaved. An incision through the scalp about 8 to 10 mm. long is made at the left of the median line and parallel with it, a little in front of a line connecting the external auditory openings. The scalp is then forcibly drawn over to the right and a hole drilled through the skull at the right of the median line. A sharp-pointed scalpel may serve the purpose of a drill. The needle is then inserted into the cerebral substance nearly to the floor of the cranial cavity and the material (0.1 to 0.5 c.c.) injected. Any blood or fluid is taken up with sterile absorbent cotton. The skin is replaced in its original position and may be dressed with cotton and collodion, although dressing may be omitted altogether.

*Inoculation into the circulating blood* is a method of special importance. In rabbits intravenous injection is easily done. The hair is removed from the ear over the marginal vein, and the vein is dilated by application of a hot towel, after which the skin is wiped dry. An assistant constricts the base of the ear to congest the vein and the needle is easily inserted into it. Other veins on the ear may be used, but they are not so easily penetrated by the needle. In rats, guinea-pigs or monkeys, intravenous injection is not so simple and it is easier to inoculate these animals

by intracardiac injection. For this purpose the animal is etherized and the precordial region is shaved and disinfected. The material to be injected is taken up into a Luer glass syringe. A second syringe, empty, with needle attached, is used to puncture the chest wall and the heart, preferably the wall of the right ventricle. The needle is introduced in the inter-costal space directly over the heart and near the border of the sternum. The appearance of blood in the previously empty syringe gives notice that the cavity of the heart has been entered. The syringe is now detached from the needle and the other syringe which contains the material to be injected is quickly substituted for it. The injection is made slowly.

**Other Sites for Inoculation.**—Many other regions are easily reached with the injection needle, such as the pleural cavity, the chambers of the eye, the spinal canal, the interior of muscles, and the substance of the testis.

**Subcutaneous Application.**—Inoculation may be accomplished without using a syringe if desired. The skin and mucous membranes may be scratched with a needle or other instrument and the infectious material applied to the slight wound thus made. A small pocket may be made under the skin by making a small incision and introducing a blade of the forceps to separate the skin from the underlying muscle; and into such a pocket one may introduce solid material, bacteria from a culture, pieces of tissue, garden soil or splinters of wood, with accompanying bacteria. The opening of the pocket is closed by cauterization or sealed with collodion.

**Alimentary and Respiratory Infection.**—Animals are sometimes infected by feeding the virus, occasionally by injection into the rectum. Infection of the respiratory tract by spraying infectious material in the air breathed by the animal is rarely employed.

**Collodion Capsules.**—Bacteria may be cultivated in the living body of an animal, without infecting the animal, when they are enclosed in collodion capsules. Their soluble products are

able to diffuse through the collodion, while the animal's fluids may pass into the sac to nourish them. These capsules were originally made by dipping the round end of a glass rod into collodion repeatedly. McCræ's method<sup>1</sup> is easier and more satisfactory. (Fig. 60.)

A piece of glass tubing is taken, and a narrow neck drawn on it near one end. This end of the tube is rounded in the flame and, while still warm, the body of a gelatin capsule is fitted over it, so that the gelatin may adhere to the glass. The capsule is now dipped into 3 per cent collodion, covering the gelatin and part of the glass. It is allowed to dry a few minutes, and is dipped again. In all, two or three coatings may be given. The capsule is filled with water and boiled in a test-tube with water. The melted gelatin is removed from the inside of the capsule



FIG. 60.—Method of making collodion capsules. (After McCræ.)

by means of a fine pipette. The capsule is partly filled with water or broth and sterilized. The capsule may now be inoculated. The narrow part of the glass tube which constitutes the neck must then be sealed in the flame, taking care that the neck be dry. The sealed capsule should be placed in bouillon for twenty-four hours. No growth should occur outside the capsule if it is tight. It may now be placed in the peritoneal cavity of an animal.

A method of making collodion sacs recommended by Gorsline<sup>2</sup> consists in the use of a glass tube, the lower end of which is rounded and closed, except a small hole, which is temporarily filled with collodion. This tube is dipped in collodion and dried, as above. It may now be filled with water. By blowing at the opposite end, the pressure through the hole in the bottom of the glass tube will cause the capsule to loosen so that it comes away easily. Sacs made in this way are soaked in water for 30 minutes, dried and attached to the glass tube by gentle heat. The joint is wound with silk thread and coated with collodion. The sac is then filled with distilled water, immersed in a tube of water and sterilized in the autoclave.

There are also various other methods recommended for making collodion sacs.

Collodion capsules are ordinarily placed free in the peritoneal cavity of the animal, by an aseptic laparotomy. The wound is sutured with silk or catgut and dressed with sterile cotton and collodion.

<sup>1</sup> *Journal of Experimental Medicine*. Vol. VI, p. 635.

<sup>2</sup> *Contributions to Medical Research*. Dedicated to Victor C. Vaughan, Ann Arbor, 1903, p. 390.

*Observation of Infected Animals.*—In nearly every case it will be well to keep a record of weight of the animal from time to time. The temperature may be observed by means of a thermometer in the rectum. It should be inserted a considerable distance, 4 to 8 centimeters in guinea-pigs. Other examinations are made in special cases, such as palpation of the lymph glands in tuberculosis and microscopic examination of the blood in anthrax, trypanosomiasis and the relapsing spirochetoses.

*Post-Mortem Examination* of experimental animals is often of great importance. The body is first soaked in bichloride solution to wet thoroughly the hair and skin. It is then fixed on a board by cords or by nails through the feet, stretched out with the ventral surface exposed. With sterile scissors an incision is carried through the skin in the median line from neck to pubis and branch incisions are carried to the extremities. The skin is reflected with aid of a scalpel and the desired examinations of the subcutaneous structures carried out. The abdominal muscle layer is then seared with a hot iron in the median line as are also the lateral walls of the thorax and with a new set of sterile instruments the seared line is incised so as to expose completely the contents of abdomen and thorax. Heart's blood is obtained by searing the epicardium and puncturing the right ventricle with a sterile glass Pasteur pipette. Any or all the organs of thorax and abdomen may then be removed to sterile glass dishes. Immediate microscopic examination for microorganisms is made by direct slide-coverglass preparations of the fresh material diluted with salt solution and by smearing the fluids and tissues on cover-glasses or slides and staining them by various methods. Cultures are also made and it is important to make plate cultures directly from the animal in all instances in which a mixed infection or possible contamination with extraneous organisms is suspected. To remove the spinal cord, the animal is turned so that the back is exposed, the skin divided by a median incision and stripped back to either side. The muscles are roughly dissected away from either side of the spinal column and the vertebral laminae are

broken through by bone-cutting forceps and the posterior sections of the vertebral arches removed from the lumbar region to the skull. The dural sheath of the cauda equina is firmly grasped by sterile forceps and the entire cord gradually lifted up as the spinal nerves are successively put on a stretch and divided by sterile scissors. Finally the cervical cord or the medulla is cut across and the cord placed in a sterile glass dish. In addition to the immediate examination it is well to place suitable pieces of tissue into fixing fluids for sectioning and histological study, by which the physical relations of the parasites and the tissue elements can be studied as well as the pathological alterations in the latter.



PART II

GENERAL BIOLOGY OF MICRO-  
ORGANISMS

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CHAPTER VII

MORPHOLOGY AND CLASSIFICATION

The minute living things included under the general term *microbe*, are exceedingly various in form and structure as well as in respect to food requirements and physiological activity. The number of different microbes is so great and so great are the difficulties involved in the accurate observation of their various features, that the biological relationships of many of the various forms to each other are not yet determined, and much of the generic and specific terminology in common use rests upon insecure foundation. Nevertheless a certain kind of order has developed in our conceptions of the grouping of micro-organisms.

**Molds and Yeasts.**—The molds are multicellular organisms characterized by the formation of a network (*mycelium*) made up of branching threads (*hyphæ*), and by their special fruiting organs. These threads vary from 2 to 7 $\mu$  in width. Within the group of molds the structure of fruiting organs is used as the most important character from which to determine relationships. The *phycomycetes*, or algo-fungi, are characterized by the presence of sexual reproduction in which the union of two cells gives rise to resting cells, zygospores and oöspores, which are enclosed in a thick wall. The *ascomycetes* are characterized by a septate mycelium and by the occurrence of a spore-sac called the ascus, which usually contains eight spores but may contain a large number in some spe-

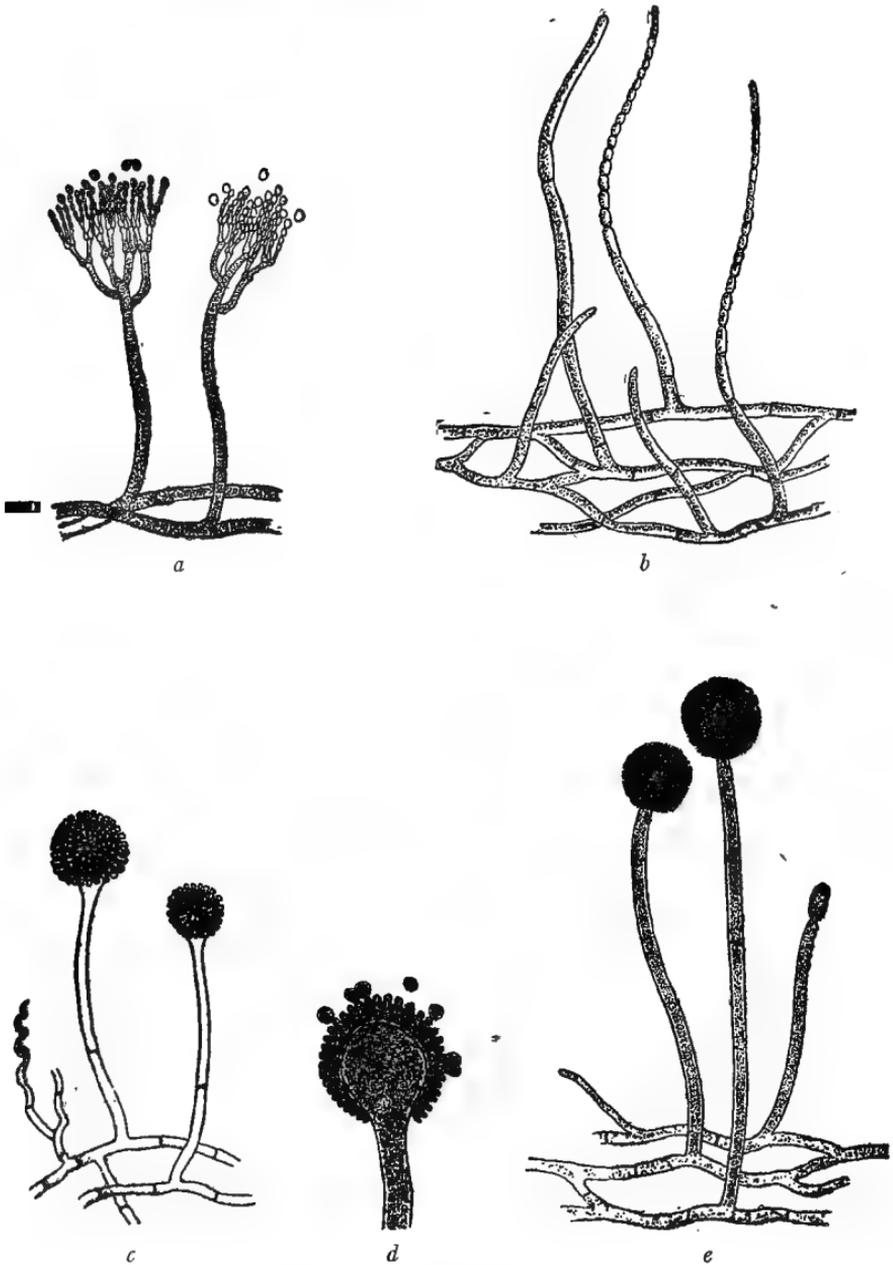


FIG. 61.—Asexual fruiting organs of common molds.  
*a.* *Penicillium glaucum.* *b.* *Oidium lactis.* *c.* *Aspergillus glaucus.* *d.* The same more highly magnified. *e.* *Mucor mucedo.* (*Baumgarten.*)

cies. The common aspergilli belong here. The *basidiomycetes* are characterized by the occurrence of a spore-bearing cell, the basidium, which bears four protuberances called sterigmata (singular *sterigma*) upon each of which is a single spore. Mushrooms and puff-balls belong to this group. Besides these three well-defined classes, there are many kinds of molds and fungi concerning which definite knowledge is still too incomplete for them to be finally placed. These are designated as imperfect fungi,

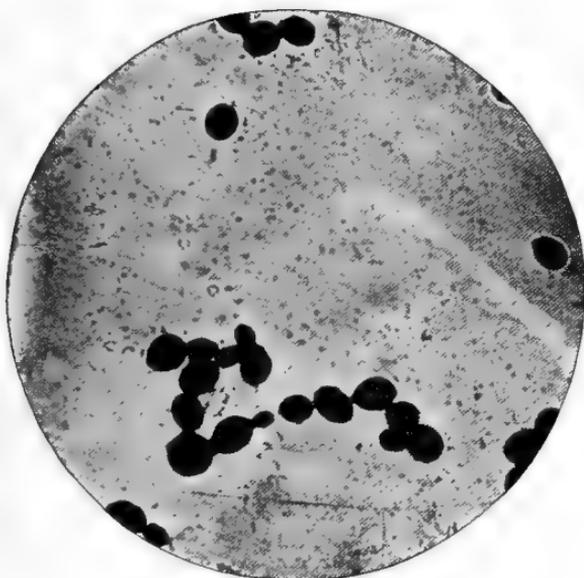


FIG. 62.—Yeast cells stained with fuchsin. ( $\times 1000$ .)

Fungi imperfecti, or perhaps best by the class name, *Hyphomycetes*. In these forms, zygospores and ascospores are unknown; the hyphae are often septate. Reproduction takes place by the formation of conidia only. These are oval or rounded cells produced by transverse division of a filament, usually as a row of conidia at the end of a hypha. The common oidium and many parasitic molds belong in this class. The molds<sup>1</sup> are especially important as causes of disease in plants. Relatively few

<sup>1</sup> For fuller discussion of molds in general see Marshall, *Microbiology*, article by Thom.

diseases of man or other animals have been shown to be due to them, although the first diseases proven to be due to micro-organisms were those caused by certain molds. The molds possess the general morphological features of plants except for the absence of chlorophyll.

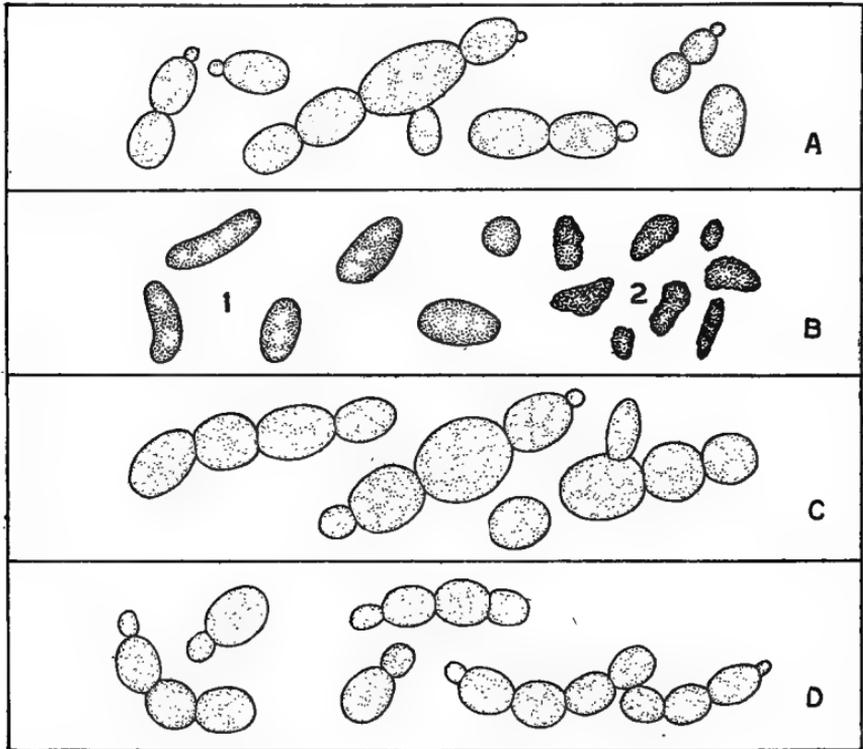


FIG. 63.—Wine and beer yeasts. A, *S. ellipsoideus*, young and vigorous; B, *S. ellipsoideus*, (1) old, (2) dead; C, *S. cerevisiae*, bottom yeast; D, *S. cerevisiae*, top yeast. (Original.)

The yeasts, in general, are ovoid, specialized cells of molds, belonging to several different genera. The true yeasts, genus *Saccharomyces*, belong to the ascomycetes. They do not grow out into long filaments but remain spherical or ovoid. The cells vary from  $2.5$  to  $12\mu$  in diameter. During active growth they reproduce by budding, a smaller portion being pinched off from the parent cell. The true yeasts also form spores inside the cell,

from four to eight typical ascospores. Yeasts are very important in the fermentation industries. Very few of them are pathogenic. Among themselves, the yeasts are subdivided into two groups, (1) those which produce ascospores (*saccharomyces* or true yeasts) and (2) those which fail to produce such spores (*torula* or wild yeasts). They are further distinguished by differences in the form of the cells, but especially by differences in physiological characters, such as the fermentation of sugars and the production of pigments.

In the yeasts there is no definite differentiation of cells. Various cell structures such as cell-wall, nucleus and cytoplasm with vacuoles and granules, can be demonstrated. The cell membrane is, as a rule, more delicate than in the molds. It sometimes secretes a gelatinous material which forms a thick capsule about the cell. The nucleus is shown by appropriate methods of staining as a single more or less sharply defined mass of chromatin. Under suitable conditions the true yeasts produce endospores, usually multiple, and as many as eight in one cell. These are spherical or ovoid masses surrounded by a definite wall, and usually about half the diameter of the yeast cell. When supplied with nutriment these spores swell and burst the mother cell, and then begin at once to multiply by budding. Dry commercial yeast cakes contain spores of yeast along with bacteria and molds; moist, "compressed," yeast contains vegetating yeast cells, also mixed with other organisms.

**Bacteria.**—Bacteria (*schizomyces*) are minute unicellular organisms 0.2 to  $4\mu$  in width, which multiply solely by simple transverse division (fission), ordinarily resulting in the production of two cells of equal size. In many instances the cells remain attached to each other so as to form long filaments.

**Trichobacteria.**—Certain of them grow into long filaments without dividing at once into shorter segments. These forms which are classed as higher bacteria or trichobacteria, suggest a very close relationship to the molds and may, perhaps, be regarded as intermediate between the molds and the lower bacteria.

Many of them exhibit a differentiation of the filament into base and apex, some of them branch in an irregular fashion, and in some there is a suggestion of the formation of special fruit organs. These higher bacteria require further study to determine their relationships. A few of them are important pathogenic agents.

**The Lower Bacteria.**—The lower bacteria, or true bacteria, are always simple in form, the transverse division producing cells, relatively short, and of nearly equal length. Long filaments are produced only by the attachment of many individual cells together, end to end. There are no special fruit organs. The special resistant form, or spore, which occurs in some forms, is produced only inside of the vegetative cell, one cell producing one spore. There are three general forms of bacteria, the sphere (*coccus*, plural *cocci*), the cylinder (*bacillus*, plural *bacilli*), and the spiral or segment of a spiral (*spirillum*, plural *spirilla*). Intermediate forms occur, so that there is not a sharp line between the groups. These three forms are generally accepted as a basis for division of the lower bacteria into three families, the coccaceæ, bacteriaceæ and the spirillaceæ.

*Spherical Bacteria.*—The *Coccaceæ* or cocci are spherical bacteria. They vary in size from about  $0.3\mu$  to  $3\mu$  in diameter.



Staphylococci. Streptococci. Diplococci. Tetrads. Sarcinae.

FIG. 64.

During the process of cell division, a coccus may become elongated somewhat, and after division, the daughter cells may be shortened so that they appear as if compressed against each other. Slightly elongated forms are included among the cocci in certain instances, and especially the lancet-shaped bacteria such as the germ of lobar pneumonia. The recognition of genera within the family is still unsettled. Morphologically five genera have been distinguished by Migula: *Streptococcus*, *Micrococcus*, *Sarcina*,

Planococcus and Planosarcina. The first three do not possess flagella and are non-motile. Streptococcus includes those forms which divide only in one plane so that a thread or chain is produced. Micrococcus includes the cocci which divide in two planes at right angles so as to produce plates, and it also includes those which divide in an irregular fashion so that no definite geometric figure results. Sarcina includes those cocci which divide in three planes at right angles to each other, in turn, so as to produce cubical masses of cells. Planococcus is similar to Micrococcus in all respects except that its members are motile and possess flagella, and Planosarcina includes the motile forms which are in other respects the same as the forms included under Sarcina.

**COCCACEÆ**—Cells spherical, without endospores.

Streptococcus—Division in one plane, forming chains of cells; non-motile; without flagella.

Micrococcus—Division in two planes, forming flat plates of cells, or irregular, forming masses of cells irregularly grouped; non-motile; without flagella.

Sarcina—Division in three planes, forming cubical or package-shaped masses of cells; non-motile; without flagella.

Planococcus—Division in two planes, forming flat plates of cells, or irregular, forming mass of cells irregularly grouped; motile; bear flagella.

Planosarcina—Division in three planes, forming cubical or package-shaped masses of cells; motile; bear flagella.

These genera have not been generally adopted by bacteriologists. The terms Streptococcus and Sarcina are, however, quite generally employed as the generic names for the organisms of their respective groups as defined by Migula, as they had been used in this way before. Micrococcus, however, is commonly employed as a general term for all the members of the family Coccaceæ, and Planococcus and Planosarcina have not been used, because bacterial forms belonging to these genera are exceedingly uncommon and it may even be questioned whether those which have been described might not better be classed with the cylin-

dricul bacteria, in which motility is of frequent occurrence. Other terms in common use as generic names for certain cocci are Diplococcus and Staphylococcus. A diplococcus is a double coccus, two spheres attached together. This grouping by twos is very common and the generic term Diplococcus is employed for those forms in which it is a prominent characteristic. The term Staphylococcus is applied to those micrococci which are grouped in an irregular mass resembling a bunch of grapes.

*Cylindrical Bacteria.*—The cylindrical bacteria, *Bacteriaceæ*, have been subdivided by Migula into three genera, *Bacterium*, *Bacillus* and *Pseudomonas*. The genus *Bacterium* includes those members of the family which are without flagella and are non-motile. *Bacillus* includes those forms possessing flagella distributed over the surface, and *Pseudomonas* is the generic term for those forms with flagella situated at the extremities only (polar flagella).

**BACTERIACEÆ**—Cells cylindrical, straight, non-motile or motile by means of flagella.

*Bacterium*—Cells without flagella, non-motile.

*Bacillus*—Cells motile with flagella distributed over the surface.

*Pseudomonas*—Cells motile with polar flagella.

These genera have not been generally adopted by bacteriologists, and there are serious reasons for dissatisfaction with such a classification of the rod-shaped bacteria. In the first place the names *Bacterium* and *Bacillus* are unfortunate. The former has long been employed as a general term designating any member of the Schizomycetes and its plural, *Bacteria*, is everywhere the common term employed in designating this large group of micro-organisms. Its use in the narrower sense by Migula has not displaced the former signification, and its use in the sense of Migula must necessarily result in confusion. The latter term, *Bacillus*, has long been used very generally by bacteriologists to designate any member of the *Bacteriaceæ* or rod-shaped bacteria, regardless of the motility or distribution

of flagella. A further serious objection is due to the lack of stability in the character selected to distinguish the genera. The flagella may disappear from bacteria ordinarily possessing them as a result of changes in environment and may be again made to appear by reversing the conditions.<sup>1</sup> Furthermore in some groups of bacteria, which seem to be closely related in respect to other characters, morphological and physiological,



FIG. 65.—Bacilli of various forms.

both motile and non-motile forms occur. On the whole the presence or absence of flagella would seem to be too fragile a character to serve as a sole distinction between genera among the rod-shaped bacteria.

The different species of rod-shaped bacteria are very numerous, several thousand different kinds having been described. They vary in width from  $4\mu$  to  $0.1\mu$  or probably less, and in length from  $60\mu$  to  $0.2\mu$ . The very large ones are non-pathogenic species.



FIG. 66.—Sporulation. *a*, First stage showing sporogenic granules; *b*, incomplete spore; *c*, fully developed spore. (After Novy.)

The form is ordinarily that of a straight cylinder of equal caliber throughout its length. Certain slightly curved forms are nevertheless included in the family, although they may perhaps be regarded as intermediate between the bacteriaceæ and the spirillaceæ. Some of the rod-shaped bacteria are of uneven caliber, especially when growing under unfavorable conditions or when spores are produced. The ends of the rod may be pointed, rounded, square-cut or concave. The bacteria may remain at-

<sup>1</sup> Passini: *Zts. f. Hyg.*, 1905, XLIX, pp. 135-160.

tached after cell-division, forming groups of two, *diplo-bacillus*, or many cells remain attached, to form long threads, *strepto-bacillus*. Endospore formation occurs almost exclusively in the bacteriaceæ and the form of the spore-bearing cell differs for different species and is fairly constant for any one species.

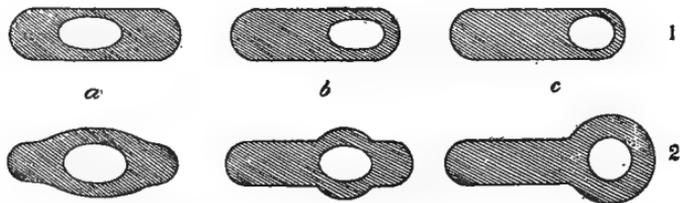


FIG. 67.—Position of spores; resultant forms (diagrammatic). *a*, Median spores; *b*, intermediate spores; *c*, terminal spores; *2a*, *b*, *c*, change in form of cells due to the presence of the spore; *2a*, clostridium; *2c*, drum-stick form. (After Novy.)

The spore, which is always single, may be located at the center of the cell, *median spore*, or at the end, *terminal spore*, or at an *intermediate* point. The spore-bearing cell may retain its normal outline or it may be bulged by the spore. The cell containing a median spore with bulging is called a *clostridium*; one with terminal spore with enlargement of the cell is spoken of as a *drum-stick* or sometimes as a *plectridium*.

*Spiral Bacteria*.—The screw-shaped bacteria, *Spirillaceæ*, have been subdivided into four genera by Migula. The genus *Spirosoma* includes those spirals which are rigid and without motility. Motile cells possessing one, two or three polar flagella are classed in the genus *Microspira*; while those possessing more than three are put in the genus *Spirillum*. The genus *Spirochæta* includes the slender flexuous forms of spirals.

**SPIRILLACEÆ**—Cells circular in cross-section but curved to form a spiral or segment of a spiral.

*Spirosoma*—Cells rigid, without flagella, motionless.

*Microspira*—Cells rigid, motile, with 1 to 3 polar flagella.

*Spirillum*—Cells rigid, motile, with polar tufts of flagella.

*Spirochæta*—Cells slender, flexuous, motile.

Two of these generic terms, *Spirillum* and *Spirochæta*, have long been used, and almost in the sense in which they are employed by Migula. *Spirillum* has frequently been applied to all the Spirillaceæ and especially to those forms which Migula includes in his first three genera, *Spirosoma*, *Microspira* and *Spirillum*. The distinction between *Microspira* and *Spirillum* seems of too slight importance to serve as a basis for the formation of two genera, and indeed the same objection exists here as in the Bacteriaceæ to the use of flagella as a basis for generic distinctions.

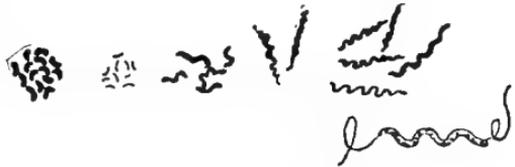


FIG. 68.—Types of spirilla.

Cell division occurs by simple transverse fission in all the spiral bacteria. Endospores are said to be formed by some species.

The group of spirochætes has received much attention in recent years and the propriety of including them in the spirillaceæ may be seriously questioned. Many investigators are inclined to regard them as more properly classed with the protozoa than with the bacteria. It is claimed that these forms multiply by longitudinal splitting and not by transverse fission, and this would at once remove them from the Schizomycetes. The observations are still in dispute and there are good observers who regard transverse fission as the mode of multiplication. Further study is necessary to settle this important question. It is possible that some of these slender spirals may multiply by both methods, or that one species may divide longitudinally and another transversely, but this does not seem probable. For the present it would seem wise to reserve judgment and avoid encumbering the group with new genera until a definite and final agreement has been reached concerning the exact morphological facts. (See page 368.)

*Structure of Lower Bacteria.*—The bacterial cell is enclosed in a relatively stiff cell membrane, which generally retains its form after plasmolysis. Under special conditions of growth many

forms of bacteria become enclosed in a gelatinous capsule. This seems to be a viscid material secreted by the cell through the cell membrane. The motile bacteria possess exceedingly slender hair-like processes, called flagella, which serve as organs of locomotion. These processes apparently take origin from the cell membrane.



FIG. 69.—Bacteria with capsules.

Bacteria without flagella are spoken of as atrichous, those with a single flagellum at one end as monotrichous, those with a flagellum at either end as amphitrichous. When there is a tuft of flagella at the end, the distribution is said to be lophotrichous, and when they are distributed all over the surface the arrangement is called peritrichous.

The internal structure of the bacterial cell has received comparatively little attention. The direct microscopic study of the living cells shows them to be finely or coarsely granular, or sometimes nearly homogeneous. No constant internal structures can be distinguished. Ordinary simple staining with the basic aniline dyes colors the bacterial cell diffusely and intensely, usually without any internal differentiation. The cell membrane between



FIG. 70.—Bacteria showing flagella.

two cells in a chain may remain relatively colorless and so be differentiated from the protoplasm on either side. At times the stainable substance is unevenly distributed in the cell, perhaps grouped at the ends of a rod, or in granules or bands. Under special conditions some bacteria show internal granules of special composition, distinguishable as pigment granules or by their microchemical reactions. Granules which stain with iodine, so-called granulose or glycogen granules, are important features of some kinds of bacteria.

The recognition of the cell nucleus has received special attention. Zettnow, more especially, has shown that the chromatin or essential nuclear substance is present in the bacterial cell as finer or larger granules, sometimes distributed pretty generally and sometimes collected together at one or more places in the cell. The Romanowsky stain and its modifications have been especially useful in differentiation of chromatin from cytoplasm.



FIG. 71.—The formation of spores. (After Fischer from Frost and McCampbell.)

Special movements of the internal granules have been described by Schaudinn as being associated with beginning cell division. For the great majority of bacteria these have not been observed,



FIG. 72.—Bacteria with spores.

and according to our knowledge, the process of cell division is extremely simple. It consists of a progressive constriction and thinning of the cell at the middle until two cells are produced. In

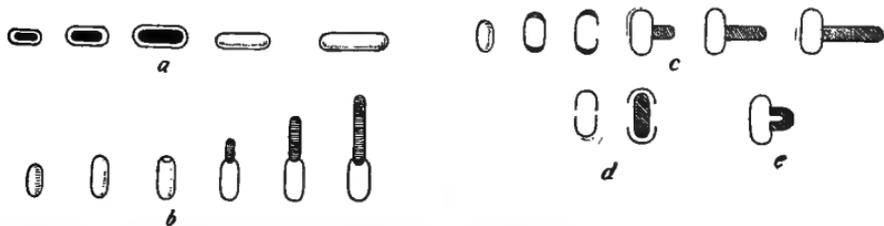


FIG. 73.—Germination of spores. *a*, Direct conversion of a spore into a bacillus without the shedding of a spore-wall (*B. leptosporus*); *b*, polar germination of *B. anthracis*; *c*, equatorial germination of *B. subtilis*; *d*, same of *B. megatherium*; *e*, same with "horse-shoe" presentation. (After Novy.)

some forms the division is completed by a sudden snapping movement.

*The formation of an endospore* begins with the accumulation of chromatin granules in one part of the cell, where they coalesce, lose their contained water and seem to become embedded in an oily or fatty substance and surrounded by a membrane. Very early in the process the spore no longer stains readily. In some forms (*Bact. anthracis*) the cell in which a spore has formed disintegrates rapidly, setting free the spore, while in others (*B. tetani*) the cell may continue its activities after formation of the spore. The spore germinates when conditions again become favorable to active growth. The new cell may burst the spore wall into halves, or at the end, or the spore wall may soften and become a part of the new growing cell.

**Filterable Viruses.**—The difficulty of accurate morphological study is so great as to appear insurmountable in the case of certain microbes which are very definitely recognizable by certain effects which they produce. This is especially true of those living things capable of passing through the fine filters which prevent the passage of small bacteria. The causes of certain diseases exhibit this character, and these have come to be known as filterable viruses. There can be little question that non-pathogenic filterable microbes also exist although they seem to have escaped observation. Accurate knowledge of the morphology of many of these forms remains to be disclosed by future investigation. Meanwhile, the efforts to classify them as bacteria or as protozoa may well be spared. The propriety of including them as living things is, however, only occasionally questioned.

**Protozoa.**—The protozoa or unicellular animals have assumed very great importance as causes of disease during the past twenty years. Fortunately for the systematist, the free-living protozoa had received considerable careful study and the larger groups of protozoa had been well defined before the interest in pathogenic properties had the opportunity to over-shadow morphological study. The number and variety of easily recognizable morphological characters presented by the protozoa are greater than those of the bacteria; and the organisms are, on the whole, larger.

These factors make for more accurate observations of morphological characters, and their more successful employment as a basis of classification.

The protozoan cell is generally larger and more complex in structure than the bacterial cell appears to be, although the dividing line is in places indefinite or even wholly obscure. In general the protozoon shows the typical structure of a single cell of the metazoon. A well-defined nucleus is usually present, sometimes several of them, although in some forms the nuclear material is more or less scattered throughout the cell. Most protozoa exhibit differentiation of the protoplasm into cell organs or organellæ, adapted to perform certain functions. In many protozoa sexual reproduction has been observed, a process involving complex morphological changes. The cells showing these evidences of complex organization resemble in most respects cells of the higher animals, and in fact a colony or group of protozoa may be regarded as representing a transition to the many-celled animals, just as, on the other hand, the bacteria were seen to be connected with the higher plants through the forms of the higher bacteria, the yeasts, the molds and algæ. Physiologically, protozoa differ from bacteria and other plants in requiring more complex nitrogenous food, but this distinction is far from absolute. Doflein divides the protozoa into two substems, (1) Plasmodroma, including those forms which move by means of pseudopodia or flagella, and which exhibit for the most part an alternation of asexual and sexual generations, and (2) Ciliophora, including those forms which move by means of cilia and in which the sexual fertilization gives rise to no special reproductive form of the organism.

The substem Plasmodroma includes three classes, (1) Mastigophora, (2) Rhizopoda and (3) Sporozoa.

**Flagellates.**—In the class *Mastigophora*, are included a great many different organisms, the one common feature being the type of locomotive apparatus, which consists of one or more flagella. The further subdivision of the class has not yet been agreed

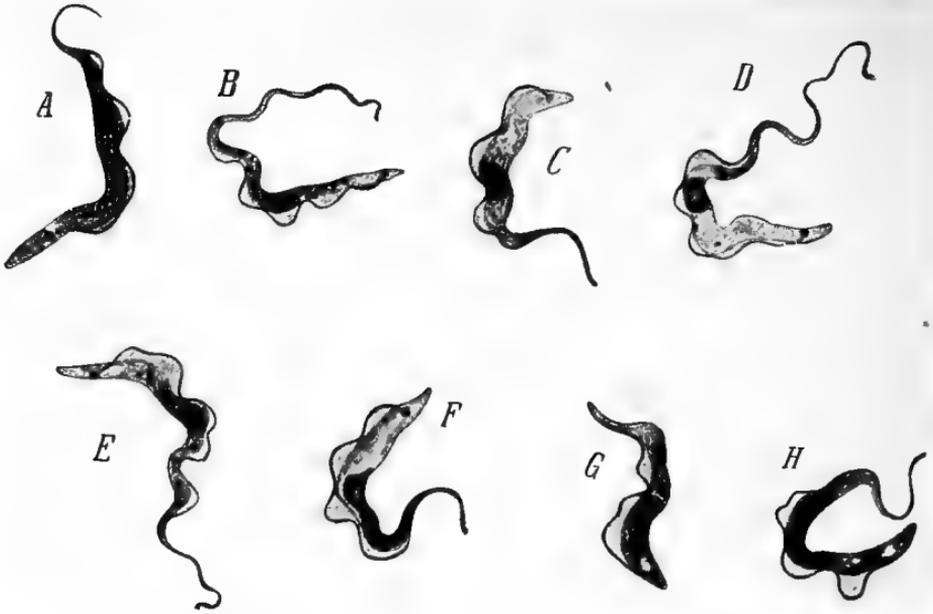


FIG. 74.—The most important trypanosomes parasitic in mammals. A, *Trypanosoma lewisi* (Kent). B, *Tr. evansi* (Steele), Indian variety. C, *Tr. evansi* (Steele), Mauritian variety. D, *Tr. brucei* (Plimmer and Bradford). E, *Tr. equiperdum* (Doflein). F, *Tr. equinum* (Voges). G, *Tr. dimorphon* (Laveran and Mesnil), H, *Tr. gambiense* (Dutton). (From Doflein after photomicrographs of Novy.)

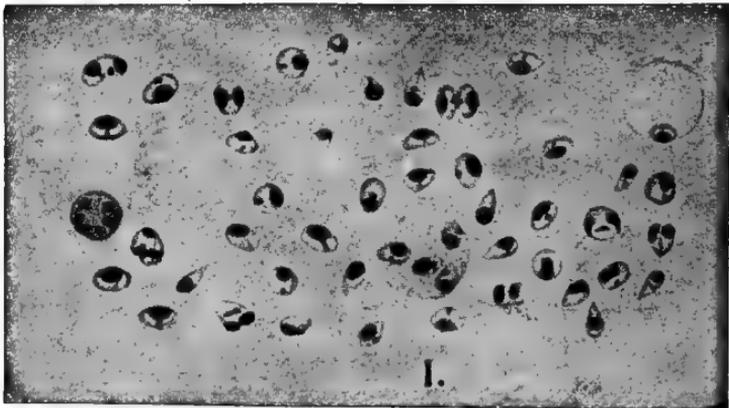


FIG. 75.—*Leishmania donovani*. Various forms obtained by spleen puncture, some free and some inside red blood cells. (From Doflein after Donovan.)

upon, not because of any lack of morphological differences upon which to base a classification, but largely on account of difficulty in estimating the relative importance and meaning of the many

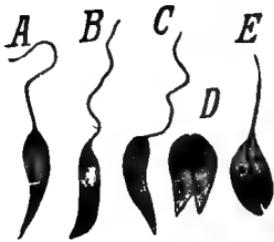


FIG. 76.—*Leishmania donovani*. Various forms of the organism in artificial culture. (From Doflein after Chatterjee.)

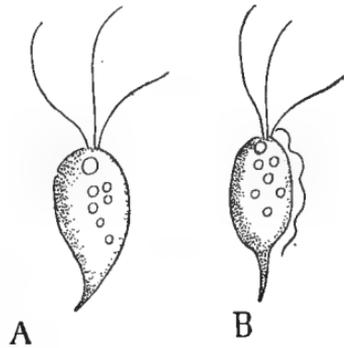


FIG. 77.—*Trichomonas hominis*. (From Doflein after Grassi.)

criteria presented. The genera of particular interest from the pathological standpoint are *Trypanosoma*, *Leishmania*, *Tricho-*

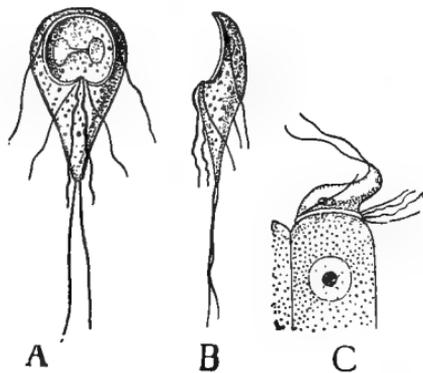


FIG. 78.—*Lamblia intestinalis*. A, Ventral aspect. B, Lateral aspect. C, Attached to an epithelial cell. (From Doflein after Grassi and Schewiakoff.)

*monas* and *Lamblia*. The members of the *Trypanosomata* are characterized by an approximately crescent-shaped body, 10 to 40 $\mu$  in length, flexible and provided with a flagellum which origi-

nates in the endoplasm near one end and passes along the border of the body and finally projects as a free whip at the other end of the cell. As it passes along the border of the cell it is enclosed in a sheath of ectoplasm, which is drawn out into a thin sheet forming an undulating membrane. Multiplication takes place by approximately longitudinal division. *Leishmania* includes a few parasitic forms, for the most part living inside the cells of the host. These organisms are oval, about  $2 \times 3\mu$ , without fla-

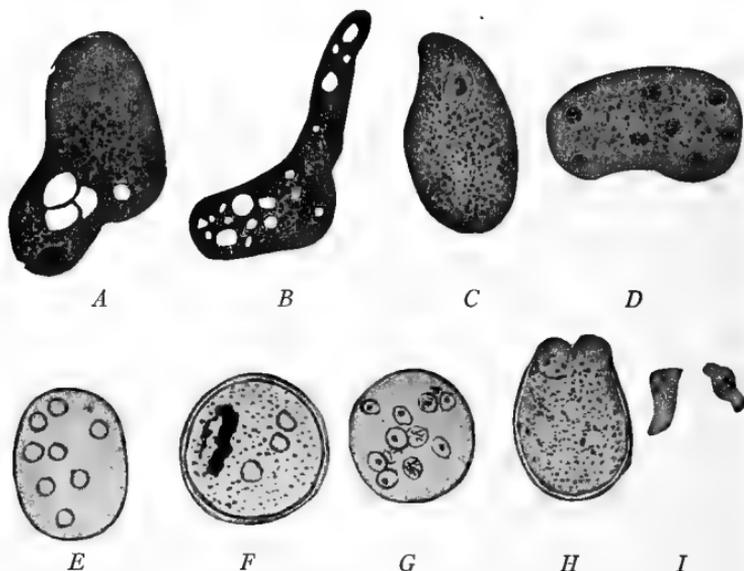


FIG. 79.—*Endamoeba coli* (Lösch). A to C, Various forms of the free amoeba. D, Stage with eight nuclei. E to G, Cysts with various numbers of nuclei. H, Opening cyst. I, Young amoebæ escaped from a cyst. (From Doflein after Casagrandi and Barbagallo.)

gellum or undulating membrane. In artificial culture outside the body, the protozoon grows larger, develops a flagellum and resembles a trypanosome. *Trichomonas* includes pear-shaped organisms 4 to  $30\mu$  in diameter, provided with three or four flagella. Isogamic and autogamic fertilization have been described, and cysts containing numerous daughter cells result from the multiplication following this process. *Lambliã* resembles trichomonas, but the cell is here shaped more like a beet, is provided

with eight flagella and is hollowed out at one side near the rounded anterior end to form a suction cavity.

**Rhizopods.**—The members of the second class, *Rhizopoda*, are characterized by their ability to send out protoplasmic processes to serve for locomotion and also to surround and engulf solid food particles. The two genera, *Amæba* and *Endamæba*, are of chiefest interest. The organisms are masses of protoplasm containing a nucleus, food granules and sometimes vacuoles, and surrounded by a slightly denser more hyaline layer of ectoplasm. The members of the genus *Amæba* are free-living saprophytic forms, while those of *Endamæba* are parasitic. Multiplication occurs by fission after a more or less complex division of the nucleus. Multiple division also occurs, more especially in an encysted condition, and subsequent to a possible autogamic fertilization.

**Sporozoa.**—The third class, *Sporozoa*, is made up entirely of parasitic forms, which at some stage in their life history multiply by division into numerous daughter cells, which are enclosed in a protective envelope to form a spore. The spores serve to distribute the species to other hosts. In cases where there are special adaptations for distribution, as for example by means of intermediate hosts, the protective envelope may be absent. An enormous number of parasitic micro-organisms are included in this group. The genera of greatest present interest from the pathological point of view are *Eimeria* (*Coccidium*), *Plasmodium*, *Babesia* (*Piroplasma*) and *Nosema*.

*The Coccidia.*—*Eimeria* includes a number of intracellular parasitic forms, perhaps better known as coccidia. The small parasite resulting from asexual division is called a merozoit. It is somewhat spindle-shaped and 5 to 10 $\mu$  long. This merozoit penetrates an epithelial cell of the host, grows at the expense of the cell to a spherical mass 20 to 50 $\mu$  in diameter, and eventually divides into numerous (sometimes as many as 200) merozoits, which become free by rupture of the host cell. Besides this asexual mode of multiplication, there is also a sexual cycle. Some of

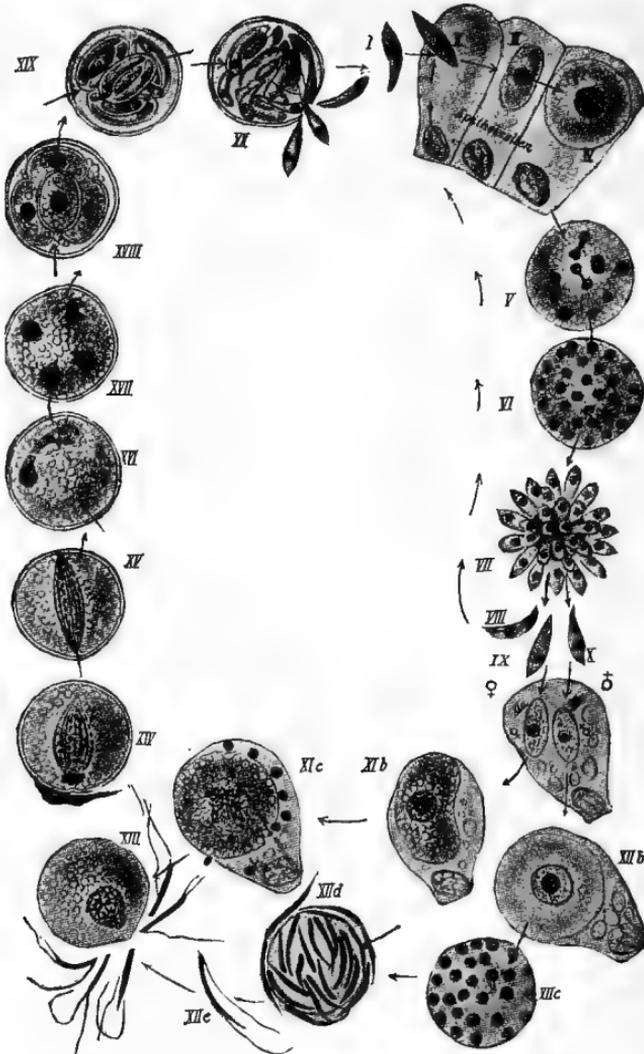


FIG. 80.—Developmental cycle of *Eimeria (Coccidium) schubergi*. I, Sporozoite; II, sporozoite penetrating a cell of the host; III and IV, stages of growth; V to VII, asexual multiplication; VIII, agamete or merozoite beginning again the asexual cycle; IX and X, agametes destined to form sexual cells (gametes); XI, a to c, development of the macrogamete; XII, a to d, development of microgametes; XIII, fertilization; XIV and XV, the fertilized cell or zygote; XVI and XVII, metagametic division of the zygote; XVIII, formation of the sporoblasts; XIX, formation of the spores and sporozoites; XX, sporozoites emerging from the spores and from the oöcyst. (From Doflein after Schaudinn.)

the growing parasites do not divide into merozoites but become differentiated into male and female cells (gametocytes). The male gametocyte gives rise to a large number of elongated motile microgametes, one of which approaches and penetrates the ripened macrogamete. The nuclei of the two gametes fuse and the fertilized cell quickly forms a protective wall around itself and then divides into eight cells which are enclosed in pairs within secondary cysts known as spores. This form of the organism passes out of the host, and after a passive existence in the external

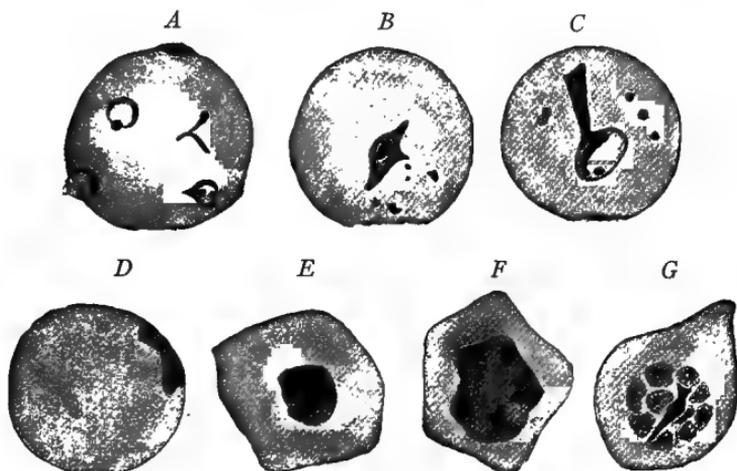


FIG. 81.—Forms in the asexual cycle of *Plasmodium falciparum*, the parasite of tropical malaria. A, Multiple infection of a red blood cell; B to E, various forms of the growing parasite; B and C show also the Maurer granulations; F, full-grown parasite with many nuclei; G, Segmentation. The pigment is shown in E, F and G. (After Doflein.)

world may gain entrance to a new host, whereupon the spore wall ruptures and the enclosed cells, sporozoites, emerge to penetrate new host cells.

*The Plasmodia.*—*Plasmodium* includes the malarial parasites, forms parasitic in red blood cells and closely analogous to the coccidia in the asexual cycle. The gametocytes are also similar to those of *Eimeria* except that the gametes are not formed within the mammalian host, but only after the blood has been drawn. The sexual cycle of development takes place in a definite secondary

host, the mosquito. In the stomach of this insect the gametes unite and the fertilized cell (oökinet) actively penetrates the epithelium and beneath it develops into a large oöcyst, 30 to 90 $\mu$  in diameter, enclosed in the elastic tunic of the stomach wall of the mosquito. As the oöcyst enlarges, the nucleus divides and eventually the cytoplasm also. The nucleus of each of these masses (sporoblasts) then divides many times. Each nucleus, together with a small amount of protoplasm, separates and then elongates into a slender thread-like sporozoit ( $14 \times 1\mu$ ). As many as 10,000

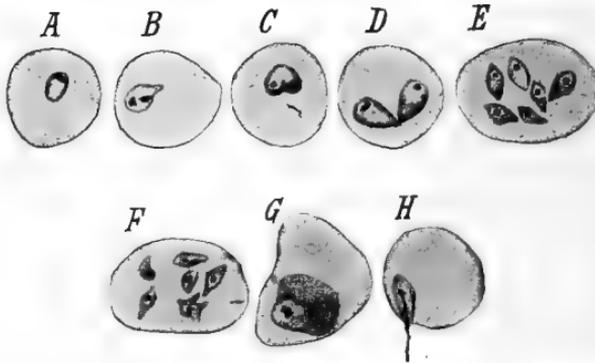


FIG. 82.—*Babesia muris*. A, Young form in a red blood cell. B, Form with two nuclei. C and D, Binary division. E and F, Multiple infection; ameboid forms in F. G, An exceptionally large individual (gametocyte?). H, Form with a thread-like process (flagellated stage?). (From Doflein after Fantham.)

of these may be produced in one oöcyst. The cyst bursts into the body cavity of the mosquito and the motile sporozoits circulate through the body of the insect and eventually assemble in the cells of the salivary glands. From these they escape with the secretion and gain entrance to the wound made by the mosquito in biting.

*Babesia*.—A number of parasites of the red blood cells are classed in the genus *Babesia* (*Piroplasma*). These resemble the members of the preceding genus very closely but multiple division (segmentation) does not seem to occur in the asexual cycle. The multiplication seems to be by longitudinal division into two daughter cells. The characteristic form is pear-shaped, but irregular amœboid forms are also common. Flagellate stages existing in the blood plasma have also been described. The sexual

cycle takes place in a tick, and is in part analogous to that described for *Plasmodium*. The stages are not fully known, but the infectivity of the tick is transmitted to the offspring in the case of the Texas-fever tick (*Rhipicephalus (Boöphilus) annulatus*).

*Nosema*.—The sporozoa above described all belong to the Telosporidia, organisms which end their individual existence at

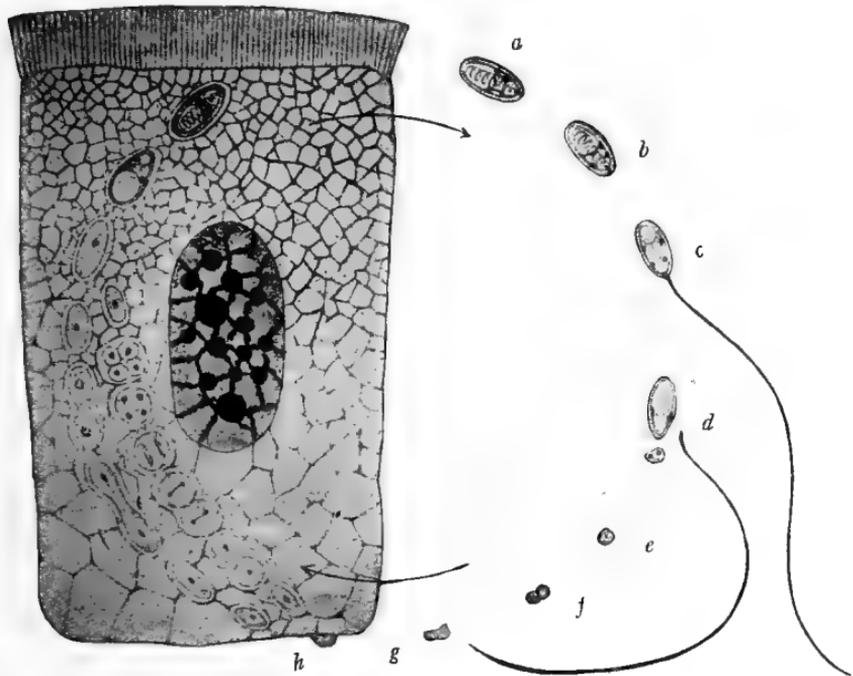
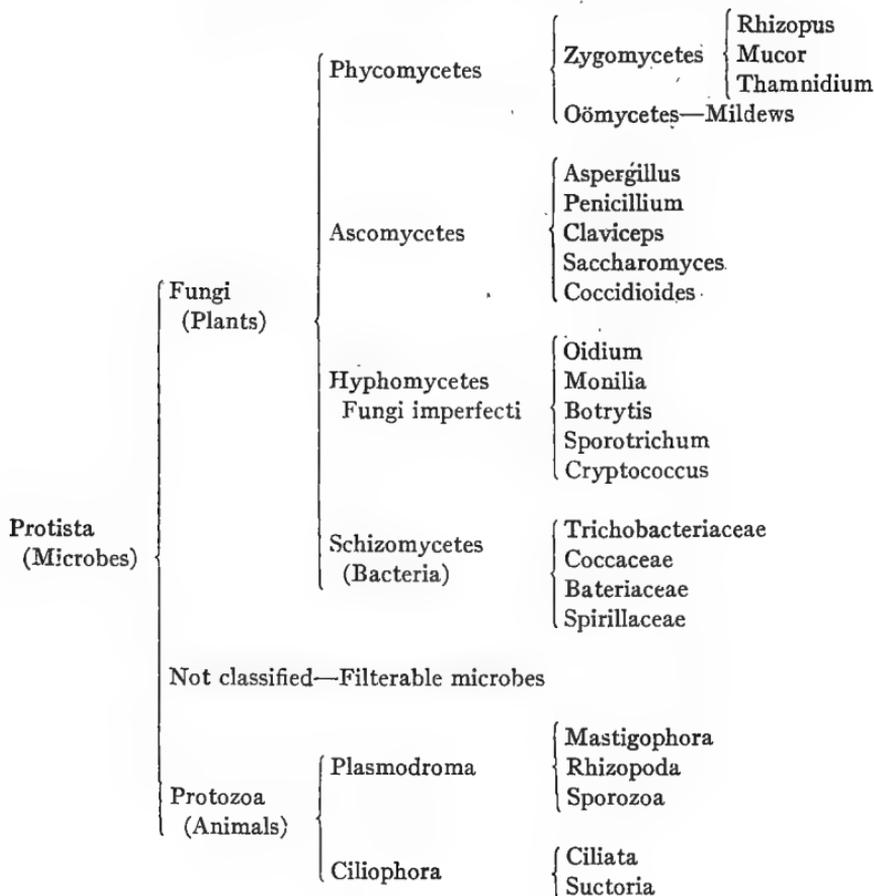


FIG. 83.—Diagram of the developmental cycle of *Nosema bombycis*. *C*, Cell of the intestinal epithelium containing asexual multiplication forms and showing their transition into spores. *a*, *b*, *c*, Spores, the last with polar thread. *d*, Ameboid form emerging from the spore to penetrate a new host cell at *h*. (From Doflein after Stenpell.)

the stage of spore formation. A second large subdivision of the sporozoa is named Neosporidia. In this group the spores are formed without terminating the existence of the individual. The parasites of this type are comparatively small and not very well known. They are often spoken of as microsporidia or psorosperms. The best-known form is *Nosema bombycis*, the cause of Pébrine in silkworms.

**Ciliates.**—The second substem of the protozoa, Ciliophora, is distinguished by the locomotive organs, numerous cilia which cover most of the body surface, and by the possession of two distinctly different nuclei, one apparently concerned with nutrition of the cell and the other definitely associated in an important manner with the sexual reproduction. Multiplication takes place by transverse division into two daughter cells or by budding. In the parasitic forms this may take place within a protecting wall (cyst). The sexual fertilization is not followed by any special kind of division. *Balantidium* is the only genus of present interest as a cause of human disease. See *Balantidium coli*, p. 454.

OUTLINE CLASSIFICATION OF MICRO-ORGANISMS



**Classification and Specific Nomenclature.**—The classification of microbes is at present in an unsatisfactory state. In part this situation may be ascribed to the difficulty of ascertaining and observing accurately the features important for classification in this group of living things. In part, however, the confusion depends upon a too little controlled activity in the creation of new names by authors unwilling to expend the time and labor to become familiar with the old names. One should not lightly create new genera and the author who creates a new genus may well be called upon to prove the necessity for its creation before the added burden is tacked on to our nomenclature. Generic names are subject to revision as are also the names of families and larger groups. It is hoped that some authoritative body such as an international committee of bacteriologists or of general biologists, will in the near future decide upon a definite scheme for the classification of the fungi and especially the schizomycetes. A committee of the Society of American Bacteriologists has published<sup>1</sup> a proposed classification which may serve to further an international agreement. At present the introduction of such a more elaborate and still somewhat unsettled classification into an elementary textbook, would seem premature.

The classification of the protozoa is in a more satisfactory state, largely because of the monumental work of Doflein<sup>2</sup> but even in this realm the careless creation of new genera and the use of different generic names for the same organism by different authors is to be regretted.

A species is properly designated by a Latin binomial, the first member of the name being the *name of the genus* and the second member the *specific name*, such, for example, as *Mucor mucedo*, *Saccharomyces cerevisiae*, *Bacillus coli*, *Spirochaeta pallida*, *Plasmodium falciparum* and *Balantidium coli*. *Bacillus* is the generic term and *coli* the specific term. A third term is allowable to

<sup>1</sup> Winslow, Broadhurst, Buchanan, Krumwiede, Rogers and Smith: The families and genera of the bacteria, *Journal of Bacteriology*, 1917, 2, p. 505.

<sup>2</sup> Doflein, F., *Lehrbuch der Protozoenkunde*, Jena, 1911.

designate a variety of a species, such use being only temporary until a decision can be reached as to the relationship of the new organism under consideration, as for example *Bacillus coli communior*. Subsequently if the new organism proves to be *Bacillus coli*, the variety name *communior* may be dropped. On the other hand if it proves to be distinct from *Bacillus coli* the old variety name should then become the specific name resulting in *Bacillus communior*. The specific name is a single and very definite term and as a rule it is either the first published name given to the organism or some emended adaptation of it, in proper grammatical agreement with the generic term employed. Thus in designating the parasite of syphilis; one may employ the term, *Spirochaeta pallida* classing it in the genus *Spirochaeta* (Ehrenberg), but if the proposed genus *Treponema* (Schaudinn) be adopted, the name becomes *Treponema pallidum*.

## CHAPTER VIII

### PHYSIOLOGY OF MICRO-ORGANISMS

**Relations of Morphology and Physiology.**—In morphological study observations are restricted to the relationship of various elements at a given time, facts relating to form and structure. From the physiological viewpoint one is more interested in the sequence of events and the relation of cause and effect. The possible suggestion that these two methods of study are independent or mutually exclusive would be most unfortunate and is really very fallacious. The sequence of events may often best be ascertained by a series of morphological observations of a microbe undergoing change of form, and certainly the form and structure of a living organism at a given time may be properly regarded as an expression and result of previous physiological activity as well the most essential element in its potentiality for future activity. All must agree that difference in behavior, that is, reaction to a definite environmental change, is really associated with a difference in structure of the living organism. The important difficulty lies in the fact that the morphological or structural difference with which this difference in reaction is correlated, may not be capable of direct observation by any known method and may be ascertainable only by means of the physiological test. On the other hand the method of experimental physiology involves the factor of environment, small and unmeasured differences in which may grossly influence the resulting phenomenon and lead to erroneous conclusions. Furthermore, the experimental conditions and the method of physiological observations may be wholly lacking in adaptation to potentialities of the organisms under observation. When properly employed, however, the method of experimental physiology yields valuable knowledge obtainable in no other way,

and it has been the most important single method in establishing our modern ideas of the relation of micro-organisms to infectious diseases, and is the method of greatest promise for the immediate future.

**Conditions of Physiological Study.**—The physiology of many organisms is subject to only very limited experimental investigation. Those organisms of very narrow biological adaptation, such as many of the parasitic protozoa, can be studied only in very close relation to their natural environment, the various important elements of which are not readily subject to experimental alteration and are largely unrecognizable. Our knowledge of these forms must therefore be derived almost exclusively from observations of form and structure, physical and chemical, as they exist and change under the natural conditions of environment, and from changes which take place in the tissues surrounding the parasite, which we may ascribe with more or less justification to their activity. Practically all that we know about the physiological activity of the very numerous microbes not yet brought into the group of artificially cultivable forms, has been deduced from morphological observations. Even observations of this kind, however, can be more successfully pursued in those forms capable of artificial culture, and artificial culture is a prime necessity for the study of cause and effect by the methods of experimental physiology. For this reason accurate knowledge of what micro-organisms do is much richer in regard to the cultivable forms such as bacteria, yeasts and molds. In fact the microbic pure culture presents the most favorable object known for the study of cellular physiology and bio-chemistry. Furthermore, the physiological activities of many microbes are of the greatest practical importance. It is not surprising, therefore, that, among the bacteria, many of which grow in artificial media under a great variety of environmental conditions, the relative ease of physiological experimentation, as compared with the difficulty of observation of the minute morphological details, and the great practical importance of the results of physiological study has lead

to an enormous development of knowledge gained by this method, which quite over-shadows our knowledge of morphology and structure in this group of organisms.

### THE INFLUENCE OF ENVIRONMENTAL FACTORS

**Moisture.**—Moisture is indispensable to the growth of micro-organisms. A few species will grow and multiply in almost pure distilled water. Drying causes the death of the majority of the vegetating cells, of some more readily than others, while the spore forms may remain alive in a dry condition for many years. The cholera germ is dead within half an hour after it has been dried on a coverglass; the typhoid bacillus survives drying for days and the tubercle bacillus for months. Spores of the anthrax bacillus survive in the dry state for years, and perhaps indefinitely.

Heim<sup>1</sup> found that pathogenic bacteria resist drying much longer when contained in pathological tissues or exudates from animals which have succumbed to the disease, than when they are taken from artificial cultures.

**Organic Food.**—One species of bacteria, *Nitrosomonas* of Winogradsky, lives, grows and multiplies without organic food, utilizing the gases of the atmosphere as its source of carbon and nitrogen. From the standpoint of nutrition this organism is among the most primitive of beings. Other bacteria are known which may grow in water containing only mineral salts and a simple sugar, utilizing large quantities of atmospheric nitrogen. These are known as nitrogen-fixing bacteria. Most of the bacteria, yeasts and molds require a small amount of nitrogenous organic matter as food, such as the amino-acids or albumoses, and many of them flourish better when furnished a fermentable carbohydrate such as dextrose. The complex organic molecules are utilized in part to build up the substance of the bacteria, but a much larger part of them is broken down into simpler and more stable substances, such as carbon dioxide, simple fatty acids, ammonia and water, with the liberation of energy. *Sapro-*

<sup>1</sup> *Zeitschrift f. Hygiene*, Apr. 4, 1905, Bd. L, No. 1, p. 123.

*phytic* organisms are those which grow on dead organic matter. Micro-organisms of still narrower adaptibility grow well in artificial culture only if they be furnished abundant protein or nucleo-protein. Some important disease-producing bacteria belong in this category, as well as many parasitic spirochetes and some of the protozoa. Such organisms are not adapted to any natural saprophytic existence, and they grow in the artificial cultures only because the dead medium is made to resemble somewhat their natural parasitic habitat. Finally there are the micro-organisms which have not yet been grown in artificial culture and whose food requirements are essentially unknown. Many of these are parasites, and are called *obligate parasites*. A few bacteria, many of the filterable agents, and most of the parasitic protozoa are included in this category.

**Inorganic Salts and Chemical Reaction.**—Phosphorus, sulphur, chlorine, calcium, sodium and potassium, in addition to carbon, hydrogen, oxygen and nitrogen, are present as constituents of the microbic protoplasm. Minute quantities of these suffice to supply the food requirements of micro-organisms and it is unnecessary to add them to culture media to serve as food. Common salt, sodium chloride, is ordinarily employed to give the artificial medium an osmotic tension approaching that of the body fluids, and calcium carbonate is sometimes used to neutralize the organic acids which may arise in the culture as a result of the bacterial growth.

The most favorable chemical reaction for most micro-organisms is that of actual slight alkalinity, not sufficiently alkaline to produce a red color with phenolphthalein and not sufficiently acid to produce a red color with litmus. Some bacteria and many of the yeasts and molds will grow well in a weakly acid medium, but most parasitic bacteria and protozoa, which can be cultivated at all, require a reaction slightly alkaline to litmus or rosolic acid. The anaërobic bacteria do best in a medium containing glucose and with a reaction quite alkaline, indeed very close to the point at which phenolphthalein becomes pink. Organisms

which produce acid or alkali are usually arrested in their growth as soon as a certain concentration is reached, and the medium may then rapidly kill the micro-organisms.

**Oxygen.**—Oxygen, either free as atmospheric oxygen or combined as in water or organic compounds, is an essential constituent of the food of all micro-organisms. The concentration of uncombined oxygen dissolved in the medium, or the partial pressure of atmospheric oxygen, is the factor ordinarily meant when oxygen requirement is mentioned. Many micro-organisms grow best in a medium freely exposed to the air. These are called *aërobes*. Some which will grow only when there is free access of oxygen are called *obligate aërobes*. There are numerous bacteria, including spirochetes, which grow only in the absence of, or in extremely weak concentration of oxygen. These are called *obligate anaërobes*. Many of the bacteria grow well in various concentrations of oxygen or in its absence. These are spoken of as *facultative anaërobes*, or sometimes as facultative aërobes if they seem to prefer the anaërobic existence. Finally there are a few organisms, some bacteria and spirochetes, and perhaps some protozoa, which seem to require a fairly definite partial pressure of oxygen, but are not adapted to growth in a medium freely exposed to the atmosphere (*B. bifidus*, *B. abortus*, *Spirochaeta rossii*, *Plasmodium falciparum*). In relation to oxygen requirement, these are designated as microaërophilic organisms.

**Temperature.**—Among the various micro-organisms are found types which are adapted for growth at different temperatures throughout a considerable range. There are some bacteria and perhaps some molds capable of growth at a temperature of  $-0.5^{\circ}$  C.; in food substances such as milk, which are not frozen at this temperature. A certain yeast is said to multiply even at  $-6^{\circ}$  C., in salted butter. Microbes which grow at very high temperatures, even up to  $80^{\circ}$  C., occur in the soil, in ensilage and sometimes in the intestine of animals. The great majority of micro-organisms grow only between  $0^{\circ}$  and  $40^{\circ}$  C. It is possible to recognize a minimum, a maximum and an intermediate optimum tem-

perature for growth of each species. Ordinarily the optimum temperature is only a few degrees below the maximum at which growth will take place. The following table from Marshall's Microbiology illustrates the relation of these temperatures.

Species	Temperatures		
	Minimum	Optimum	Maximum
<i>Penicillium glaucum</i> .....	1.5°	25°-27°	31°-36°
<i>Aspergillus niger</i> .....	7°-10°	33°-37°	40°-43°
<i>Saccharomyces cerevisia</i> I.....	1°-3°	28°-30°	40°
<i>Saccharomyces pasteurianus</i> I.....	0.5°	25°-30°	34°
<i>Bacterium phosphoreum</i> .....	below 0°	16°-18°	28°
<i>Bacillus subtilis</i> .....	6°	30°	50°
<i>Bacterium anthracis</i> .....	10°	30°-37°	43°
<i>Bacterium ludwigii</i> .....	50°	55°-57°	80°

Heating above the maximum temperature for growth injures the microbe and exposure for a short time kills it. A temperature of 60° C. for 20 to 30 minutes destroys most vegetative forms of bacteria. Cooling, on the other hand, merely checks and inhibits growth. Freezing destroys some of the germs contained in a liquid but many of them remain alive. Still lower temperatures seem to be entirely without further effect. Bacteria gradually die in frozen material.

**Germicides.**—Unfavorable environmental factors, germicides and antiseptics have been considered in an earlier Chapter (Chapter II).

**Microbic Variation.**—A microbic species is very stable in its characters when maintained under fairly constant conditions in its normal habitat. Change in environment brings about rather quickly change in some of the characters of a bacterial species. The alterations in virulence or ability to produce disease, which may be produced by methods of artificial culture, are perhaps best known. It would seem that the descendants of a single cell are not all identical, but they vary among themselves within fairly

narrow limits in respect to a great many characters, fluctuating about a mean type which is that best adapted to the environment. With a change in surrounding conditions, this mean or normal type may no longer be best adapted, but a variation slightly removed in respect to certain characters may flourish better and become the mean type about which the fluctuating variants group themselves. Thus the pure culture seems to respond to environmental change. Whether the fluctuating variations are due to small differences in the immediate surroundings of the individual microbes, or whether they arise as a result of a property of variability inherent in protoplasm, may be disputed, but the latter view is more commonly held by biologists.

#### THE PRODUCTS OF MICROBIC GROWTH

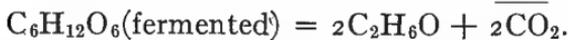
The effects resulting from the growth of a micro-organism depend on the one hand upon the nature of the organism and on the other upon the environment, more especially the medium in which it grows and the conditions of temperature and oxygen supply. Apparently slight variations in the latter may influence the results to a marked degree.

**Physical Effects.**—Heat is evolved by many actively growing bacterial cultures and is especially evident in the fermentation of such substances as ensilage and manure. Perhaps some of the heat may result directly from microbic activity, but the most of it appears to arise from secondary chemical reactions in which the microbic products sometimes play a part. Microbes which produce heat are designated as *thermogenic*. Light is also emitted by some microbic cultures. Here it seems certain that the light is produced by the oxidation of a bacterial product and not emitted directly by the micro-organisms. These phosphorescent or *photogenic* organisms occur in salt water and on fish and they have rarely been found in other places.

**Chemical Effects.**—These are the most important results of microbic growth. As we have just seen, the production of heat and light is probably due to a secondary reaction entered

into by some of the chemical products of growth. Almost all the other important practical effects of the growth of micro-organisms are due to chemical changes produced by them. *Primary products* are those which are produced inside the cell by its living protoplasm. These include all the synthetic products such as the substance of the germ itself, the complex bodies which it forms from simpler substances, such as its enzymes and its toxins, and also the simpler chemical substances which result from internal cellular metabolism, the proper excretions of the cell. The *secondary products* are those which result from the action of a primary product, such as an enzyme, upon some material outside the cell. The distinction is clear enough in theory but practically it is often obscure.

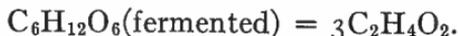
**Enzymes.**—Fermentation in its broad sense means the chemical changes brought about by living cells or their products. In its more restricted sense, it applies to the splitting of carbohydrates by the action of microbes, which is accompanied by the evolution of gas. Organisms which cause active fermentation are spoken of as *zymogenic*. Dextrose,  $C_6H_{12}O_6$ , is a readily fermentable carbohydrate and is decomposed in various ways by different microbes. In some instances a large proportion of it is converted into alcohol and carbon dioxide according to the following equation:



Other kinds of micro-organisms produce little alcohol or gas but abundant lactic acid. The reaction may be represented roughly by this equation:



In other instances acetic acid may be produced:



These equations are only an approximate indication of the reactions which take place, as it is very doubtful that the whole molecule of dextrose is ever converted into a single simpler

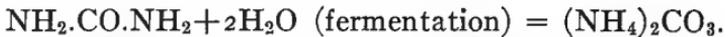
compound by fermentation, but they will serve to indicate the nature of the reactions involved and to suggest the variety of products which may arise from the decomposition of complex organic substances. Some of these fermentative changes take place to a large extent inside the microbic cell. Such is the case in the alcoholic fermentation produced by *saccharomyces*. The sugar-splitting or glycolytic ferments are found in the cultures of many bacteria and molds. Less common are the diastatic ferments capable of changing starch to dextrose, the inverting ferments which change saccharose and lactose into glucose and other hexoses, and the acetic ferments capable of causing the oxidation of alcohol to produce vinegar.

The fermentation or decomposition of proteins usually gives rise to evil-smelling gases. This decomposition is called putrefaction, and the organisms which cause it are called *saprogenic* or putrefactive organisms. The nature of the products is much influenced by the amount of oxygen available and the foulest gases are produced especially in the absence of oxygen. Proteolytic ferments of the same general nature as trypsin are produced by many microbes. A few form rennet-like enzymes. Proteolytic ferments which act in the presence of acid, like pepsin, are produced by some molds and by some bacterial species.

The decomposition of the complex protein molecules gives rise to an enormous variety of intermediate products before the ultimate analysis into ammonia, carbon dioxide, water, sulphates and phosphates is accomplished. Many of these intermediate products are very unstable and of unknown chemical composition. Some of them are highly poisonous. Brieger and his followers were able to separate a number of the complex substituted ammonia and ammonium compounds in a pure state and these particular bodies are known as putrefactive alkaloids, or as *ptomains*. A simple ptomain is trimethylamin,  $N(CH_3)_3$ ; a more complex one cadaverin,  $H_2N \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot NH_2$ . Some of the ptomains are poisonous. These various decomposition products are for the most part secondary products resulting from the action

of enzymes upon the decomposing material. Many of them are so unstable that their presence in a decomposing substance is influenced by access of air, temperature and moisture, and they may quickly disappear or decompose.

Micro-organisms also form fat-splitting or steatolytic enzymes, and enzymes capable of transforming urea into ammonium carbonate.



Various inorganic substances undergo chemical change under the influence of microbic activity and some of these changes appear to be due to enzymes. Specific examples will be considered in the section on the soil bacteria.

The *toxins* of bacteria are primary products built up by the cell. The true bacterial toxins are of unknown chemical composition, are labile like enzymes and stimulate the production of antitoxins when they are injected into animals. They are the most poisonous substances at present known. Analogous substances have been found in some plants, ricin in the castor bean and abrin in the jequirity bean, and the poisonous property of some kinds of snake venom is due to the presence of substances similar in nature to the bacterial toxins. These substances will be considered more fully in a later chapter devoted to the relation of parasitic microbes to their hosts.

#### MUTUAL RELATIONS OF A MICROBE AND ITS ENVIRONMENT

**Morphological Characters.**—It is evident that the phenomena of growth taking place in a microbic pure culture depend not only upon the particular kind of microbe present but also in a very important way upon the chemical and physical structure of the medium, the access of air and the temperature. Variations in these latter may even bring about considerable alteration in the form and structure of the individual cells. A common effect of high temperature is the shortening of individual bacilli and spirilla because of more rapid division and complete separation of the

daughter cells. The presence of unfavorable influences, such as antiseptics or bacterial waste products in the medium, may cause marked irregularities in the shape and size of the cells, so-called involution forms. The ability to form endospores may be lost through growth at high temperature. The form which a micro-organism presents in a given instance may not, therefore, be regarded as essentially typical without regard to the conditions under which it has been produced.

The morphology of cell-groups is even more obviously dependent upon the conditions of the environment and the physiological properties of the micro-organism. A slow scanty growth on a given medium does not necessarily mean that the organism essentially lacks vigor. It may mean that the medium is not well adapted to the requirements. Diffuse growth through a semi-solid medium may be merely an expression of the motility of an organism. A great variety of different culture media have been employed to bring out more or less characteristic features in the gross appearance of cultures, and these appearances often depend upon the grouping of the cells or upon their fermentative activity or both. Although the characters of a cell-group of micro-organisms are really morphological characters of the same general nature as the morphological characters of higher plants and animals, to which so much significance is attached; in the case of micro-organisms in an artificial environment, such as a culture medium, the gross appearance or the cell-grouping is more properly regarded as a feature of physiological rather than morphological significance. Nutrient gelatin is a medium well adapted, in the case of those microbes which will grow in it, for showing physiological differences in the appearance of cell-groups or colonies, and perhaps a greater variety of appearances may be obtained upon this medium than any other. Unfortunately its use entails certain difficulties, the most important of which is the necessity for experience and care in the interpretation of the appearances observed. Important features in the appearance of the colonies and other cell-groups are brought out by the use of various other media.

**Physiological Tests.**—Specific tests for a simple physiological character require less skill and care in their observation, and are widely used. Cultivation in a fermentation tube of sugar broth as a test of ability to form gas from the sugar, titration of sugar-broth cultures to ascertain the ability to produce acid from various sugars, chemical test for the presence of indol and of ammonia in a culture in peptone solution, observation of the ability to hemolyze or discolor blood mixed with the medium, and the ability to ferment glycerin, these are some of the valuable simpler tests. Cultivation in milk is a somewhat more complex test, as a variety of fermentable substances is offered the microbe, increasing the difficulties of interpretation but also increasing the variety of phenomena which may occur.

A convenient outline to use in making morphological and physiological observations upon bacteria and in recording the results, has been prepared by a committee of the Society of American Bacteriologists. Many features of this will be found of assistance in the study of new or unknown bacteria, especially saprophytic forms. A copy of the revised descriptive chart is inserted along with a copy of the earlier chart of 1907.

# DESCRIPTIVE CHART

## FOR USE IN BACTERIOLOGICAL INSTRUCTION

Recommended by the Committee on the Chart for Identification of Bacterial Species at the 1917 meeting of the  
**SOCIETY OF AMERICAN BACTERIOLOGISTS**

H. J. CONN  
 H. A. HARDING  
 I. J. KLIGLER  
 W. D. FROST  
 M. J. PRUCHA  
 K. N. ATKINS  
 } *Committee*

Name of organism.....  
 Source.....  
 Date of isolation.....

Culture No.....

Studied by.....

**INVIGORATION OF CULTURE**

Date.....  
 Series No.....

**BRIEF CHARACTERIZATION**

Group No.....

Medium used.....  
 Temperature.....°C. Number of transfers.....  
 Length of each incubation.....days.

**GROUP NUMBER**

As each of the determinations listed below is made, check the proper figure. When complete place the entire group number in the space above.

**MORPHOLOGY**

NOTE—Underscore required terms.	Sketches
<b>VEGETATIVE CELLS, Medium used.....</b> temp.....age.....days. Form, <i>spheres, short rods, long rods, filaments, commas, short spirals, long spirals, curved.</i> Arrangement, <i>single, pairs, chains, fours, cubical packets.</i> Limits of Size.....Size of Majority..... Ends, <i>rounded, truncate, concave</i> <b>CAPSULES, present on.....</b> How stained.....	
<b>SPORANGIA, present, absent. Medium used.....</b> temp.....age.....days. Form, <i>elliptical, short rods, spindled, clavate, drumsticks.</i> Limits of Size.....Size of Majority..... <b>ENDOSPORES, present, absent.</b> Location of Endospores, <i>central, polar.</i> Form, <i>spherical, elliptical, elongated.</i> Limits of Size..... Size of Majority..... Wall, <i>thick, thin.</i> Sporangium wall, <i>adherent, not adherent.</i>	
<b>MOTILITY</b> In broth..... On agar..... <b>FLAGELLA, No..... Attachment, <i>polar, bipolar, peritrichiate.</i> How stained.....</b>	
<b>IRREGULAR FORMS.</b> Present on.....in.....days at.....°C. Form <i>spindled, cuneate, filamentous, branched, or.....</i>	
<b>STAINING REACTIONS.</b> I: 10 watery fuchsin, gentian violet, carbol fuchsin. Loeffler's alkaline methylene blue. Special Stains. Gram..... Acid fast..... Neisser.....	

100.0	Endospores produced
200.0	Endospores not produced
10.0	Aerobic (Strict)
20.0	Facultative anaerobic
30.0	Anaerobic (Strict)
1.0	Gelatin liquefied
2.0	Gelatin not liquefied
0.1	Acid and gas from dextrose
0.2	Acid without gas from dextrose
0.3	No acid from dextrose
0.4	No growth with dextrose
0.01	Acid and gas from lactose
0.02	Acid without gas from lactose
0.03	No acid from lactose
0.04	No growth with lactose
0.001	Acid and gas from saccharose
0.002	Acid without gas from saccharose
0.003	No acid from saccharose
0.004	No growth with saccharose
0.0001	Nitrates reduced with evolution of gas
0.0002	Nitrates reduced without gas
0.0003	Nitrates not reduced
0.00001	Fluorescent
0.00002	Violet chromogens
0.00003	Blue chromogens
0.00004	Green chromogens
0.00005	Yellow chromogens
0.00006	Orange chromogens
0.00007	Red chromogens
0.00008	Brown chromogens
0.00009	Pink chromogens
0.00000	Non-chromogenic
0.000001	Diastatic action on starch, strong
0.000002	Diastatic action on starch, feeble
0.000003	Diastatic action on starch, absent
0.0000001	Acid and gas from glycerin
0.0000002	Acid without gas from glycerin
0.0000003	No acid from glycerin
0.0000004	No growth with glycerin

<b>VEGETATIVE CELLS</b>	Diameter over 1μ
	Diameter 0.5-1μ
	Diameter under 0.5μ
	Length over two diams.
	Chains (4 or more cells)
<b>ENDOSPORES</b>	Filaments
	Capsules
	Motile
<b>CULTURAL FEATURES</b>	Gram's Stain
	Central
	Polar
	Diameter > Diam. of rod
	Round
<b>PHYSIOLOGY</b>	Oval to cylindrical
	Absent
	Abundant
	Absent
	Shining
	Wrinkled
	Chromogenic
	Punctiform
	Round (over 1 mm.)
	Rhizoid
Filamentous	
<b>AGAR STROKE</b>	Curled
	Punctiform
	Round (over 1 mm.)
	Irregular
	Filamentous
<b>AGAR COLONIES</b>	Acid curd
	Rennet curd
	Casein peptonized
	Acid without curdling
	Unchanged
<b>GEL. COLONIES</b>	Fluorescent
	Luminous
	Animal pathogen
	Plant pathogen
<b>MILK</b>	Saprophyte

The genus according to the system of Migula is given its proper symbol which precedes the number thus:

BACILLUS COLI (Esch.) Mig. becomes B. 222.111202

# CULTURAL CHARACTERISTICS

	..... hours	Changes, ..... days	Changes, ..... days	SKETCHES		
<b>Agar Stroke</b>  <b>Incubation Temperature</b> .....°C.	Growth, <i>scanty, moderate, abundant, none.</i> Form of growth, <i>filiform, echinulate, beaded, spreading, arborescent, rhizoid.</i> Elevation of growth, <i>flat, effuse, raised, convex.</i> Lustre, <i>glistening, dull.</i> Topography, <i>smooth, contoured, rugose.</i> Optical Characters, <i>opaque, translucent, opalescent, iridescent.</i> Chromogenesis..... Photogenic. Fluorescent. Odor, <i>absent, decided, resembling.....</i> Consistency, <i>butyrous, viscid, membranous, brittle.</i> Medium, <i>grayed, browned, reddened, blued, greened.</i>					
<b>Gelatin Stab</b>  <b>Temperature</b> .....°C.	Growth, <i>uniform, best at top, best at bottom.</i> Line of puncture, <i>filiform, beaded, papillate, villous, arborescent.</i> Liquefaction, <i>none, crateriform, napiform, infundibuliform, saccate, stratiform;</i> begins in.....d. complete in.....d. Depth of liquefaction in tube of 10 mm. diameter evenly inoculated at 20° C. for 30 days.....mm. Medium, <i>fluorescent, browned.</i>					
<b>..... Medium (solid)</b>  <b>Temperature</b> .....°C.						

	..... hours	Changes, ..... days	Changes, ..... days		..... hours	Changes, ..... days	Changes, ..... days
<b>Nutrient Broth</b>  <b>Temperature</b> .....°C.	Surface growth, <i>ring, pellicle, flocculent membranous, none.</i> Clouding, <i>slight, moderate, strong transient, persistent, none, fluid turbid.</i> Odor, <i>absent, decided, resembling.....</i> Sediment, <i>compact, flocculent, granular, flaky, viscid on agitation, abundant, scant, none.</i>			<b>..... Medium (liquid)</b>  <b>Temperature</b> .....°C.			

		SKETCHES			
		Surface Colony		Deep Colony	
<b>Agar Colonies</b>  <b>Temperature</b> .....°C.	Growth, <i>slow, rapid.</i> Form, <i>punctiform, circular, irregular, mycelioid, filamentous, rhizoid.</i> Surface, <i>smooth, rough, concentrically ringed, radiate.</i> Elevation, <i>flat, effuse, raised, convex, pulvinate, umbonate.</i> Edge, <i>entire, undulate, lobate, erose, filamentous, curled.</i> Internal structure, <i>amorphous, finely-, coarsely-granular, filamentous, curled, concentric.</i>	..... days	..... days	..... days	..... days
<b>Gelatin Colonies</b>  <b>Temperature</b> .....°C.	Growth, <i>slow, rapid.</i> Form, <i>punctiform, circular, irregular, mycelioid, filamentous.</i> Elevation, <i>flat, raised, convex, pulvinate, crateriform (liquefying).</i> Edge, <i>entire, undulate, lobate, erose, filamentous, floccose, curled.</i> Liquefaction, <i>cup, saucer, spreading.</i> Internal structure, <i>amorphous, finely-, coarsely-granular, filamentous, curled, concentric.</i>	..... days	..... days	..... days	..... days

Source..... Date of Isolation..... Name..... Group No.....  
 Date of Revivification..... Determined: When?..... By whom?..... Culture No.....

**DETAILED FEATURES**

NOTE—Underscore required terms. Observe notes.  
 VEGETATIVE CELLS, Medium used.....  
 temp..... age..... days.  
 Form, *spheres, short rods, long rods, filaments, commas, short spirals, long spirals, spindled, cuneate, clavate, curved.*  
 Arrangement, *single, pairs, chains, fours, cubical packets.*  
 Limits of Size.....  
 Size of Majority.....  
 Ends, *rounded, truncate, concave.*  
 Agar  
 Hanging-Block { Orientation (grouping).....  
 Chains (No. of elements).....  
 Orientation of Chains, *parallel, irregular.*  
 Postfission movements, *loop forming, folding, snapping, slipping.*  
 SPORANGIA, *present, absent.* Medium used.....  
 temp..... age..... days.  
 Form, *elliptical, short rods, spindled, clavate, drumsticks.*  
 Limits of Size..... Size of Majority.....  
 Agar  
 Hanging-Block { Orientation (grouping).....  
 Chains (No. of elements).....  
 Orientation of Chains, *parallel, irregular.*  
 ENDOSPORES, *present, absent.*  
 Location of Endospores, *central, polar.*  
 Form, *spherical, elliptical, elongated.*  
 Limits of Size.....  
 Size of Majority.....  
 Wall, *thick, thin.*  
 Sporangium wall, *adherent, not adherent.*  
 Germination, *equatorial, oblique, polar, bipolar, by stretching, by absorption of spore wall.*  
 CAPSULES, *present on.....*  
 ZOOGLOEA, *Pseudozoogloea.*  
 FLAGELLA, No..... Attachment, *polar, bipolar, lophotrichiate, peritrichiate.* How Stained.....  
 INVOLUTION FORMS, on..... in..... days at.....°C.  
 STAINING REACTIONS.  
 1:10 watery fuchsin, gentian violet, carbol fuchsin, Loeffler's alkaline methylene blue.  
 Special Stains.  
 Gram..... Glycogen.....  
 Fat..... Acid fast.....  
 Neisser.....  
 Metachromatic granules, sporogenous granules.  
 NUTRIENT BROTH.  
 Surface growth, *ring, pellicle, flocculent, membranous, none.*  
 Clouding, *slight, moderate, strong, transient, persistent, none, fluid, turbid.*  
 Odor, *absent, decided, resembling.....*  
 Sediment, *compact, flocculent, granular, flaky, viscid on agitation, abundant, scant, none.*  
 AGAR STROKE.  
 Growth, *invisible, scanty, moderate, abundant, none.*  
 Form of growth *filiform, echinulate, beaded, spreading, plumose, arborescent, rhizoid.*  
 Elevation of growth, *flat, effuse, raised, convex.*  
 Lustre, *glistening, dull, cretaceous.*  
 Topography, *smooth, contoured, rugose, verrucose.*  
 Optical Characters, *opaque, translucent, opalescent, iridescent.*  
 Chromogenesis..... Photogenic. Fluorescent.  
 Odor, *absent, decided, resembling.....*  
 Consistency, *slimy, butyrous, viscid, membranous, coriaceous, brittle.*  
 Medium, *grayed, browned, reddened, blued, greened.*  
 AGAR COLONIES.  
 Growth, *slow, rapid, temperature.....*  
 Form, *punctiform, circular, irregular, ameiboid, mycelioid, filamentous, rhizoid.*  
 Surface, *smooth, rough, concentrically ringed, radiate, striate.*  
 Elevation, *flat, effuse, raised, convex, pulvinate, umbonate.*  
 Edge, *entire, undulate, lobate, erose, lacerate, fimbriate, floccose, curled.*  
 Internal structure, *amorphous, finely-, coarsely-granular, grumose, filamentous, floccose, curled.*  
 GELATIN COLONIES.  
 Growth, *slow, rapid.*  
 Form, *punctiform, circular, irregular, ameiboid, mycelioid, filamentous, rhizoid.*  
 Elevation, *flat, effuse, raised, convex, pulvinate, crateriform (liquefying).*  
 Edge, *entire, undulate, lobate, erose, lacerate, fimbriate, filamentous, floccose, curled.*  
 Liquefaction, *cup, saucer, spreading.*

**GELATIN STAB.**

Growth, *uniform, best at top, best at bottom.*  
 Line of puncture, *filiform, beaded, papillate, villous, plumose, arborescent.*  
 Liquefaction, *none, crateriform, napiform, infundibuliform, saccate, startiform;* begins in..... d, complete in..... d.  
 Depth of liquefaction in tube of 10 mm. diameter, evenly inoculated at 20°C. for 30 days..... mm.

**POTATO.**

Growth, *scanty, moderate, abundant, transient, persistent.*  
 Form of growth, *filiform, echinulate, beaded, spreading, plumose, arborescent, rhizoid.*  
 Elevation of growth, *flat, effuse, raised, convex.*  
 Lustre, *glistening, dull, cretaceous.*  
 Topography, *smooth, contoured, rugose, verrucose.*  
 Chromogenesis..... Pigment in water *insoluble, soluble;* other solvents.....  
 Odor, *absent, decided, resembling.....*  
 Consistency, *slimy, butyrous, viscid, membranous, coriaceous, brittle.*  
 Medium, *grayed, browned, reddened, blued, greened.*

**POTATO STARCH JELLY.**

Growth, *scanty, copious, absent.*  
 Diastasic action, *absent, feeble, profound.*  
 Medium stained.....  
 Dextrin, *present, absent.*

**TEMPERATURE RELATIONS.**

Optimum temperature for growth 20°C.: or 37°C.:  
 or.....°C.  
 Maximum temperature for growth.....°C.  
 Minimum temperature for growth.....°C.

**COHN'S SOLUTION.**

Growth, *copious, scanty, absent.*  
 Medium *fluorescent, nonfluorescent.*

**USCHINSKY'S SOLUTION.**

Growth, *copious, scanty, absent.*  
 Fluid, *viscid, not viscid.*

**LOEFFLER'S BLOOD SERUM.**

Stroke *invisible, scanty, moderate, abundant.*  
 Form of growth, *filiform, echinulate, beaded, spreading, plumose, arborescent, rhizoid.*  
 Elevation of growth, *flat, effuse, raised, convex.*  
 Lustre, *glistening, dull, cretaceous.*  
 Topography, *smooth, contoured, rugose, verrucose.*  
 Chromogenesis.....  
 Medium, *grayed, browned, reddened, blued, greened.*  
 Liquefaction begins in..... d., complete in..... d.

**MILK.**

Clearing without coagulation.  
 Coagulation *prompt, delayed, absent.*  
 Extrusion of whey begins in..... days.  
 Peptonization begins on..... d., complete on..... d.  
 Coagulum, *slowly peptonized, rapidly peptonized.*  
 Reaction, id..... 2d..... 4d..... 10d..... 20d.....  
 Consistency, *slimy, viscid, unchanged.*  
 Medium, *browned, reddened, blued, greened.*

**LITMUS MILK.**

Acid, *alkaline, acid then alkaline, no change.*  
 Prompt reduction, *no reduction, partial slow reduction.*

**INDOL PRODUCTION, feeble, moderate, strong, absent.**

**HYDROGEN SULPHIDE, feeble, moderate, strong, absent.**

**AMMONIA PRODUCTION, feeble, moderate, strong, absent, masked by acids.**

**NITRATE IN NITRATE BROTH. Reduced, not reduced.**

Presence of nitrites..... Ammonia.....  
 Presence of nitrates..... free nitrogen.....

**NITROGEN. Obtained from peptone, asparagin, glycocoll, urea, ammonia salts, nitrogen.**

**SILICATE JELLY (Fermi's Solution).**

Growth, *copious, scanty, absent.*  
 Medium stained.....

Best media for long-continued growth.....

Quick test for differential purposes.....

Endospores produced 100.  
 Endospores not produced 200.

Aerobic (Strict) 10. Facultative anaerobic 20.  
 Anaerobic (Strict) 30.

Gelatin liquefied 1.  
 Gelatin not liquefied 2.

Acid and gas .1. Acid without gas .2.  
 No acid .3. No growth .4 with dextrose.

Acid and gas .01. Acid without gas .02.  
 No acid .03. No growth .04 with lactose.

Acid and gas .001. Acid without gas .002.  
 No acid .003. No growth .004 with saccharose.

Nitrates reduced with gas .0001.  
 Nitrates reduced without gas .0002.  
 Nitrates not reduced .0003.

Fluorescent .00001. Violet .00002. Blue .00003.  
 Green .00004. Yellow .00005. Orange .00006. Red .00007.  
 Brown .00008. Pink .00009. Non-chromogenic .00000.

Diastasic action on potato starch, strong .000001  
 Feeble .000002. Absent .000003.

Acid and gas .000001. Acid without gas .000002  
 No acid .000003. No growth .000004 with glycerin.

1. Fermentation-tubes containing peptone-water or sugar-free bouillon.  
 At.....°C.

Gas production, in per cent

(H  
CO<sub>2</sub>)

Growth in closed arm

Amount of acid produced d.

**BRIEF CHARACTERIZATION**

MORPHOLOGY	Diameter over 1μ
	Chains, filaments
	Endospores
BIOCHEMICAL FEATURES	Capsules
	Zoogloea, Pseudozoogloea
	Motile
CULTURAL FEATURES	Involution forms
	Gram's Stain
	Cloudy, turbid
Broth	Ring
	Pellicle
	Sediment
Agar	Shining
	Dull
	Wrinkled
Gel Plate	Chromogenic
	Round
	Proteus-like
Gel Stab	Rhizoid
	Filamentous
	Curled
Potato	Surface-growth
	Needle-growth
	Moderate, absent
Liquefaction	Abundant
	Discolored
	Starch destroyed
Milk	Grows at 37°C.
	Grows in Cohn's Sol.
	Grows in Uschinsky's Sol.
DISTRIBUTION	Gelatin
	Blood-serum
	Casein
Milk	Agar, mannan
	Acid curd
	Rennet curd
Milk	Casein peptonized
	Indol
	Hydrogen sulphide
Milk	Ammonia
	Nitrates reduced
	Fluorescent
Milk	Luminous
	Animal pathogen, epizoon
	Plant pathogen, epiphyte
Milk	Soil
	Milk
	Fresh water
Milk	Salt water
	Sewage
	Iron bacterium
Milk	Sulphur bacterium

Committee on Revision of Chart Identification of Bacterial Species.  
 Prepared by H. A. HARDING, H. J. CONN, I. J. KLIGLER, W. D. FROST, M. J. PRUEHA  
 DESCRIPTIVE CHART  
 SOCIETY OF AMERICAN BACTERIOLOGISTS  
 Endorsed by the Society for General Use at the Annual Meeting  
 Dec. 31, 1914

# PHYSIOLOGY

## FERMENTATION

Temperature.....°C.

Fermentation-tubes containing..... .....and:	DEXTROSE	LACTOSE	SACCHAROSE	GLYCERIN				
Growth in closed arm								
Gas percentage								
$\frac{H}{CO_2}$								
Acid in..... days								
Acid in..... days								
Acid in..... days								

## CHROMOGENESIS

Nutrient broth	
Nutrient gelatin	
Nutrient agar	
Potato	

## MILK

Temperature.....°C.

REACTION	COAGULATION	PEPTONIZATION
1 day.....	1 day.....	1 day.....
2 days.....	2 days.....	2 days.....
4 days.....	4 days.....	4 days.....
7 days.....	7 days.....	7 days.....
10 days.....	10 days.....	10 days.....

## LITMUS MILK

Temperature.....°C.

REACTION	COAGULATION	PEPTONIZATION	REDUCTION
1 day.....	1 day.....	1 day.....	1 day.....
2 days.....	2 days.....	2 days.....	2 days.....
4 days.....	4 days.....	4 days.....	4 days.....
7 days.....	7 days.....	7 days.....	7 days.....
10 days.....	10 days.....	10 days.....	10 days.....

## NITRATE REDUCTION

Medium.....

Temperature.....°C.

Nitrite: 1 day.....; 2 days.....; 4 days.....; 7 days.....; 10 days.....  
 Gas: 1 day.....; 2 days.....; 4 days.....; 7 days.....; 10 days.....

## DIASTATIC ACTION

Breadth of clear zone on starch agar plates
in..... days:
in..... days:
in..... days:

## TEMPERATURE RELATIONS

Optimum temperature for growth.....°C.  
 Maximum temperature for growth.....°C.  
 Minimum temperature for growth.....°C.

## DESCRIPTIVE CHART—SOCIETY OF AMERICAN BACTERIOLOGISTS

Prepared by F. D. Chester, F. P. Gorham, Erwin F. Smith, Committee on Methods of Identification of Bacterial Species.

Endorsed by the Society for general use at the Annual Meeting, Dec. 31, 1907.

### GLOSSARY OF TERMS

**AGAR HANGING-BLOCK**, a small block of nutrient agar cut from a poured plate, and placed on a cover-glass, the surface next the glass having been first touched with a loop from a young fluid culture or with a dilution from the same. It is examined upside down, the same as a hanging drop.

**AMEBOID**, assuming various shapes like an ameba.

**AMORPHOUS**, without visible differentiation in structure.

**ARBORESCENT**, a branched, tree-like growth.

**BEADED**, in stab or stroke, disjointed or semi-confluent colonies along the line of inoculation.

**BRIEF**, a few days, a week.

**BRITTLE**, growth dry, friable under the platinum needle.

**BULLATE**, growth rising in convex prominences, like a blistered surface.

**BUTYROUS**, growth of a butter-like consistency.

**CHAINS**,  
Short chains, composed of 2 to 8 elements.  
Long chains, composed of more than 8 elements.

**CILLIATE**, having fine, hair-like extensions like cilia.

**CLOUDY**, said of fluid cultures which do not contain pseudozoogloae.

**COAGULATION**, the separation of casein from whey in milk. This may take place quickly or slowly, and as the result either of the formation of an acid or of a lab ferment.

**CONTOURED**, an irregular, smoothly undulating surface, like that of a relief map.

**CONVEX**, surface the segment of a circle, but flattened.

**COPROPHYL**, dung bacteria.

**CORIACEOUS**, growth tough, leathery, not yielding to the platinum needle.

**CRATERIFORM**, round, depressed, due to the liquefaction of the medium.

**CRETACEOUS**, growth opaque and white, chalky.

**CURLED**, composed of parallel chains in wavy strands, as in anthrax colonies.

**DIASTASIC ACTION**, Same as **DIASTATIC**, conversion of starch into water-soluble substances by diastase.

**ECHINULATE**, in agar stroke a growth along line of inoculation, with toothed or pointed margins; in stab-cultures growth beset with pointed outgrowths.

**EFFUSE**, growth thin, veily, unusually spreading.

**ENTIRE**, smooth, having a margin destitute of teeth or notches.

**EROSE**, border irregularly toothed.

**FILAMENTOUS**, growth composed of long, irregularly placed or interwoven filaments.

**FILIFORM**, in stroke or stab-cultures a uniform growth along line of inoculation.

**FIMBRIATE**, border fringed with slender processes, larger than filaments.

**FLOCCOSE**, growth composed of short curved chains, variously oriented.

**FLOCCULENT**, said of fluids which contain pseudozoogloae, *i.e.*, small adherent masses of bacteria of various shapes and floating in the culture fluid.

**FLUORESCENT**, having one color by transmitted light and another by reflected light.

**GRAM'S STAIN**, a method of differential bleaching after gentian violet, methyl violet, etc. The + mark is to be given only when the bacteria are deep blue or remain blue after counter-staining with Bismark brown.

**GRUMOSE**, clotted.

**INFUNDIBULIFORM**, form of a funnel or inverted cone.

**IRIDESCENT**, like mother-of-pearl. The effect of very thin films.

**LACERATE**, having the margin cut into irregular segments as if torn.

**LOBATE**, border deeply undulate, producing lobes (see undulate).

**LONG**, many weeks, or months.

**MAXIMUM TEMPERATURE**, temperature above which growth does not take place.

**MEDIUM**, several weeks.

**MEMBRANOUS**, growth thin, coherent, like a membrane.

**MINIMUM TEMPERATURE**, temperature below which growth does not take place.

**MYCELIOD**, colonies having the radiately filamentous appearance of mold colonies.

**NAPIFORM**, liquefaction with the form of a turnip.

**NITROGEN REQUIREMENTS**, the necessary nitrogenous food. This is determined by adding to *nitrogen-free* media the nitrogen compound to be tested.

**OPALESCENT**, resembling the color of an opal.

**OPTIMUM TEMPERATURE**, temperature at which growth is most rapid.

**PELLICLE**, in fluid bacterial growth either forming a continuous or an interrupted sheet over the fluid.

**PEPTONIZED**, said of curds dissolved by trypsin.

**PERSISTENT**, many weeks, or months.

**PLUMOSE**, a fleecy or feathery growth.

**PSEUDOZOOGLOEAE**, clumps of bacteria, not dissolving readily in water, arising from imperfect separation, or more or less fusion of the components, but not having the degree of compactness and gelatinization seen in zoogloae.

**PULVINATE**, in the form of a cushion, decidedly convex.

**PUNCTIFORM**, very minute colonies, at the limit of natural vision.

**RAISED**, growth thick, with abrupt or terraced edges.

**RHIZOID**, growth of an irregular branched or root-like character, as in *B. mycoides*.

**RING**, Same as **RIM**, growth at the upper margin of a liquid culture, adhering more or less closely to the glass.

**REPAND**, wrinkled.

**RAPID**, developing in 24 to 48 hours.

**SACCATE**, liquefaction the shape of an elongated sack, tubular, cylindrical.

**SCUM**, floating islands of bacteria, an interrupted pellicle or bacterial membrane.

**SLOW**, requiring 5 or 6 days or more for development.

**SHORT**, applied to time, a few days, a week.

**SPORANGIA**, cells containing endospores.

**SPREADING**, growth extending much beyond the line of inoculation, *i.e.*, several millimeters or more.

**STARTIFORM**, liquefying to the walls of the tube at the top and then proceeding downward horizontally.

**THERMAL DEATH-POINT**, the degree of heat required to kill young fluid cultures of an organism exposed for 10 minutes (in thin-walled test-tubes of a diameter not exceeding 20 mm.) in the thermal water-bath. The water must be kept agitated so that the temperature shall be uniform during the exposure.

**TRANSIENT**, a few days.

**TURBID**, cloudy with flocculent particles; cloudy plus flocculence.

**UMBONATE**, having a button-like, raised center.

**UNDULATE**, border wavy, with shallow sinuses.

**VERRUCOSE**, growth wart-like, with wart-like prominences.

**VERMIFORM-CONTOURED**, growth like a mass of worms, or intestinal coils.

**VILLOUS**, growth beset with hair-like extensions.

**VISCID**, growth follows the needle when touched and withdrawn, sediment on shaking rises as a coherent swirl.

**ZOOGLLOEAE**, firm gelatinous masses of bacteria, one of the most typical examples of which is the *Sireptococcus mesenterioides* of sugar vats (*Leuconostoc mesenterioides*) the bacterial chains being surrounded by an enormously thickened firm covering inside of which there may be one or many groups of the bacteria.

### NOTES

(1) For decimal system of group numbers see Table I. This will be found useful as a quick method of showing close relationships inside the genus, but is not a sufficient characterization of any organism.

(2) The morphological characters shall be determined and described from growths obtained upon at least one solid medium (nutrient agar) and in at least one liquid medium (nutrient broth). Growth at 37°C. shall be in general not older than 24 to 48 hours, and growths at 26°C. not older than 48 to 72 hours. To secure uniformity in cultures, in all cases preliminary cultivation shall be practised as described in the revised Report of the Committee on Standard Methods of the Laboratory Section of the American Public Health Association, 1905.

(3) The observation of cultural and bio-chemical features shall cover a period of at least 15 days and frequently longer, and shall be made according to the revised Standard Methods above referred to. All media shall be made according to the same Standard Methods.

(4) Gelatin stab-cultures shall be held for 6 weeks to determine liquefaction.

(5) Ammonia and indol tests shall be made at end of 10th day, nitrite tests at end of 5th day.

(6) Titrate with  $\frac{N}{20}$  NaOH, using phenolphthalein as an indicator: make titrations at same times from blank. The difference gives the amount of acid produced.

The titation should be done after boiling to drive off any CO<sub>2</sub> present in the culture.

(7) Generic nomenclature shall begin with the year 1872. (Cohn's first important paper.)

Species nomenclature shall begin with the year 1880. (Koch's discovery of the poured plate method for the separation of organisms.)

(8) Chromogenesis shall be recorded in standard color terms.

### TABLE I.

#### A NUMERICAL SYSTEM OF RECORDING THE SALIENT CHARACTERS OF AN ORGANISM. (GROUP NUMBER)

100.0	Endospores produced
200.0	Endospores not produced
10.0	Aërobic (Strict)
20.0	Facultative anaërobic
30.0	Anaërobic (Strict)
1.0	Gelatin liquefied
2.0	Gelatin not liquefied
0.1	Acid and gas from dextrose
0.2	Acid without gas from dextrose
0.3	No acid from dextrose
0.4	No growth with dextrose
0.01	Acid and gas from lactose
0.02	Acid without gas from lactose
0.03	No acid from lactose
0.04	No growth with lactose
0.001	Acid and gas from saccharose
0.002	Acid without gas from saccharose
0.003	No acid from saccharose
0.004	No growth with saccharose
0.0001	Non-chromogenic
0.0002	Nitrate reduced with evolution of gas
0.0003	Nitrates not reduced
0.0004	Nitrates reduced without gas formation
0.0005	Fluorescent
0.0006	Violet chromogens
0.0007	Blue chromogens
0.0008	Green chromogens
0.0009	Yellow chromogens
0.0010	Orange chromogens
0.0011	Red chromogens
0.0012	Brown chromogens
0.0013	Pink chromogens
0.0014	Non-chromogenic
0.0015	Diastasic action on potato starch, strong
0.0016	Diastasic action on potato starch, feeble
0.0017	Diastasic action on potato starch, absent
0.0018	Acid and gas from glycerine
0.0019	Acid without gas from glycerine
0.0020	No acid from glycerine
0.0021	No growth with glycerine

The genus according to the system of Migula is given its proper symbol which precedes the number thus: (7)

BACILLUS COLI (Esch.) Mig.	becomes B.	222.111102
BACILLUS ALCALIGENES Petr.	becomes B.	212.333102
PSEUDOMONAS CAMPETRIS (Pam.) Sm.	becomes Ps.	211.333151
BACTERIUM SUICIDA Mig.	becomes Bact.	222.232203

DETAILED FEATURES

NOTE.—Underscore required terms. Observe notes and glossary of terms on opposite side of card.

- I. MORPHOLOGY (2)
1. Vegetative Cells, Medium used ..... temp. .... days  
 Form, round, short rods, long rods, short chains, long chains, filaments, commas, short spirals, long spirals, clostridium, cuneate, clavate, curved.  
 Limits of Size ..... Size of Majority .....  
 Size of Majority .....  
 Ends, rounded, truncate, concave.
- Agar Hanging-block { Orientation (grouping) .....  
 Chains (No. of elements) .....  
 Short chains, long chains  
 Orientation of Chains, parallel irregular.
2. Sporangia, medium used ..... temp. .... days  
 Form, elliptical, short rods, spindled, clavate, drum-sticks.  
 Limits of Size ..... Size of Majority .....  
 Size of Majority .....  
 Agar Hanging-block { Orientation (grouping) .....  
 Chains (No. of elements) .....  
 Orientation of Chains, parallel irregular.
- Location of Endospores, central, polar.
3. Endospores.  
 Form, round, elliptical, elongated.  
 Limits of Size .....  
 Size of Majority .....  
 Wall, thick, thin.  
 Sporangium wall, adherent, not adherent.  
 Germination, equatorial, oblique, polar, bipolar, by stretching.
4. Flagella No. .... Attachment polar, bipolar, peritrichiate. How Stained .....
5. Capsules, present on .....
6. Zoogloea, Pseudozoogloea.
7. Involution Forms, on ..... in ..... days at ..... °C.
8. Staining Reactions.  
 1:10 watery fuchsin, gentian violet, carbol fuchsin, Löffler's alkaline methylene blue.  
 Special Stains  
 Gram ..... Glycogen .....  
 Fat ..... Acid fast .....  
 Neisser .....

- II. CULTURAL FEATURES (2)
1. Agar Stroke.  
 Growth, invisible, scanty, moderate, abundant.  
 Form of growth, filiform, echinulate, beaded, spreading plumose, arborescent, rhizoid.  
 Elevation of growth, flat, effuse, raised, convex.  
 Luster, glistening, dull, cretaceous.  
 Topography, smooth, contoured, rugose, verrucose.  
 Optical Characters, opaque, translucent, opalescent, iridescent.  
 Chromogenesis (2) .....  
 Odor, absent, decided, resembling. .....  
 Consistency, slimy, butyrous, viscid, membranous, coriaceous, brittle.  
 Medium grayed, browned, reddened, blued, greened.
2. Potato.  
 Growth, scanty, moderate, abundant, transient, persistent.  
 Form of growth, filiform, echinulate, beaded, spreading, plumose, arborescent, rhizoid.  
 Elevation of growth, flat, effuse, raised, convex.  
 Luster, glistening, dull, cretaceous.  
 Topography, smooth, contoured, rugose, verrucose.  
 Chromogenesis (2) ..... Pigment in water insoluble, soluble; other solvents. .....  
 Odor, absent, decided, resembling. .....  
 Consistency, slimy, butyrous, viscid, membranous, coriaceous, brittle.  
 Medium grayed, browned, reddened, blued, greened.
3. Löffler's Blood Serum.  
 Stroke, invisible, scanty, moderate, abundant. Form of growth, filiform, echinulate, beaded, spreading, plumose, arborescent, rhizoid.  
 Elevation of growth flat, effuse, raised, convex.  
 Luster, glistening, dull, cretaceous.  
 Topography, smooth, contoured, rugose, verrucose.  
 Chromogenesis (2) .....  
 Medium grayed, browned, reddened, blued, greened.  
 Liquefaction begins in ..... d., complete in ..... d.
4. Agar Stab.  
 Growth uniform, best at top, best at bottom; surface growth scanty, abundant; restricted, wide-spread.  
 Line of puncture, filiform, beaded, papillate, villous, plumose, arborescent; liquefaction.

- III. PHYSICAL AND BIOCHEMICAL FEATURES
- |   |          |            |         |         |          |        |
|---|----------|------------|---------|---------|----------|--------|
| 1. Fermentation-tubes containing peptone-water or Sugar-free bouillon and | Dextrose | Saccharose | Lactose | Maltose | Glycerin | Mannit |
| Gas production, in per cent   |          |            |         |         |          |        |
| (H <sub>2</sub> )   |          |            |         |         |          |        |
| (CO <sub>2</sub> )  |          |            |         |         |          |        |
| Growth in closed arm  |          |            |         |         |          |        |
| Amount of acid produced 1d.   |          |            |         |         |          |        |
| Amount of acid produced 2d.   |          |            |         |         |          |        |
| Amount of acid produced 4d.   |          |            |         |         |          |        |

5. Gelatin Stab.  
 Growth uniform, best at top, best at bottom.  
 Line of puncture, filiform, beaded, papillate, villous, plumose, arborescent.  
 Liquefaction, cratersform, napiform, infundibuliform, saccate, stariform; begins in ..... d., complete in ..... d.
6. Nutrient Broth.  
 Surface growth, ring, pellicle, flocculent, membranous, none.  
 Clouding slight, moderate, strong; transient, persistent; none; fluid turbid.  
 Odor, absent, decided, resembling. .....  
 Sediment, compact, flocculent, granular, flaky, viscid on agitation, abundant, scant.
7. Milk.  
 Clearing without coagulation.  
 Coagulation prompt, delayed, absent.  
 Extrusion of whey begins in ..... days.  
 Coagulum slowly peptonized, rapidly peptonized.  
 Peptonization begins on ..... d., complete on ..... d.  
 Reaction, 1d. .... 2d. .... 4d. .... 10d. .... 20d. ....  
 Consistency, slimy, viscid, unchanged.  
 Medium browned, reddened, blued, greened.  
 Lab ferment, present, absent.
8. Litmus Milk.  
 Acid, alkaline, acid then alkaline, no change.  
 Prompt reduction, no reduction, partial slow reduction.
9. Gelatin Colonies.  
 Growth, slow, rapid.  
 Form, punctiform, round, irregular, ameboid, mycelioid, filamentous, rhizoid.  
 Elevation, flat, effuse, raised, convex, pulvinate, crateriform (liquefying).  
 Edge, entire, undulate, lobate, erose, lacerate, fimbriate, filamentous, floccose, curled.
10. Agar Colonies.  
 Growth slow, rapid (temperature .....):  
 Form, punctiform, round, irregular, ameboid, mycelioid, filamentous, rhizoid.  
 Surface smooth, rough, concentrically ringed, radiate, striate.  
 Elevation, flat, effuse, raised, convex, pulvinate, umbonate.  
 Edge, entire, undulate, lobate, erose, lacerate, fimbriate, floccose, curled.  
 Internal structure, amorphous, finely, coarsely-granular, grumose, filamentous, floccose, curled.
11. Starch Jelly.  
 Growth, scanty, copious.  
 Diastasic action, absent, feeble, profound.  
 Medium stained .....
12. Silicate Jelly (Fermi's Solution.)  
 Growth copious, scanty, absent.  
 Medium stained .....
13. Cohn's Solution.  
 Growth copious, scanty, absent.  
 Medium fluorescent, non-fluorescent.
14. Uschinsky's Solution.  
 Growth copious, scanty, absent.  
 Fluid viscid, not viscid.
15. Sodium Chloride in Bouillon,  
 Per cent inhibiting growth .....
16. Growth in Bouillon over Chloroform, unrestrained, feeble, absent.
17. Nitrogen. Obtained from peptone, asparagin, glyco-coll, urea, ammonium salts, nitrogen.
18. Best media for long-continued growth .....
19. Quick tests for differential purposes .....

IV. PATHOGENICITY.

1. Pathogenic to Animals.  
*Insects, crustaceans, fishes, reptiles, birds, mice, rats, guinea pigs, rabbits, dogs, cats, sheep, goats, cattle, horses, monkeys, man.* .....

2. Pathogenic to Plants:  
 .....

3. Toxins, soluble, endotoxins.

4. Non-toxin forming.

5. Immunity bactericidal.

6. Immunity non-bactericidal.

7. Loss of virulence on culture media: prompt, gradual, not observed in. ..... months.

BRIEF CHARACTERIZATION  
 Mark + or O, and when two terms occur on a line erase the one which does not apply unless both apply.

MORPHOLOGY (2)	Diameter over 1μ		
	Chains, filaments		
	Endospores		
	Capsules		
	Zoogloea, Pseudozoogloea		
	Motile		
	Involution forms		
	Gram's Stain		
	Broth	Cloudy, turbid	
		Ring	
Pellicle			
Agar	Sediment		
	Shining		
	Dull		
Gal. Plate	Wrinkled		
	Chromogenic		
	Round		
Gel. Plate	Proteus-like		
	Rhizoid		
	Filamentous		
Gel. Stab.	Curled		
	Surface-growth		
	Needle-growth		
Potato	Moderate, absent		
	Abundant		
	Discolored		
BIOCHEMICAL FEATURES	Starch destroyed		
	Grows at 37°C.		
	Grows in Cohn's Sol.		
	Grows in Uschinsky's Sol.		
	Liquefac-tion	Gelatin (2)	
		Blood-serum	
		Casein	
	Milk	Agar, mannian	
		Acid curd	
		Rennet curd	
Casein peptonized			
DISTRIBUTION	Indol (2)		
	Hydrogen sulphide		
	Ammonia (2)		
	Nitrates reduced (2)		
	Fluorescent		
	Luminous		
	Animal pathogen, epizoon		
	Plant pathogen, epiphyte		
	Soil		
	Milk		
Fresh water			
Salt water			
Sewage			
Iron bacterium			
Sulphur bacterium			





## CHAPTER IX

### THE DISTRIBUTION OF MICRO-ORGANISMS AND THEIR RELATION TO SPECIAL HABITATS

**General Distribution.**—Micro-organisms are very generally distributed over the surface of the earth and in its waters, and are carried about as dust in the air. They flourish abundantly in the digestive canals of animals and on their body surfaces. Wherever there is organic matter, the dead remains of animal and plant life, there are micro-organisms in abundance living upon the dead material and, if the temperature and moisture be suitable, transforming it into simpler chemical substances. In the soil, bacteria, yeasts, molds and protozoa are fairly numerous, especially in fertile soils near the surface. Their number rapidly diminishes in the deeper layers, and at a depth of six to twelve feet they are very scarce or entirely absent. The surface waters of the earth contain large numbers of bacteria and protozoa, especially numerous where organic matter is abundant. The air contains considerable numbers of molds and bacteria suspended as dust. The deep layers of the soil and water below impervious rock strata are free from micro-organisms. The surfaces of snow-covered mountains and of the frozen polar regions of the earth, as well as the atmosphere in these regions, are practically free from microbes. The atmosphere over large bodies of water during calm weather, the air in damp cellars, in sewers and in undisturbed rooms is germ-free, because the suspended dust particles settle out and do not escape again into the air unless swept up by air currents, which must be rather violent to remove them from moist surfaces.

The environment and the surfaces of growing plants and animals are rich in micro-organisms, especially bacteria, but the interior of the living tissues is generally germ-free in health. To this statement there are certain exceptions, namely, the occurrence of a few bacteria in the liver, the thoracic duct and the blood of animals during active digestion, which are, however, soon destroyed by the healthy tissue; and the invasion of the root tissues of leguminous plants by *Ps. radicicola* and the growth of the bacterium within the plant tissues, which results not in injury to the host but in a definite improvement of its nutrition by enabling it to utilize atmospheric nitrogen.

**Micro-organisms of the Soil.**—The germ-content of soil depends chiefly upon the amount of organic matter present. They may be present in millions per gram of soil. Bacteria, molds and protozoa are the most numerous. Their relation to soil fertility seems to be important, and they probably play a large part in preparing the organic matter of the soil for use as food by plants. A great many soil bacteria decompose protein and set free ammonia, and the urea bacteria are especially important in the transformation of urea and of animal manures into ammoniacal compounds. The transformation of ammoniacal compounds into nitrates, so-called nitrification, is accomplished by the nitrifying bacteria, of which a few species have been obtained in pure culture, *Nitrosomonas* of Winogradski which produces nitrite from ammonia, and his genus *Nitrobacter* which oxidizes nitrites to nitrates. Very many species of soil bacteria are able to change nitrogen in the opposite direction, reducing nitrates to nitrites and further to ammonia or to free nitrogen gas. Of special interest are the soil bacteria which are able to fix atmospheric nitrogen, that is, absorb nitrogen from the air and combine it so as to make it available for plant food. The various species of the genus *Azotobacter* (*A. chroococcum*, *A. beyerincki*) accomplish this as they grow in the presence of dextrose, and the organism of the root tubercles, *Pseudomonas radicicola*, fixes nitrogen as it grows within the tissues of the legume roots. Numerous soil bacteria

ferment sugars, starches and fats, and there are several known species capable of dissolving cellulose.<sup>1</sup>

**Pathogenic Soil Bacteria.**—Certain pathogenic bacteria are of common occurrence in the soil. Whether this is their normal habitat or whether they gain entrance to the soil with animal excrement, may be questioned. At any rate the pathogenic anaërobes, *B. edematis*, *B. tetani*, and *B. welchii*, are likely to occur in garden soil, and it seems probable that they actually multiply there to some extent. *Bact. anthracis* also occurs in the soil of fields where the disease has prevailed, and it is not improbable that this organism multiplies in the ground at times. Other pathogenic bacteria, such as those of typhoid and cholera, seem to be rather quickly eliminated in the struggle for existence under the conditions found in surface soils.

**Micro-organisms of the Air.**—Micro-organisms exist in the air only as floating particles of dust, or as passengers on small droplets of moist spray, or as parasites on or in winged aerial creatures. Those floating as dust are derived from the earth's surface, and most of the living germs usually found in this condition are the spores of molds. Living tubercle bacilli are unquestionably suspended in the air as dust, especially in the dry sweeping of floors where careless consumptives have lived. The spores of anthrax bacilli may also be suspended in the air where hides or wool of anthrax animals are handled. Other pathogenic bacteria may at times float as dust, but their presence in the air in this condition is apparently rather uncommon, and should be expected only in the fairly recent environment of cases of the disease. The moist droplets, expelled from the mouth and nose in speaking, in coughing and especially in sneezing, may remain suspended in the air for many minutes and be distributed to considerable distances. After drying the solid material may still float as dust. Pathogenic micro-organisms may readily be transmitted from person to person in this way.

<sup>1</sup> For a discussion of the microbiology of the soil, see Monograph by Lipman in Marshall's *Microbiology*, 1920.

In a rough way one may obtain some knowledge of the character of the organisms in the air of a given locality by removing the cover of a Petri dish containing sterilized gelatin or agar for a few minutes, replacing it, and allowing the organisms to develop. In most cases a large proportion of the growths that appear will be molds. Yeasts are also common, and among the bacteria the micrococci are abundant. Chromogenic varieties are likely to be present.

A few studies of this character will show that the number of organisms that are present depends chiefly upon whether the air is quiet or has recently been disturbed by drafts, gusts of wind, or sweeping. These facts are of fundamental importance in laboratory work, if we wish to avoid contaminations. Among various devices that have been proposed for the accurate study



FIG. 84.—Sedgwick-Tucker aërobioscope.

of the organisms of the air, the Sedgwick-Tucker aërobioscope is the simplest and most accurate. It consists of a glass tube, one end of which is drawn out so as to be smaller than the other. The small end contains a quantity of fine granulated sugar; both ends are plugged with cotton, and the instrument is sterilized. A definite quantity of air is to be aspirated through the large end, after removing the cotton, and this may be done by means of a suction-pump applied to the other end, or by siphoning water out of a bottle, the upper part of which is connected with the end of the aërobioscope by means of a rubber tube. The sugar acts as a filter and sifts out of the air the micro-organisms which are contained in it. Liquefied gelatin or agar may be introduced into the large end of the instrument by means of a bent funnel; and, after replacing the cotton, it is mixed with the sugar which dissolves. The culture-medium may be spread around the inside of the larger portion of the tube after the manner of an Esmarch

roll-tube. The microbes which are filtered out by the sugar will develop as so many colonies upon the solidified medium.

Many important micro-organisms, and certainly some germs of disease, are borne through the air by the winged insects, and to a less extent by birds. The microbes are found not only on the feet and outer body surfaces of these carriers, but they also occur on and in the mouth parts, in the alimentary canal and sometimes in the interior of the animal's body tissues. Certain pathogenic micro-organisms (*Plasmodium*, *Trypanosoma*) are known to be transmitted from one person to another almost exclusively by biting insects, and the importance of these carriers in air-borne disease of both animals and plants, is being recognized more and more.

**Micro-organisms of Water and Ice.**—The water of rivers, lakes and the ocean always contains bacteria. The number or organisms varies greatly in different places and under different conditions. The number of different species found in water is also very large. Some of these, the natural water bacteria, including many bacilli which produce pigment and some cocci and spirilla, seem to live in surface water as their natural habitat. With the addition of putrescible material these forms are increased in number and certain of them (*Proteus* group, fluorescing bacteria) become numerous. Soil bacteria are numerous in waters during floods and after rain, and they may persist for some time. Intestinal bacteria occur in waters which receive sewage or are otherwise contaminated with excreta. They persist only a relatively short time. Certain intestinal protozoa, *Endamæba*, *Balantidium*, seem also to occur in waters at times. Ground-water<sup>1</sup> contains few or no bacteria under normal conditions, and is therefore suitable for a source of water-supply, when a sufficient amount is available. The possibility of contamination of the ground-water from unusual or abnormal conditions

<sup>1</sup> Ground-water is the water which—originally derived from rain or snow—sinks through superficial porous strata, like gravel, and collects on some underlying, impervious bed of clay or rock.

should always be eliminated before it is taken for drinking water. Numerous epidemics of typhoid fever have been traced to contamination of wells. The location of wells with reference to privy-vaults and other possible sources of contamination should be chosen with the greatest care.

The ordinary bacteria of water are harmless, as far as is known.<sup>1</sup> Bad odors and tastes in drinking water that is not polluted with putrid material are usually due to minute green plants (algæ).<sup>2</sup> The diseases most commonly disseminated by water are typhoid fever and Asiatic cholera, and probably also dysentery. The spirillum of cholera will usually die in natural water (not sterilized water) inside of two or three weeks; the bacillus of typhoid fever will usually die in two or three weeks. Under exceptional circumstances these organisms may perhaps maintain their vitality for a longer period. They appear, however, to be less hardy than the ordinary water bacteria. As we now understand these diseases, the organisms causing them will be present only in a water-supply which has been recently contaminated by the excreta from a case of the disease. Notwithstanding the rapid death of these organisms in water, they may exist long enough to infect individuals habitually drinking the water. Many epidemics of cholera and typhoid fever have been traced to water polluted with the discharges from cases of these diseases, and in a few instances the relation of the contaminated water supply to the epidemic has been established beyond a reasonable doubt.

By *self-purification* of water is meant the removal, through natural processes, of contaminating organisms such as might occur from the discharge of sewage into it. It depends upon the sedimentation of the contaminating material in the form of mud, upon the growth of the ordinary water-plants and protozoa,

<sup>1</sup> See Fuller and Johnson, "The Classification of Water Bacteria," *Journal of Experimental Medicine*, Vol. IV, p. 609.

<sup>2</sup> "Contamination of Water Supplies by Algæ." G. T. Moore in Yearbook U. S. Department of Agriculture, 1902.

upon the exhaustion of the food supply by the growth of bacteria themselves, upon the destructive influence of direct sunlight, and the dilution of the contamination by a large volume of water.<sup>1</sup> It is not usually to be relied upon as a means of freeing the water-supply from pathogenic bacteria.

*Storage of Water.*—When water is kept in large reservoirs, the solid particles in it, including bacteria, tend to fall to the bottom. The number of bacteria in a water-supply may be considerably reduced in this way. The use of large storage reservoirs also provides for the dilution of any sudden excess of pollution, and if the water is held in storage the pathogenic germs present disappear for the most part in a few days or weeks.

*Filtration.*—Water may be completely sterilized by passing it through the Pasteur-Chamberland filters of unglazed porcelain, or through the more porous Berkefeld filters. Such filters are effective only when frequently cleaned and baked, and in practical purification of water for household purposes they usually fail because of the intelligent care they require. Other types of domestic filters are generally worse than useless.

Filtration on a large scale is commonly employed in the purification of water supplies of cities. By this method 98 per cent to 99 per cent of the bacteria in water may be removed.

*Slow Sand Filtration.*<sup>2</sup>—The filter consists of successive layers of stones, coarse and fine gravel. The uppermost layers are of fine sand. The whole filter is from 1 to 2 meters thick. The sand should be 60 cm. to 1.2 meters in thickness. The accumulated deposit from the water and a little of the fine sand must be removed from time to time, but the layer of fine sand must never be allowed to become less than 30 cm. in thickness. The first water coming from the filter is discarded. The actual filtration is done largely by the slimy sediment which collects on the surface of the layer of fine sand. The filterbeds may

<sup>1</sup> See Jordan, *Journal of Experimental Medicine*. Vol. V, p. 271.

<sup>2</sup> For a full discussion see *Journal American Medical Association*. Oct. 3 to 31, 1903.

be several acres in extent, and in cold climates should be protected by arches of brick or stone. They require renewal occasionally. This kind of filtration has come largely into use since the cholera epidemic of 1892-93, and it appears to be very effective. It is important to use storage basins in connection with sand filtration.

The results obtained by filtration depend greatly upon the intelligence displayed in operation, and must be controlled by frequent examinations of the water.

*Mechanical Filtration.*—This method of filtration is also called the American system. It is more rapid than the preceding method and does not require a large area for filter beds. Although sand is required also, filtration is accomplished by a jelly-like layer of aluminium hydroxide. This product is formed by adding to the water a small quantity of aluminium sulphate or of alum. The carbonates in the water decompose the aluminium salt and produce aluminium hydroxide. It precipitates as a white, flocculent deposit, entangling solid particles, including bacteria, as coffee is cleared with white of egg. Only a trace of aluminium should appear in the water. This method of filtration has not been tested so extensively as slow sand filtration, but seems likely to prove efficient. With water poor in carbonates, these may have to be added.<sup>1</sup>

Whipple and Longley<sup>2</sup> found that the efficacy of mechanical filters with the addition of alum depends somewhat upon the character of the alum. They find that the alum shall be shown by analysis to contain 17 per cent of alumina ( $\text{Al}_2\text{O}_3$ ) soluble in water, and of this amount at least 5 per cent shall be in excess of the amount necessary theoretically to combine with the sulphuric acid present. It shall not contain more than 1 per cent of insoluble substances, and shall be free from extraneous debris of all kinds. It must not contain more than 0.5 per cent of iron ( $\text{Fe}_2\text{O}_3$ ) and the iron shall be preferably in the ferrous state.

<sup>1</sup> See Fuller, *Journal American Medical Association*, Oct. 31, 1903.

<sup>2</sup> *Journ. Infect. Diseases*, Supplement No. 2, Feb., 1906, pp. 166-171.

*Chemical Disinfection.*—Various methods for the purification of water by means of chemicals have been proposed. The use of copper sulphate to disinfect drinking water was recommended by Moore and Kellerman,<sup>1</sup> and various investigators tested the value of their recommendation. Clark and Gage<sup>2</sup> came to the conclusion from their investigation that the treatment of water with copper sulphate or the storing of water in copper vessels has little practical value. Others also have come to practically the same conclusion. While the addition of copper sulphate is of use in preventing the growth of the algæ, which sometimes grow so abundantly as to choke up water pipes, and is of benefit in this direction, the weight of evidence appears to be against its efficacy for purifying water for drinking purposes. More effective chemical disinfection has been obtained by means of ozone generated by electricity. More recently, calcium hypochlorite and free chlorine have been employed for this purpose with considerable success, and have almost completely displaced other substances as chemical disinfectants of drinking water.

*Physical Disinfection.*—The most effective and surest method of disinfecting drinking water is by boiling it or by distillation.

*Bacteriological Examination of Water.*—For bacteriological examination samples from the water-supply of a city may be drawn from the faucet, but the water should first be allowed to run for half an hour or longer. From other sources the supply should be collected in sterilized tubes or bottles, taking care to avoid contamination. These samples should be examined as promptly as possible, for the water bacteria increase rapidly in number after the samples have been collected. When transportation to some distance is unavoidable the samples should be packed in ice, but even this precaution does not preserve the original bacteriological condition of the water at the time of collection; for more or less change probably takes place at all temperatures. If the temperature is too low, and the water

<sup>1</sup> U. S. Dep. Agriculture, Bu. Plant Ind. *Bulletin* 64, 1904.

<sup>2</sup> *Journ. Inf. Diseases*, Sup. No. 2, Feb., 1906, pp. 175-204.

## MICRO-ORGANISMS OF FOOD

**Milk.**—Milk is the natural food of young mammals, and naturally it is taken directly from the mammary gland into the digestive tract of the young mammal. For many centuries however, the milk of certain animals has been extensively used as a commercial food for man. The principal animals furnishing commercial milk are the cow, goat and mare. The chemical composition of milk is different in different animals, in the same animal at different periods of lactation, and even that obtained at different stages of a single milking shows considerable variation. In general cow's milk has the following composition.

	Variation	Average
Fat.....	3-6	4 per cent.
Lactose.....	1-3	2 per cent.
Protein.....	5-8	7 per cent.
Water.....	84-88	87 per cent.

It is an excellent medium for the growth of most bacteria and is commonly used in the laboratory for this purpose.

There are about 200 species and varieties of bacteria which commonly occur in milk. They are derived in part from the udder itself. Bacteria are always present in the milk ducts of the udder and are fairly abundant in the first portions of milk drawn, so that milk very carefully drawn from healthy animals may contain 200 to 400 bacteria per cubic centimeter. Milk from diseased udders may be very rich in pathogenic micro-organisms. As the milk is drawn, many micro-organisms usually gain entrance to it from the atmosphere, the hands of the milker and the utensils with which it comes in contact. From the body of the cow, particles of dust and hairs drop into the milk, carrying with them the flora of the intestine and of the skin of the cow. From the milker, the material on the hands and possibly also from the nose and mouth may reach the milk. The utensils, unless sterilized before use, contribute the microbic flora of the previous milkings, of the water used for cleansing and from the

person who handles them. From the air, the milk may receive further contamination (1) from flies coming to drink or perhaps to drown without a clean bill of health from their port of last departure, (2) from particles suspended as dust and containing micro-organisms derived from manure, from hay and straw, and from soil, and (3) moist droplets expelled from the mouth and nose of the milkers and of the cattle. The subsequent handling of the milk may add further kinds of bacteria from human sources. Modern dairy practice in vogue in the production of the higher grades of milk eliminates some of these sources of contamination and minimizes the importance of the rest, but nevertheless fresh milk of even the better grades contains a great variety of micro-organisms, and often as many as 10,000 to 100,000 per cubic centimeter when it leaves the producer's dairy.

The usual milk flora derived from these various sources may be classed under the following heads:

A. Lactic acid bacteria.

1. *Bacterium (streptococcus?) acidi lactici*
2. *Bacillus coli* and *B. lactis aërogenes*.
3. Long rods of *B. bulgaricus* type.
4. *Streptococcus pyogenes*.
5. *Micrococcus acidi lactici*.
6. Acid formers which liquefy gelatin.

B. Gelatin-liquefying bacilli.

7. Rapidly liquefying types—*B. subtilis*.
8. Slowly liquefying types.

C. Pigment-forming bacteria.

D. Anaërobic bacteria—*B. welchii*, putrefactive anaërobes.

E. Special types causing peculiar fermentations, such as slimy consistency, bitter taste and peculiar odors.

F. Pathogenic organisms—typhoid, tuberculosis, scarlatina, diphtheria, diarrhea, septic sore throat, foot-and-mouth disease, dysentery.

G. Other fungi—Molds, Oidia, Yeasts, Actinomyces.

The development of these various microbes in the milk depends very much upon the temperature at which it is kept. At  $0^{\circ}$  to  $10^{\circ}$  C. the acid-forming bacteria grow very slowly or not at all, and the milk may remain practically unchanged for many days or even weeks. Eventually some of the liquefying bacilli or the slime-producing types may gain the upper hand and change the consistency and flavor. Between  $10^{\circ}$  and  $21^{\circ}$  the *Bact. acidi lactici* is almost certain to gain the dominance and rapidly to suppress the other types, and it produces the normal souring of milk. Between  $21^{\circ}$  and  $35^{\circ}$  C. the organisms of the *B. coli* and *B. lactis aërogenes* groups are likely to predominate and at temperatures from  $37^{\circ}$  C. to  $40^{\circ}$  C. the *B. bulgaricus* is likely to gain the ascendancy, after a few days at any rate. These may be regarded as the normal fermentations of unheated milk of very good quality. The other microbes in the milk are not destroyed by these fermentations but their development is usually held in check somewhat.

Shortly after the coagulation of the milk, which occurs when the lactic acid reaches a concentration of about 0.45 per cent, the living bacteria begin to diminish in number, and gradually *Oidium lactis* and other molds become prominent, although acid-resisting forms such as *B. bulgaricus* still continue to grow. Organisms of these kinds seem to be specially concerned in the ripening of acid curd in cheese making. Finally the acidity may disappear as a result of the activity of molds, and putrefactive bacteria find the opportunity to develop.

If the milk be pasteurized, the bacteria which form lactic acid are killed, and when fermentation occurs it is likely to be different from the normal souring. At a high temperature, the stormy butyric-acid fermentation due to *B. welchii* may be observed. At a lower temperature, a slow putrefaction due to spore-forming putrefactive anaërobes in conjunction with other bacteria may occur. These fermentations are ordinarily inhibited by the lactic acid produced in the normal souring of milk.

Alcoholic fermentation of milk occurs as a rule only when

special ferments are purposely added to produce this result. Kumyss and Kefir are fermented milks produced in this way. The starter or ferment contains yeasts as well as bacteria.

The pathogenic micro-organisms in milk are derived in part from unhealthy cows—tuberculosis, foot-and-mouth disease, septic sore throat (?)—but in a larger measure from the people who handle the milk or from utensils—tuberculosis, typhoid fever, scarlatina, diphtheria, diarrheas, dysentery, septic sore throat (?). The bacteria of typhoid fever, diphtheria and dysentery are known to multiply in milk. The microbes of tuberculosis and foot-and-mouth disease may persist in butter and cheese for several weeks at least.

Leaving out of consideration the question of specific pathogenic micro-organisms, the presence of more than 500,000 bacteria per cubic centimeter in the milk regularly fed to infants and young children is undoubtedly harmful, and especially so in warm weather. Doubtless many factors contribute to the causation of the summer diarrheas and the summer mortality of children, but there can no longer be any question that a milk rich in living bacteria as food for these children is one of the very important causes of their illness and death.

Milk for infant feeding should come from clean, healthy (tuberculin-tested) cows, should be handled by clean healthy workmen, in clean stables and rooms and with clean, sterilized utensils. It should be bottled at the producing dairy, promptly chilled to 10° C. or below, and maintained at this temperature until delivered at the home. At this time the living bacteria should not exceed 30,000 per cubic centimeter. In the home, the milk should be kept cold. It must be handled only with utensils sterilized by boiling in water. Boiled water is employed in making the necessary dilutions. If the milk supply is not above suspicion the milk should be pasteurized by heating to 60° C. for 20 minutes. The dilution is prepared and filled into separate bottles sufficient in number so that one may be used for each feeding during the succeeding 24 hours. Each bottle

is chilled in cool water, then ice water, and finally stored in the refrigerator. Immediately before feeding it is warmed by partial immersion in warm water.

**Other Foods.**—Other foods, meats, fish, eggs, vegetables and fruits, undergo decompositions due to more or less definite types of micro-organisms, and the activities of these are delayed or prevented by modern methods of preserving foods, in some instances very successfully, and in other cases with limited success.<sup>1</sup> Any food, and especially that eaten without cooking, may serve as a passive carrier of pathogenic micro-organisms. Salads, green vegetables and fresh fruits may undoubtedly act in this way during epidemics. Oysters taken from sewage-polluted beds have been found to convey typhoid fever. Meats derived from mammals may contain specific germs causing disease in both animals and man, such as tuberculosis, anthrax and foot-and-mouth disease. The flesh of bovine animals suffering with enteritis at the time of slaughter seems to be particularly liable to develop poisonous properties, and the ill effects observed in these instances may have been due to a specific infection. Paratyphoid fever is sometimes traced to such meat as a cause.

Meats and fish are rich in protein and their decomposition by saprophytic bacteria may give rise to various poisonous substances, as has been mentioned on page 177. The usual course of putrefaction, however, goes on without very strong poisons being produced, as we may judge from the habitual use of partly decomposed foods of this sort. Virulent poisons are occasionally encountered and some of these are due to the presence of specific microbes, *B. botulinus* of Van Ermengen, *B. enteritidis* of Gaertner and the paratyphoid and paracolon bacilli.

<sup>1</sup> For a discussion of the microbiology of foods and of food preservation see Marshall's Microbiology for agricultural and domestic science students, 1920.

## CHAPTER X

### PARASITISM AND PATHOGENESIS

**The Parasitic Relation.**—The presence in a living organism of one or several organisms of another species, which live as parasites upon the first, is a phenomenon of common occurrence in nature. Those organisms such as the bacteria, which are too small to harbor visible internal parasites, are subject to the parasitic ravages of larger beings such as amebæ and other protozoa, which engulf them bodily and digest them. Man, who is wont to complain of his parasitic ailments, takes all his protein, fat and carbohydrate from the bodies of plants and other animals. Parasitism in the larger sense is a well-nigh universal characteristic of living beings. Parasitism in a narrower sense usually applies to the existence of a smaller organism, the parasite, in or on the body of a larger, the host, a relation in which the host furnishes the parasite its necessary food. In many instances the advantages of the relation are wholly one-sided, but in others the two organisms seem to be of mutual benefit. In the latter case, the condition is called symbiosis. The infection of the roots of the clover with *Pseudomonas radiculicola*, which promotes the nitrogenous nutrition of the plant, is an example of this relation. In other instances the two organisms living in close association seem neither to help nor injure each other. They are then called commensals or companions at the same table. Internal parasites occur in all the higher animals and plants, and have been found even in the bodies of protozoa. Representatives of all the great classes of micro-organisms are found among the internal parasites, and many more highly organized animals and plants also lead parasitic lives. Man, alone, is subject to

infestation with parasitic insects and numerous worms, in addition to an enormous variety of microbes. Whether a parasitic organism is to be regarded as a symbiont, a commensal or a pathogenic agent depends upon the effect which it produces upon its host. A pathogenic organism is one whose presence results in definite injury to the host.

**Pathogenesis.**—In human pathology the phenomena of disease have for centuries been the object of careful study and speculation, and in many instances the phenomena commonly associated together have long been regarded as a complex result of a single primary cause, and the condition in which such phenomena are observed has been regarded as a single morbid entity or a definite disease. Even the most ancient records indicate that such recognition had long been common knowledge. A beginner in parasitology or pathology may be inclined to ascribe a causal relation to a parasite which he observes in the body of a sick individual; in fact this has been done repeatedly. The logical requirements for the proof of such a relationship were first formulated by Henle, as has been mentioned in the historical sketch in the introductory chapter. They were reformulated by Koch, who, for the first time, was able to comply with them in respect to a bacterial disease. They may be stated as follows:

1. The organism must be present in all cases of the particular disease.
2. The organism must be isolated from the diseased body and propagated in pure culture.
3. The pure culture of the organism when introduced into susceptible animals must produce the disease.
4. In the disease thus produced, the organism must be found distributed as in the natural disease.

Although we may very properly consider a micro-organism as the probable cause of a disease with which it is associated, without satisfying all of the above requirements, experience has served to emphasize more and more the wisdom of reserving final

judgment wherever these rules or similar stern logical requirements have not been satisfied.

**Infectious Disease.**—An infectious disease is a disease due to the entrance of a living micro-organism and its growth in the body. Although conservative bacteriologists are sometimes loth to accept a disease as infectious until Koch's rules have been satisfied, most are agreed that a disease, which can be reproduced indefinitely by the inoculation of healthy individuals in series with material taken from a preceding case, is due to a living cause. The proof that a disease is due to a living cause may therefore precede the identification of the causal organism, often by many years.

**Possibility of Infection.**—Whether a parasitic organism will be able to enter and multiply in a new host and cause disease depends upon a number of circumstances, the most important of which may be considered under four heads, namely, the quality of the microbe, the resistance of the host, the quantity of invading parasites, and the path of entrance. The course and ultimate result of an infection depend also to a marked degree upon these same factors. In general the qualifications of the micro-organism depend first upon the experience of its ancestry under the same or similar environmental conditions, factors inherent in its species, and second, upon its very recent history, factors affecting the virulence and general vigor of the individual microbe. Thus the tubercle bacillus is qualified by inheritance for a parasitic existence, while the common yeast cell is not. Yet, the tubercle bacillus, when cultivated for a long time on artificial media may lose its former ability to grow in the animal body. The factors affecting the pathogenic properties of a microbe will be considered in the succeeding chapter.

**Susceptibility and Resistance.**—Among the important things in the nature and condition of the host, we need also to consider both racial and individual characters. Certain species of animals have harbored certain parasites for so long that the latter have become adapted to growth in the particular species of host. In

some instances the adaption is very narrow and the parasite may be able to exist naturally only in the one-host species, as for example *Spirochæta pallida*. Individual resistance of different hosts of the same species is variable. Age is one important factor: there are the children's diseases, measles, chickenpox; the diseases of active adult life, pulmonary tuberculosis, typhoid fever; and the diseases of the aged, pneumonia, carcinoma. Hunger and thirst have been shown experimentally to reduce the resistance to infection: pigeons, which are normally immune to anthrax become susceptible when starved. The effect of fatigue is well known: a white-rat, normally immune to anthrax, succumbs to it after prolonged work in the treadmill. Abnormal chilling of hens removes their immunity to anthrax and abnormal heating of frogs affect them in a similar way. Chemical poisoning also increases susceptibility to infection, and cachexia and malnutrition are well-known predisposing factors to such infections as tuberculosis. Traumatism is very important, not only for its general effect upon the resistance of the host, but especially in the reduction of local resistance by destruction or injury of tissue (wounds). There are certain locations where resistance to infection is naturally lower, such as the ends of growing bones and the interior of the parturient uterus.

**Number of Invaders.**—The quantity of infectious material introduced is of importance in determining whether infection will or will not occur. Very few species of microbes are capable of causing disease when only a single individual organism is introduced into the body. A large number of microbes entering at the same time seems to overburden the defensive powers of the body so that some of the parasites succeed in establishing themselves and multiplying.

**Modes of Introduction.**—There are various avenues by which micro-organisms may enter the body to produce disease. Infection of the ovum in the ovary with spirochetes and protozoa is known to occur in some insects, and Rettger has shown that this phenomenon occurs in the hen infected with *Bacterium pul-*

*lorum*. The human ovum also seems occasionally to be infected with *Spirochaeta pallida* in this way. It may also become infected with the same organism derived from the seminal fluid. The developing fetus is sometimes invaded by pathogenic microorganisms introduced through the placental circulation. The organisms of tuberculosis, small-pox, typhoid fever and the pyogenic cocci are known to be transmitted, somewhat uncommonly to be sure, in this way. As a rule the germ must be circulating in the blood of the mother in considerable numbers, or there must be actual infectious lesions of the placenta before placental transmission occurs. After birth non-pathogenic microbes gain access to the entire surface of the body and penetrate the various canals opening to the exterior to certain normal limits. Pathogenic germs may be introduced with the food and drink, which is the common natural mode of infection with cholera and typhoid fever in man and with tuberculosis in hogs and cattle. The barrier presented by the activity of the gastric juice is frequently passed in safety by the ingested microbes. Inhalation is probably the most common way in which tuberculous infection<sup>1</sup> reaches the lungs in man, although there is conclusive evidence that tuberculosis in this location may be derived from the alimentary tract through the blood stream. Experimentally, guinea-pigs are much more susceptible to infection with tubercle bacilli by inhalation than by ingestion. Mere application of the infectious agents to the epithelial surface of the skin or mucous membranes results in infection in many instances and, indeed, infection by ingestion and inhalation may be regarded as examples of this. The mucous membranes of the urethra and the eye, and also of the rectum in young children, are especially susceptible to infection with the gonococcus. The unbroken skin may be infected with staphylococci, which seem to penetrate through the hair follicles and sebaceous glands, giving rise to boils and car-

<sup>1</sup> McFadyean, *Journal Royal Institute of Public Health*, 190, Vol. XVIII, pp. 703-724.

buncles; but to most microbes the uninjured skin presents an effective barrier.

The question whether infectious agents may penetrate epithelium and gain the lymph or blood-vessels beyond without causing a local lesion, has received considerable attention and it seems to be established as certainly possible in the intestine during the absorption of fat, and it may perhaps occur in other locations.

Infection through wounds, even minute breaks in the epithelial covering, is very common. Such wounds made by insects are the common portals of entry for the germs of malaria, plague, yellow fever, relapsing fever and many more diseases. Larger wounds nearly always become infected with pyogenic cocci unless they are properly cared for. The introduction of infectious material into the subcutaneous tissue may occur accidentally in deep wounds and is a common mode of inoculation in the laboratory. Infection with the anaërobic bacillus of tetanus frequently occurs in this type of wound.

Infections of the peritoneal cavity, pleural cavities and cavities of the joints result from penetrating wounds, by the entrance of bacteria from contiguous tissues, as through the intestinal wall into the peritoneal cavity, and through the blood and lymph channels.

**Local Susceptibility.**—The invading parasite is favored by conditions of local susceptibility such as tissue destruction, presence of necrotic tissue and foreign bodies, and also by the presence of other infectious microbes. Small-pox and staphylococcus, tetanus and the pus cocci, scarlet fever and streptococcus, are common examples of such mixed infections. In some instances one infection predisposes to another. For example, measles is likely to favor the development of tuberculosis; the caseous tubercle is likely to be invaded by the streptococcus. These subsequent invasions are spoken of as *secondary infections*.

**Local and General Infections.**—The invading microbes may remain localized near the point of entrance, as for example in tetanus and diphtheria. In such cases the general effects may be

due to disturbance in function of the local tissue, such as laryngeal obstruction in diphtheria, or to the absorption into the lymph and blood of poisons produced at the infected site. Such absorption results in toxemia with symptoms due to poisoning of distant tissue elements. On the other hand, the infectious agent may pass quickly to the blood stream without appreciable local reaction and multiply there, as in malaria, trypanosomiasis and streptococcus *bacteremia*. Again there may first develop an intense local reaction, with subsequent extension to the blood stream with fatal issue, as in malignant pustule (anthrax). In other instances repeated temporary invasions of the blood occur, with numerous localized abscesses in various parts of the body, a condition to which the name *pyemia* has been applied. Of particular interest are those general infections of the blood stream, which finally fade away, but leave behind localized infections in the joints, on the heart valves, in the central nervous system, or other parts of the body. Sleeping sickness, syphilis, acute articular rheumatism and generalized gonococcus infection belong in this category.

**Transmission of Infection.**—The manner in which an infectious agent passes from its host to a new victim varies considerably. In general it may be said to occur (1) by direct contact or close association, transmission by *contagion*, (2) through the agency of intermediate dead objects as passive carriers, transmission by *fomites*, or (3) through the agency of a living or dead object, in which the parasite undergoes development or multiplication, transmission by *miasm*. These terms have been employed in the past to designate rather hypothetical objects not to say abstract ideas, and their application to the facts learned by modern research is, perhaps, not desirable. Nevertheless, they may be made to fit the observed phenomena in a way. Thus, syphilis and gonorrhoea are transmitted by contagion; diphtheria and small-pox by contagion and by fomites; tetanus and anthrax by fomites and perhaps also miasm; plague by contagion, fomites and miasm (through the rat and flea); malaria, trypanosomiasis

and yellow fever by miasm. All of these are doubtless infectious diseases but some of them are not naturally spread by contact at all. In studying each disease it will be necessary to consider the avenues by which the parasite leaves the patient, its existence in the external world and the means of gaining access to its new victim.

**Healthy Carriers of Infection.**—A person or animal may harbor virulent infectious agents without showing symptoms of disease, and may serve as a source of infection to others. This was clearly recognized in the sixteenth century by Fracastorius as a factor in the spread of syphilis. Only recently has its importance in other diseases been emphasized.

## CHAPTER XI

### THE PATHOGENIC PROPERTY OF MICRO-ORGANISMS

**Adaptation of Parasitism.**—In order to live as a parasite, an organism must be adapted to grow under the conditions met with in the body of the host, but in order to produce disease it must also injure the host. The most perfect adaptation of parasitism is probably exhibited by those micro-organisms which do not injure the host, the symbionts and commensals, as it is obviously to the interest of the parasite to keep its host alive. An adaptation of this kind usually requires that the microbe shall either grow very slowly, or shall be so situated that the excessive numbers resulting from its multiplication may readily pass out of the host or be disposed of in some way; otherwise the host would be physically crowded out. This sort of adaptation is illustrated by the normal intestinal bacteria. Parasites which invade the tissues of the body rarely show such adaptation. It is, perhaps, approached to some extent by the slow-growing bacilli of leprosy and tuberculosis. In most instances of parasitism, however, there is more or less of a struggle between the invader and the host for the possession of the field, and the phenomena of disease are incident to this combat.

**Virulence.**—The ability of the parasite to injure its host, is designated as virulence. Virulence depends in part upon growth vigor, but also upon other factors largely unknown. A great deal is known about specific methods of changing the virulence of micro-organisms, and various procedures are commonly employed with this object in view. A diminution in virulence is called attenuation and an increase in virulence, exaltation. Attenuation was first observed by Pasteur in a culture of *Bacterium avisepticum* (chicken cholera) grown in broth in the presence

of air. Pneumococci and streptococci also attenuate rapidly in artificial culture. Even those bacteria which retain their virulence in ordinary cultures become attenuated when grown at unusually high temperatures ( $42^{\circ}$  C.) or in the presence of anti-septics, both of which methods have been employed in attenuating the anthrax bacillus. Attenuation also results sometimes from parasitism in hosts of another species. Variola and vaccinia present a conspicuous example of this. Mere dessication of a virus seems to attenuate it in some instances (rabies) but this is somewhat doubtful. Many pathogenic agents become somewhat attenuated upon long residence in the same host in chronic infections. Exaltation of a virus, on the other hand, is accomplished by rapid passage through susceptible animals in series. When the organism is too attenuated to produce an infection alone, it may be aided by the admixture of other organisms (mixed infection) or by the presence of irritating foreign bodies (splinters, stone dust) or by mechanical protection in collodion capsules.

**Microbic Poisons.**—The weapons which the pathogenic agent employs to injure its host are various. The physical mass of the invaders may be injurious, more particularly by obstructing blood-vessels, as in estivo-autumnal malaria in man and anthrax in the mouse. Usually, however, the offensive weapons are chiefly chemical poisons. The soluble toxins, or true toxins are substances of unknown chemical composition, produced inside bacterial cells and passed out to their surroundings. These so-called extracellular toxins include the most poisonous substances known. Brieger and Cohn obtained a toxin, still impure, from tetanus bacilli, of which five one hundred millionths of a gram (.0000005 gram) killed a mouse weighing 15 grams. At this rate .00023 of a gram would kill a man weighing 70 Kilos. The soluble toxins elaborated by the diphtheria and tetanus bacilli have been studied most, and many of our ideas concerning toxins in general have been derived from these studies. These poisons are rapidly destroyed by heat, resembling

enzymes in this respect. They differ from enzymes in that they are used up in combining with tissue. Thus tetanus toxin may be completely neutralized by the addition of brain tissue, and either diphtheria or tetanus antitoxin may be quantitatively neutralized by its specific antitoxin. Ehrlich in his study of the reactions of diphtheria toxin showed that on standing it loses much of its poisonous property without any diminution in its ability to combine with diphtheria antitoxin, and to this less poisonous substance he gave the name toxoid. From this observation he concluded that the toxin molecule contains at least two very definite atomic groups. One of these is comparatively stable and serves for attachment of the toxin molecule to the cell attacked by it, and is called the haptophorous group or simply haptophore. The other recognizable chemical group disintegrates more readily and is that which bears the poisonous property. To this he gave the name of toxophorous group or toxophore. In their reactions toxins behave in part like feebly dissociated chemical compounds, as has been shown by Arrhenius and Madsen, but the reactions by which they combine are only slightly or not at all reversible and, moreover, take place in variable proportions. Bordet very aptly compares the reactions of toxin to the union of a dye with a stainable material. Bacteria also produce poisons which are part of their own body substance, and set free only upon their death and disintegration. These are spoken of as intracellular toxins. Injurious substances may also be produced from the tissue of the host by a secondary action outside the cell of the parasite, but these secondary products play a very minor rôle.

**Defensive Mechanisms.**—The defensive armor of parasites seems also to be in part physical and in part chemical, and perhaps we may regard the physiological adaptation to slow growth as a defensive mechanism because it tends to avoid exciting the opposition of the host. The physical structure seems to be protective in case of the waxy bacteria (tubercle and leprosy) and the capsules of other bacteria may serve a similar purpose

(pneumococcus). There is some indication that micro-organisms may produce special chemical substances to neutralize the agencies which the host employs against them. These defensive substances have been designated by Bail as aggressins. Ehrlich has also found evidence of the acquirement of immunity to chemical substances by certain pathogenic microbes, especially trypanosomes and spirochetes, and he ascribes this property of the parasites to an alteration of their cell-chemistry.

## CHAPTER XII

### REACTION OF THE HOST TO INFECTION

**Facts and Theories.**—The host reacts to the presence of a pathogenic agent by a number of alterations in its physiological activities. Some of these alterations are gross and well known as the clinical manifestations of an infectious disease; others require special search for their detection; while some, doubtless a considerable number, still pass unobserved. Many of these changes are susceptible of very accurate observation, and in most instances the observed facts are well established. A clear understanding of the relation of the various facts to each other involves some imaginative reasoning, and various hypotheses have been advanced to explain the phenomena observed, and to fill in the gaps in our knowledge. The student may need to be on his guard not to confuse facts susceptible of observation with hypothetical deductions based upon such observations. Both have their peculiar value. An understanding of the phenomena of pathological physiology must be based upon correct ideas of normal physiology and accurate knowledge has not fully replaced hypothesis in this latter field.

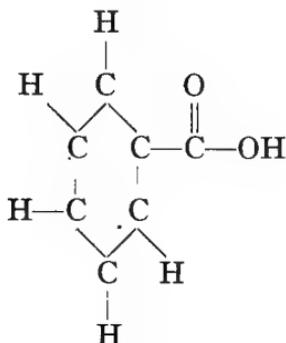
**Physiological Hyperplasia.**—Under normal conditions each cell of the human body is in close association with other cells and with the body fluids, and is subject to the physical and chemical stimulation of cells and fluids. One of the effects is apparently to restrain the proliferative activity of the cells. When certain of these cells are destroyed, or even certain parts of them, this restraint is removed, and the natural tendency to proliferation asserts itself, resulting in the production of new cells or of new parts to replace the old, and usually more than compensates for the loss. This somewhat hypothetical conception, due to Carl

Weigert, serves to explain tissue hyperplasia and repair following exercise or local destruction of tissue. Examples of these phenomena will occur to the reader.

**Phagocytosis and Encapsulation.**—The mere physical mass of a parasite within the tissue acts as a foreign body and it becomes surrounded by tissue elements. If it is minute, certain cells of the body (*phagocytes*) flow around and ingest it, as was first observed by Metchinkoff. If it is larger, the connective tissue cells proliferate and surround it, and eventually contract into a firm capsule. Further, the tissues produce enzymes capable of dissolving many foreign substances introduced in this way (parenteral digestion). If the foreign body is insoluble, it will remain encapsulated, or, if sufficiently minute, it may be transported considerable distances inside wandering cells and eventually be deposited in a lymph gland. The wholly passive parasite or the dead body of a micro-organism is therefore either digested and dissolved, ingested by cells, or encapsulated in fibrous tissue. Most infectious agents are not passive in this way, as we have seen, but tend actively to grow and multiply, to absorb and utilize food material, and, most important of all, to produce various substances which stimulate or poison the cells of the host. Against these the physical measures of ingestion (phagocytosis) and encapsulation are often inadequate defenses and may be entirely useless.

**Chemical Constitution of the Cell.**—Ehrlich has compared the living body cell to a complex chemical molecule; in fact it may be said that he regards the living cell as an enormous molecule, a chemical unit of great complexity. Certain atom groups within this molecule are pictured as relatively very stable and they constitute the chemical nucleus (not to be confused with the anatomic nucleus). Grouped about this chemically stable center are very many, more labile atom groups which readily enter into chemical reaction with substances in the surrounding medium. The conception is derived directly from well-known facts in organic chemistry. For example when benzoic acid,  $C_6H_5 \cdot COOH$ , reacts with other chemicals the reaction takes place at the reactive group,

or side-chain, rather than in the nucleus. The graphic formula may illustrate this point better. The six carbon atoms in the ring



are stable, and a strong chemical reagent, such as phosphorus pentachloride, reacts with the side-chain without attacking the ring. So in the living cell, Ehrlich assumes, as a working hypothesis, the existence of a wonderfully complex but comparatively stable chemical nucleus, with abundant and various more reactive side-chains. These latter serve to combine with food materials in the surrounding lymph, and these are then utilized in the cell by an intramolecular rearrangement of atoms which is always in progress. Useless atomic groups formed in the metabolism of the cell are detached and passed off as excretions. These reactions of intra-molecular rearrangement and molecular disintegration also find their analogues in carbocyclic chemistry.

**Antitoxins.**—Von Behring and Kitasato (1890-91) showed that animals injected with small non-fatal doses of toxin of the tetanus bacillus, produce as a result of this treatment a something which circulates in solution in the blood plasma, which is capable of neutralizing the poisonous properties of the tetanus toxin. Soon afterward von Behring obtained analogous results with the toxin of diphtheria. The protective substances in the blood were called antitoxins. The exact chemical composition of these substances is unknown. They accompany the pseudoglobulin fraction of the plasma in its chemical analysis,<sup>1</sup> but the

<sup>1</sup> Banzhaf, *Johns Hopkins Hospital Bull.*, 1911, Vol. XXII, pp. 106-109

union here is probably a mere physical adsorption or very unstable chemical combination. Ehrlich explains the formation of antitoxin on the basis of his side-chain theory as follows. The molecule of toxin attacks the body cell at one of its side-chains or receptors which is best adapted to this reaction. In the re-

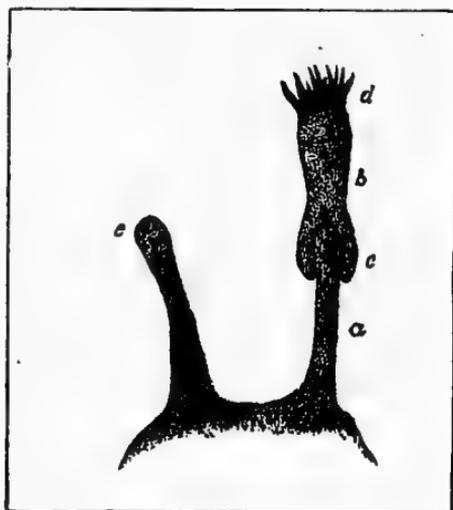


FIG. 87.—Receptor of the first order uniting with toxin. (*Journal of the American Medical Association*, 1905, p. 955.) a, Cell receptor; b, toxin molecule; c, haptophore of the toxin molecule; d, toxophore of the toxin molecule; e, haptophore of the cell receptor.

sulting intra-molecular re-arrangement the toxin reveals itself as a disturbing element, causing destruction of that portion of the cell to which it has become attached. In recovering from this disturbance the cell overcompensates by forming an excessive number of the particular kind of side-chain destroyed, and some of the excess side-chains are detached, and circulate in the blood, ready to react with toxin entirely apart from the cell which has produced them. These constitute Ehrlich's receptors of the first order and their sole

effect upon the toxin is that of combining with it. The free receptors circulating in the blood give it its antitoxic property.

**Precipitins.**—Other chemical products of bacterial growth are attacked and rendered insoluble by products of the body cells. Kraus<sup>1</sup> (1897) showed that animals injected with cultures of bacteria produce a substance, or substances, which circulates in the blood and is capable of causing a precipitate when mixed with the clear filtrate of the cultures of the same bacteria. The parenteral introduction of any foreign protein in solution stimu-

<sup>1</sup> *Wiener klin. Wochenschr.*, 1897, X, p. 736.

lates the production of a substance which will precipitate it.<sup>1</sup> These substances, which are called precipitins, resemble enzymes in many respects. Thus, the precipitin produced by the injection of a milk, causes a change in the milk very similar to that caused by rennet. Rennet, however, coagulates milk from various animals while the milk precipitin is specific, within certain limits, for the one kind of milk. Precipitation results only when the blood serum (precipitin) is combined with the proper amount of the culture filtrate or other protein solution (precipitinogen)—when too large an excess of one or the other is used no precipitate occurs. Ehrlich explains the formation of precipitins on the basis of his side-chain theory in the same way as the production of antitoxins was explained. The foreign protein stimulates the body cells to produce specific receptors

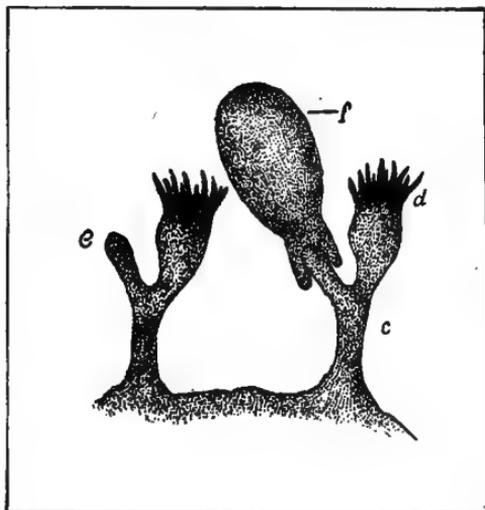


FIG. 88.—Receptors of the second order and some substance uniting with one of them. (*Journal of the American Medical Association*, 1905, p. 1113.) *c*, Cell receptor of the second order; *d*, toxophore or zymophore group of the receptor; *e*, haptophore of the receptor; *f*, food substance or product of bacterial disintegration uniting with the haptophore of the cell receptor.

capable of combining with it. In this instance, however, the receptor not only combines with the foreign material, but also brings about a definite change in it which is evidenced by the phenomenon of precipitation. The side-chain therefore contains at least two distinct atomic groups, one of which serves to combine with the precipitinogen, and is specific in nature, and another which brings

<sup>1</sup> Specific precipitin tests have been employed to some extent in determining the source of blood stains and of meats. See Citron, *Immunity*, translated by Garbat, Phila., 1914, p. 125.

about the change evidenced by formation of the precipitate. The former of these chemical groups is called the combining or haptophorous group or haptophore, and the latter is called the ferment-bearing or zymophorous group or zymophore. This type of side-chain is Ehrlich's receptor of the second order. It is represented in the figure as possessing one smooth branch which serves for simple attachment, the haptophore, and one branch equipped with saw-teeth to suggest its property of producing chemical change, the zymophore. The precipitin present in the blood plasma is supposed to consist of such receptors which have become detached from the cell producing them.

**Agglutinins.**—Gruber and Durham (1896) found that the blood of animals suffering from certain infections has the power of causing the bacteria involved to clump together and lose their motility when it is added to a broth culture or a suspension of the bacteria in salt solution. The phenomenon has been observed in connection with many bacteria, not only motile but also non-motile species, but the most important examples are the typhoid, paratyphoid, cholera and dysentery organisms. In typhoid and paratyphoid fever the agglutination test is used as an aid in diagnosis of the disease by testing patient's serum against known cultures, and the test with known serum is important in the identification of cultures of any of these bacteria. Agglutinins are comparatively stable substances although they decompose rapidly at  $70^{\circ}$  to  $75^{\circ}$  C. When dried they keep for a long time. In Ehrlich's theory, the agglutinins are classed as receptors of the second order, along with the precipitins.

*The Phenomenon of Agglutination.*—Clear fluid blood serum to be tested for specific agglutinins is diluted with broth or with salt solution to make mixtures containing one part of the serum in 5, 10, 20, 40, 80 and 160 parts of the mixture. This is conveniently done by means of the Wright capillary pipette, or graduated pipettes may be employed. To each dilution of serum an equal amount of a very young (preferably two to six hours old) broth culture, or a suspension of an active young agar cul-

ture in broth or salt solution, is added. The reaction may be observed by mixing small quantities (loopfuls) on a large cover-glass and studying the mixture microscopically as a hanging drop, or by mixing larger quantities in small tubes and incubating them at  $37^{\circ}$  C. Control specimens free from serum and containing normal serum should be set up at the same time for comparison; as many bacteria may be agglutinated somewhat by normal serum in a dilution of one to ten, and sometimes the organisms in the culture, especially if it is too old, may be already grouped together somewhat or may spontaneously clump during the experiment. Some practice is necessary before one can estimate agglutinins reliably and, on the whole, accuracy is more easily attained with the macroscopic test. For agglutination tests requiring only moderate accuracy, dried blood may be used, the dilutions being prepared by comparison of colors with an empirical standard.

**Bactericidal Substances, Alexin.**—Nuttall (1886) showed that normal blood is capable of killing bacteria and that this germicidal property is destroyed by heating the blood to  $55^{\circ}$  C. for thirty minutes. Buchner confirmed these observations and showed further that the germicidal property is resident in the serum and not exclusively in the cells of the blood as taught by Metchnikoff. To this germicidal substance Buchner gave the name alexin, and he ascribed the normal resistance to infection exhibited by the healthy animal, as well as the heightened resistance of the immunized animal, to this substance. It will have been noted that, historically, these discoveries followed Metchnikoff's first observations on the phagocytes, and preceded the discovery of antitoxins, agglutinins and precipitins, and thus presented the first proof of the existence of soluble anti-infectious agents. These bactericidal substances are now considered to be identical with the bacteriolysins and will be considered with them under the more general heading of cytolytins.

**Cytolytins.**—Pfeiffer (1896) found that guinea-pigs, when injected repeatedly with non-fatal doses of cholera germs, reacted

to this treatment by producing a something which would dissolve these bacteria. This new property was present in the blood and also in the peritoneal fluid. The substance was called bacteriolysin. Subsequent investigators have shown that bacteriolysins can be produced for a great variety of micro-organisms, although in none can the reaction be better demonstrated than in the cholera vibrio originally employed by Pfeiffer. Lysins, or dissolving substances, have been produced for very many other kinds of cells also, of which those for red blood cells (hemolysins) are perhaps the most important. It seems to be possible to produce a lysin (cytolysin) for any kind of cells by injecting these cells into an appropriate animal.

Cytolysins, including bacteriolysins, are active only when comparatively fresh. Upon standing for a day at room temperature, or upon heating to 56° C. for 30 minutes, the cytolytic power disappears. This power is, however, restored in a remarkable manner if the cytolysin and the cells to be dissolved are injected together into a normal animal, for example into the peritoneal cavity of a guinea-pig, or if a fresh normal blood serum be added to the mixture in the test-tube. The experiment results as follows:

Immune serum	+ cholera germs	= Bacteriolysis.
Immune serum (old or heated)	+ cholera germs	= No bacteriolysis.
Normal serum	+ cholera germs	= No bacteriolysis.
Immune serum (old or heated)	+ normal serum + cholera germs	= Bacteriolysis.

This experiment proves that the cytolytic property of the serum depends upon the presence of at least two recognizably different substances, one of which is present in fresh normal serum and in fresh immune serum but is destroyed on standing or by heating, and a second which is present in the immune serum and which is not destroyed so readily.

Ehrlich explains the formation of cytolysins by the same kind of reasoning as was applied to antitoxins and precipitins. The resulting side-chain would be considered of the same sort

as in the latter class of substances, that is a receptor of the second order with a haptophorous group by which to combine with the foreign cell, and a zymophorous group to bring about its solution, were it not for the observed facts given in the experiment outlined above, which demonstrate the presence of two distinct substances in the cytolytic complex. A new picture is here necessary and it is furnished by making a joint in the arm of the receptor of the second order in which the fermentative property is supposed to reside, separating off the zymophorous group as a separate sub-

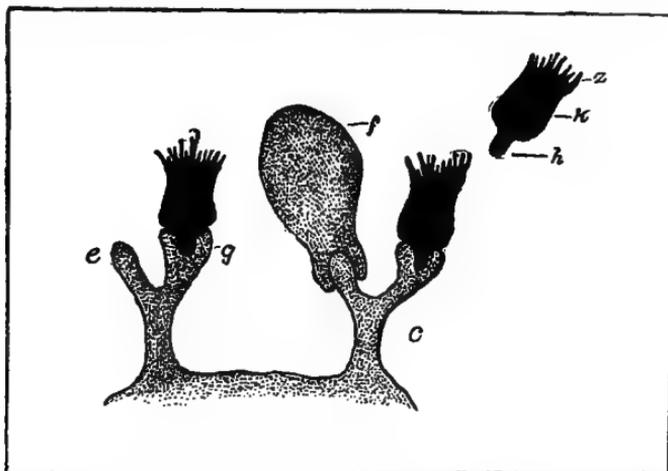


FIG. 89.—Receptors of the third order. (*Journ. A. M. A.*, 1905, p. 1369.) *c*, Cell receptor of the third order—an amboceptor; *e*, one of the haptophores of the amboceptor with which the foreign body, *f*, (antigen) may unite; *g*, the other haptophore of the amboceptor with which complement, *k*, may unite; *h*, haptophore of the complement; *z*, zymophore of the complement.

stance and leaving a branched figure with two combining or haptophorous elements, one capable of combining with the foreign cell and the other capable of combining with the cytolytic ferment of normal serum and so bringing its action to bear upon that particular cell. The receptor of the third order is called, in accordance with this conception of its relationships, amboceptor, because it acts as a receptor at two points. It is also called intermediary body, immune body and sensitizer. The other component of the lytic complex, which is thermolabile and which is

present in normal serum is called complement or cytase and by some authors (Bordet) alexin.<sup>1</sup> It will be noted that only a part of the cytolysin is produced by the body in its reaction to invasion, namely, the immune body.

**Deviation of Complement.**—Neisser and Wechsberg observed that the bactericidal power of a given immune serum (bacteriolytic amboceptor), when combined with a constant amount of normal serum (complement) and a constant amount of a bacterial suspension (antigen), increased progressively with progressive dilution of the immune serum to a certain point, after which it diminished again. The following data taken from Citron illustrate the experiment:

Typhoid culture (antigen)	Immune serum (amboceptor)	Fresh serum 1 : 2 (complement)	Colonies produced by plating after 3 hrs. at 37° C.
0.5 c.c. 1/5000.....	1/100 c.c.	0.5 c.c.	Many thousand
0.5 c.c. 1/5000.....	1/5000 c.c.	0.5 c.c.	Many thousand
0.5 c.c. 1/5000.....	1/20000 c.c.	0.5 c.c.	200
0.5 c.c. 1/5000.....	1/30000 c.c.	0.5 c.c.	0
0.5 c.c. 1/5000.....	1/50000 c.c.	0.5 c.c.	60
0.5 c.c. 1/5000.....	1/200000 c.c.	0.5 c.c.	Many thousand

Neisser and Wechsberg have undertaken to explain this result by supposing that the excessive number of amboceptors present in the more concentrated solutions of immune serum hinders cytolysis because some of them combine with the antigen by means of their cytophile groups while others are combining with the complement by means of their complementophile groups, and as a result the mixture contains combinations of amboceptor with antigen, and of amboceptor with complement, but practically no combinations of the three elements together. There are grave reasons for questioning the accuracy of this assumption,

<sup>1</sup> This use of the term alexin would seem to be undesirable, for Buchner employed the term to designate the whole bactericidal or cytolytic complex before the possibility of recognizing two separate elements was clearly recognized.

as it has been shown by Bordet that amboceptor does not unite with complement in the absence of antigen. It seems more probable that some other factor, such perhaps as a marked agglutination of the bacteria in the stronger solutions, may serve to protect them from the bacteriolytic action.

**Fixation of Complement.**—As has been mentioned, it is possible to produce cytolytins for red blood cells. This is commonly done by injecting the washed blood corpuscles of a sheep (0.1 c.c. + 0.5 c.c. salt solution) into a rabbit intravenously three or four times at intervals of five days. The serum of the rabbit becomes

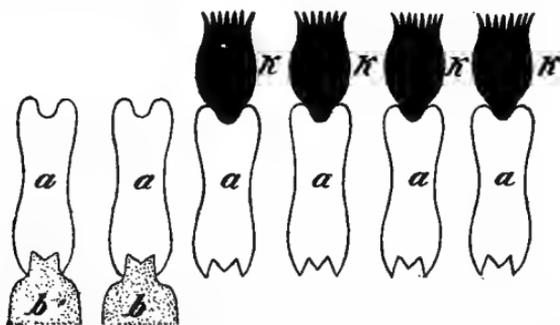


FIG. 90.—Illustrating the conception of deviation of complement. *a*, Amboceptor; *b*, antigen; *k*, complement.

strongly hemolytic for sheep's cells. The blood is drawn from the carotid artery, the serum separated, rendered perfectly clear and after heating to 56° C. for 30 minutes is stored in hermetically sealed ampoules containing 1 c.c. each, in a low temperature refrigerator. When this *hemolytic amboceptor* is diluted to the proper point, which must be ascertained by trial and error, it will just cause the complete hemolysis of a definite quantity of washed sheep's corpuscles (0.2 c.c. of a 5 per cent suspension) when combined with 0.1 c.c. of a 10 per cent solution of fresh normal serum of guinea-pig (complement). The mixture of this quantity of the immune serum, which may now be called one unit of hemolytic amboceptor, with 0.2 c.c. of freshly prepared 5 per cent suspension of washed sheep's corpuscles produces a reagent which serves for the detection of complement and the approxi-

mate estimation of its amount in an unknown mixture. By the use of such a reagent it is possible to show that complement is destroyed or used up in various specific cytolytic, proteolytic, and precipitin reactions. Thus Bordet and Gengou mixed together typhoid bacilli (antigen), heated typhoid-immune serum (amboceptor) and fresh normal serum (complement) and incubated the mixture. After an hour the hemolytic amboceptor and sheep's blood cells were added and incubation continued. No hemolysis resulted, showing that the complement added in the first place had been used up, "fixed," as a result of a reaction with the typhoid bacilli and typhoid amboceptor. This is the phenomenon of fixation of complement. Obviously it lends itself to use as a test for the presence of a specific antigen or for the presence of specific amboceptor. Its more definite application will require subsequent mention.

**Opsonins.**—Wright and Douglas (1903) showed that blood serum contains a something which affects bacterial cells, soaked in the serum, in such a way that they are more readily ingested by the living leukocytes. To this substance they gave the name "opsonin" (*opsono*, I prepare victuals for). Substances of this sort are present in normal blood, but are increased as a reaction following infection. It would seem that more than one substance may act upon bacterial cells in this manner, for Neufeld has shown that the opsonic power of normal serum may be destroyed by heating to 56° C., while the similar property of immune serum remains after this treatment. It is not yet conclusively proven that opsonins are separate substances entirely distinct from bacteriolysins and agglutinins, but it has been shown that opsonic power of a serum does not correspond in its concentration to that of the other antibodies, and some other element must, therefore, be a factor. Hektoen considers the opsonins to be distinct bodies, different from lysins and agglutinins. The study of opsonins has done much to bring about harmony between the followers of Metchnikoff, with their tendency to emphasize the importance of phagocytosis, and the followers

of Buchner and Ehrlich, who fixed their attention largely upon the substances dissolved in the body fluids.

**Anti-aggressins, Specific Proteolysins.**—Various substances produced in the body as a result of infection show particular ability to combat the effects of the soluble products of the parasite to which the name aggressins has been given (see page 212). Knowledge of these substances and their behavior is still somewhat incomplete, but they seem to be particularly concerned with the parenteral digestion of foreign proteins, a process in which cytolysis may be regarded as a beginning stage. Whereas, however, cytolysis is concerned with the disintegration of formed material, these substances now under consideration act particularly upon proteins already in solution. In many instances the products of the first stages in this parenteral digestion are toxic (disintegration of tuberculin and of egg-white), and some of the symptoms of infectious disease, such as fever, have been ascribed to them. In their general characters these lytic substances are wholly analogous to the cytolysins and their action is due to at least two components, an amboceptor and a complement.

**Source and Distribution of Antibodies.**—The exact source of the antibodies dissolved in the body fluids is unknown. All agree that they are derived from cells. Metchnikoff regards the phagocytic cells as the important source; Ehrlich does not specify, but it would seem, in accordance with his theory, that any cell capable of being affected by the foreign substance should be capable of throwing off cell receptors (antibodies) to combine with it. Many investigators consider antibody formation to be a common property of many kinds of cells, but more especially of relatively undifferentiated cells such as those of the connective tissue.

Antibodies are present in greatest concentration in the blood and lymph. They are absent or present only in small amount in the serous fluids of the pleural, pericardial, peritoneal and joint cavities, and in the cerebrospinal fluid.<sup>1</sup> Parasites in

<sup>1</sup> See Flexner, Harbin Lectures, *Journ. of the State Medicine*, March, April, May 1912.

these locations are less readily influenced by antibodies circulating in the blood, so that localized infections may continue in these regions in spite of a considerable concentration of antibodies in the body generally.

**Allergy.**—Allergy is a term invented by Von Pirquet to designate the condition of altered reactivity on the part of the body which comes about as a result of infection. A few of the phenomena which may be included under this term have been considered above in this chapter. Many of these alterations in bodily function are manifestly of advantage to the host in limiting the activities of the parasite, neutralizing its poisonous products, and even in destroying and removing the parasite itself. Some of them, such as specific precipitation, seem to serve no important purpose, while others, such as cytolysis and proteolysis, actually lead sometimes to results very harmful to the host, although their usual effect is favorable. Many of the recognized weapons which the body employs in its battle against parasites are still imperfectly understood, and there are doubtless many factors involved in this relation which are not yet capable of definite recognition. Of those agents mentioned above, the phagocytes are ready for immediate defense as soon as the body is invaded by the parasite. Hyperplasia and encapsulation require more time, probably one to four weeks. The chemical antibodies, antitoxins, agglutinins, cytolysins and opsonins, although possibly present in small amounts in the normal body fluids, become definitely increased in from eight to twelve days after the entrance of the parasite, an interval approximately equal to the incubation period of some infectious diseases. These various agents have much to do in determining the manifestations and course of the disease as well as the final outcome, and as we shall see, they also play a part in immunity.

## CHAPTER XIII

### IMMUNITY AND HYPERSUSCEPTIBILITY. THEORIES OF IMMUNITY

**Immunity.**—Immunity is that condition of a living organism which enables it to escape without contracting a disease when fully exposed to conditions which normally give rise to that disease. Immunity may depend upon many different factors, or upon only one of a great variety. In general, we shall find that it depends very largely upon those factors which we have already considered in the preceding chapters, such as the possession of anatomical structures or habits of life which render invasion by the particular parasite impossible, or the possession of a body structure, physically or chemically not adapted for the growth of the particular disease virus, or the ability to harbor the particular parasite as a commensal without suffering injury, or the ability to react against the invading parasite and destroy it by phagocytosis or by cytolysis, neutralize its poisons by antitoxins, or limit its activity by encapsulation. Immunity is ordinarily considered under two heads, Natural Immunity, or that present as a part of the individual's birthright, and Acquired Immunity, that which follows as the result of some experience of the individual.

**Immunity of Species.**—Natural immunity to certain diseases is possessed by certain species of animals. Where the morphology and physiology is quite different from that of the usual victims of the disease, immunity might be expected. Thus cold-blooded vertebrates, fish, amphibians and reptiles, are immune to many diseases of mammals, apparently because of the different temperature of their tissues. In other instances the difference in resistance between two species of animals seems to be correlated

with difference in food habits. Thus the carnivorous mammals are relatively unsusceptible to anthrax and tuberculosis, diseases natural to the herbivora. Many infectious diseases of man are not readily transmissible to animals, for example, typhoid fever, syphilis, pneumonia, and in some instances it has so far been impossible to infect animals, as for example with scarlet fever and gonorrhoea.<sup>1</sup>

**Racial Immunity.**—Within a species there is moreover a racial difference in resistance to natural infection. Thus the pure-bred dairy cattle are more susceptible to tuberculosis than other cattle, and Yorkshire swine are relatively less susceptible to swine erysipelas. In man, the relation of race to susceptibility is not very clear. The examples of supposed racial immunity have not proved to be so definite as has been assumed at first. Thus the supposed immunity of African natives to syphilis has vanished with their increasing contact with civilization and with this accompanying disease. In the case of malaria the supposed racial immunity of negroes seems to be an acquired immunity due to severe attacks of the disease in childhood. There is, however, some evidence that prolonged contact with a disease through many generations may result in a relative resistance, so that the disease assumes a milder form in such a race of people—a sort of inherited acquired immunity. Such considerations have been brought forward to explain the relatively high resistance to tuberculosis shown by the Hebrews as compared with the American Indians.

**Individual Variations.**—Individual variations in resistance to infection are commonly observed. They may depend in part upon age, condition of nutrition, fatigue, exposure or intoxication, but they are ascribed also to differences in anatomical structure (shape of the thorax in tuberculosis). Individuals especially susceptible to a disease are said to possess an idiosyncrasy for it. The physiological mechanisms upon which variations in individual resistance depend are not clearly understood.

<sup>1</sup> Kolle und Wassermann, II *Auflage*, Bd. IV, p. 693 (1912).

Doubtless, the number and activity of the white blood cells and the nature and amount of bactericidal substances in the blood play a part in some instances.

**Acquired Immunity.**—Acquired immunity results from some experience affecting the individual, either an infection which the individual has survived or some artificial procedure of immunization. There are recognized two different kinds of acquired immunity, first, active immunity which is due to the activity of the cells of the individual immunized, and second, passive immunity which is produced by introducing into the body, material (blood serum) from another animal, which contains substances conferring at once an immunity upon the new individual.

**Active Immunity.**—Active immunity may be acquired by an attack of the disease. This immunity may endure for a lifetime in some instances (yellow fever, small-pox, scarlet fever) or for many years (typhoid fever) or it may be very evanescent (erysipelas, pneumonia, influenza). Some diseases were at one time so universal that few escaped them, and individuals used to be purposely exposed or inoculated in order to contract the disease and gain the resulting immunity. Inoculation of small-pox seems to have been practised in China about 1000 A. D. and in India as early as the twelfth century, and it was introduced into Europe in 1721 by Lady Montague and was employed very extensively in Europe and America during that century.

Active immunity may also be produced without causing a definite attack of the disease. This may be accomplished in a variety of ways. Fully virulent micro-organisms may be introduced into a part of the body unfavorable to their development. The subcutaneous injection of cholera cultures according to the method of Ferran and Haffkine has proven to be practically without danger, and results in immunity. The same principle is utilized in immunizing cattle against pleuro-pneumonia.<sup>1</sup> Introduction of virulent organisms in very minute doses has been

<sup>1</sup> Kolle und Wassermann, II *Auflage*, Bd. I, S. 928 (1912).

employed to immunize against rabies (Högyes method),<sup>6</sup> and against tuberculosis by Webb. In most diseases these methods are regarded as too dangerous for extensive use.

Living virus, altered in its virulence, was first used by Edward Jenner, when he inoculated with cow-pox (vaccinia) and induced immunity to small-pox. Cow-pox is doubtless due to the organism which causes small-pox, attenuated by its life in the body of the cow. Viruses artificially cultivable are attenuated by a variety of procedures, and are employed to induce immunity. Pasteur's vaccine for anthrax, for chicken cholera and possibly the treatment of rabies with dried spinal cord, are examples of the application of this principle. Virus of extraordinary virulence is sometimes injected after previous treatment with attenuated organisms, in order to confer a higher degree of immunity. Thus Pasteur employed the most virulent rabies virus obtainable, *virus fixé*, in the immunization against rabies.

Living virus, of full virulence, but apparently influenced in some way by the body fluid containing it, is employed in immunizing against rinderpest and against Texas fever. The bile of an animal dying of rinderpest is injected subcutaneously in doses of 10 c.c. into cattle. Kolle has shown that the virus can be separated from such bile in fully virulent condition; so it appears that some constituents of the bile restrain the activity of the virus. In Texas fever, blood of young animals containing relatively few of the parasites is used to inject new animals.

Immunization by injection of dead microbic substance is now extensively employed in the prophylaxis of cholera, typhoid fever and plague. As a result of such injections there is a marked increase in specific agglutinins and bacteriolysins in the blood. The principle of general immunization is also employed with some success in the treatment of subacute, chronic or recurrent local infections, the production of antibodies and their circulation in the blood and lymph exerting a favorable effect upon the local lesions. The emulsions of dead bacteria employed are called bacterial vaccines.

The soluble products of bacterial growth are injected into animals to immunize them, especially in the case of diphtheria and tetanus, the bacteria of which produce very powerful soluble toxins. As a result of this treatment antitoxins are produced and circulate in the blood of the animal.

Bacterial extracts, either those contained in inflammatory exudates, the so-called aggressins of Bail, or extracts obtained by soaking bacteria in blood serum or in distilled water, the so-called artificial aggressins of Wassermann and Citron, have proved of value in experimental immunization of animals against many different bacteria. It is claimed that the reactions to injection are exceptionally mild, while the resulting immunity is more solid. Certain products of the disintegration of typhoid bacilli have been obtained by Vaughan, which possess considerable immunizing power, but apparently only slight toxicity. None of these bacterial extracts has yet passed beyond the experimental stage in the immunization of man against a disease.

A certain slight grade of immunity may be secured in some instances by procedures which seem to bear no relation to the specific micro-organisms in question. Thus the injection of cultures of *B. prodigiosus* and *B. pyocyaneus* results in an increased resistance to infection with anthrax. Similar increased resistance has been observed to follow a simple surgical procedure, such as section of the sciatic nerve. The explanation of these results is not clear, but perhaps the effect may be attributed to a general stimulation of the body defenses, especially the phagocytes.

**Passive Immunity.**—Passive immunity is produced by injecting into the body a fluid taken from another animal, which contains antitoxins, bacteriolysins, opsonins or other substances known as immune bodies. The animal which furnishes the immune bodies must be first actively immunized, and it possesses an active immunity. If its blood plasma be drawn and injected into a child, the child acquires a borrowed immunity without the necessity of any active participation of its own cells in the production of immune bodies. The possibility of producing such

passive immunity has been demonstrated in a number of diseases. In some instances the effect of the serum is antitoxic (diphtheria and tetanus), in others it is bacteriolytic (cholera), while in other instances the nature of the dominant antibodies is not definitely known.

**Combined Active and Passive Immunity.**—Various procedures have been devised to produce immunity by introducing at, or nearly at, the same time the infectious agent or its products and the serum of an immune animal containing protective substances. The combination of immune blood with virus of full strength is used in immunizing animals against rinderpest, foot-and-mouth disease and hog cholera, all being diseases due to filterable agents; and also in immunizing hogs against hog erysipelas (*B. rhusiopathiæ*). The combined injection of attenuated virus and immune serum is employed especially in Sobernheim's method of preventive inoculation against anthrax. Besredka has employed dead bacteria combined with their specific immune serum in immunizing against typhoid fever, plague and cholera.

**The Mechanisms of Immunity.**—Certain biological factors in the phenomenon of immunity are now clearly recognizable and readily demonstrable. The activity of the phagocytes, first emphasized by Metchnikoff and believed by him to be the sole important factor in the defense of the body, is easily observed in immunity to many diseases. The dependence of phagocytic activity upon dissolved substances in the body fluids (opsonins) is also demonstrated beyond doubt. Phagocytosis is, perhaps, the factor of most general operation in immunity to all sorts of disease. The antitoxins stand forth prominently as powerful factors in immunity to two important diseases, diphtheria and tetanus, and the bacteriolysins are undoubtedly of greatest importance in the case of Asiatic cholera, and probably also in typhoid and plague. In most instances the immunity seems to depend upon several different factors, phagocytosis, opsonins, bacteriolysins, antitoxins, and perhaps substances of unknown nature. In some instances of immunity there is no particular excess of these immune bodies demonstrable in the blood, and

nearly always an immunity remains long after such an excess has disappeared. It would seem that the ability of the cells of the body to respond promptly to invasion is often developed by experience with one such invasion, and that this ability may remain for a long time as a factor in immunity.

**Hypersusceptibility or Anaphylaxis.**—If a guinea-pig be injected with a small amount of a protein, such as egg-albumen or blood serum of the horse, and then after an interval of ten to twenty days be injected with a second dose of the same protein of good size (0.5 to 5 grams), the animal usually develops symptoms of nervous intoxication and often dies within a half hour. Inasmuch as normal guinea-pigs withstand enormous doses of such protein substances, it is evident that the first injection has brought about some change in the animal, an altered reactivity, which results in the intoxication after the second dose. That this phenomenon of hypersusceptibility or anaphylaxis (= *against protection*) bears a definite relation to immunity may be illustrated by an experiment in which typhoid bacilli are substituted for the soluble protein. If a guinea-pig be immunized by repeated doses of the killed micro-organisms he is able to resist inoculation with an ordinarily fatal dose of the living germs, which are quickly killed and dissolved by the specific bacteriolysins in the body fluids. However, if such an immune guinea-pig be injected with a proper dose of dead organisms, which would not kill a normal animal, he may quickly succumb. The ability of the body fluids of the immune animal to disintegrate the bacterial cells rapidly would seem to be the factor upon which depends not only its immunity to the small dose of living germs, but also its exaggerated sensitiveness to dead germ substance. The products of the rapid parenteral digestion of the foreign protein would seem to be the cause of the symptoms of intoxication. The essential unity of the substances upon which immunity and anaphylaxis depend has been emphasized by Von Pirquet<sup>1</sup> and his co-workers.

<sup>1</sup> Von Pirquet: *Allergy. Archives of Internal Medicine*, 1911, Vol. VII, pp. 259-288; pp. 383-436.

**Theories of Immunity.**—Early theories of immunity were based upon meager observations. The idea that an attack of a disease left behind in the body something which prevented the subsequent entrance of that disease was formulated by Chauveau in 1880 as the so-called retention hypothesis. In the same year Pasteur expressed the idea that an attack of a disease removed something from the body and so exhausted the soil as far as that particular disease was concerned. Neither of these ideas was new at that time, and neither of them pretended to any very definite or specific application to phenomena observed in immunity, but only to the general phenomenon of immunity itself. The discovery of phagocytosis by Metchnikoff in 1884 was the first observation of a definite phenomenon which appeared to explain the facts of immunity. The phagocytic theory, which grew out of this observation, was an attempt to ascribe immunity in general to this one phenomenon of phagocytosis. With the observation of the bactericidal substances in solution in the blood plasma by Nuttall and by Buchner, of the antitoxins by von Behring and the bacteriolysins by Pfeiffer, there developed attempts to ascribe all the observed facts of immunity to these factors, resulting in the alexin theory and the antitoxin theory of immunity. More intimate study of the dissolved immune bodies lead to the formulation of a hypothesis to explain their formation, composition and action, the side-chain theory of Ehrlich, which has been of great value as a working hypothesis and as a central conception about which to arrange the observed facts relating to these dissolved substances. The elementary concepts of this theory have been given in the preceding chapter.

In brief, Ehrlich pictures the living cell as a chemical unit possessing numerous and varied combining groups or side-chains capable of uniting with substances in contact with the cell. The toxin molecule is conceived as a substance containing at least two distinct chemical groups, one which serves for attachment to the side-chain of the cell and the other serving to bear the poisonous properties. The union of the toxin with the cell results in

destruction of the side-chains attacked, and in regenerating these the cell over-compensates, the excess side-chains, receptors of the first order (see page 216), being set free into the blood and constituting the antitoxin, which is capable of neutralizing toxin there or in the test-tube. The assumption of two chemical groups in the toxin molecule is strengthened by the observation that diphtheria toxin changes on standing so that its poisonous property is much diminished without corresponding loss of ability to combine with antitoxin. Such changed toxin, in which the haptophorous group persists while the toxophorous group has degenerated, is called toxoid. In order to explain the formation and structure of agglutinins and precipitins, Ehrlich assigned a more complex composition to the side-chains which constitute these substances, leading to the conception of a receptor of the second order (see page 217), with its haptophorous and zymophorous groups. In the case of the cytolytins, a further amplification of the idea was necessary to explain the observed fact that the cytolysis is due to two components, one of which is a thermolabile, normal constituent of the blood and not increased as a result of immunization, the other being a thermostable substance which is produced as a result of the immunization process. This latter immune body, the receptor of the third order, was therefore pictured as a double receptor (amboceptor) capable of attaching on the one hand the foreign body (antigen) and on the other the normal component necessary to complete the lytic complex, to which component the name complement was given.

With the recognition of opsonins by A. E. Wright in 1903, the opposing theories of the French and the German schools began to be reconciled, and the relatively simple and largely hypothetical theories of immunity began to give way to a more exact and necessarily more complex science of immunology. Bordet and his pupils deserve credit for leading the reaction against too slavish adherence to theory in the study of immunity. Our modern ideas are no longer confined within the scope of any one theory and it is necessary to recognize the existence of a great

variety of phenomena in the interaction of the host cells and their secretions on the one hand with the parasites and their chemical products on the other. The elementary conceptions of immunology and the primary language of the science are derived from the old theories, especially from Ehrlich's theory, and these theories are an essential part of the introduction to immunology.<sup>1</sup>

<sup>1</sup> For a concise presentation in English of facts and practical experiments relating to immunity, the student is referred to Citron, *Immunity*, translated by Garbat, Philadelphia.

## PART III

# SPECIFIC MICRO-ORGANISMS

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### CHAPTER XIV

#### THE MOLDS AND YEASTS AND DISEASES CAUSED BY THEM

**Mucor Mucedo.**—This is the most common species of mucor, especially about barns and on manure. It produces a network of threads (mycelium) in the substratum, and zygospores are produced here by the union of two cells. The aërial hyphæ are vertical, about 10 cm. in length and bear a spherical spore sac (sporangium) at the end. The sporangium is at first yellow, later brown and finally black and covered with crystals. The contained spores are 4 to 6 $\mu$  wide by 7 to 10 $\mu$  long. It is saprophytic and may be found as a contamination on culture media.

**Mucor Corymbifer.**—Lichtheim found this mold growing on a bread-infusion gelatin as an accidental contamination. The growth is at first white and later gray. The spore-bearing hyphæ are long and irregularly branched, and each branch bears a pear-shaped sporangium 10 to 70 $\mu$  in diameter. The contained spores are small ( $2 \times 3\mu$ ). Intravenous injection of the spores into rabbits causes severe nephritis and death in two or three days. The mold has been found growing as a parasite in the auditory canal.

More than a hundred species of *Mucor* have been described and several of them cause disease and death when injected into animals.

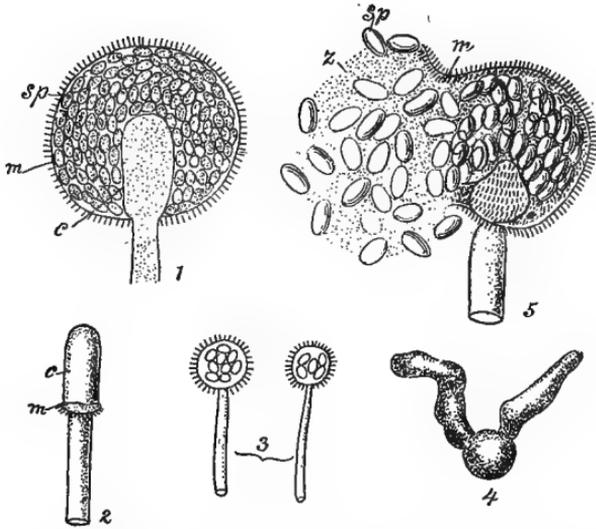


FIG. 91.—*Mucor mucedo*. 1, A sporangium in optical longitudinal section: *c*, columella; *m*, wall of sporangium; *sp*, spores. 2, A ruptured sporangium with only the columella (*c*) and a small portion of the wall (*m*) remaining. 3, Two smaller sporangia with only a few spores and no columella. 4, Germinating spore. 5, ruptured sporangium of *Mucor mucilaginus* with deliquescing wall (*m*) and swollen interstitial substance (*z*); *sp*, spores. (From Jordan after Brefeld.)

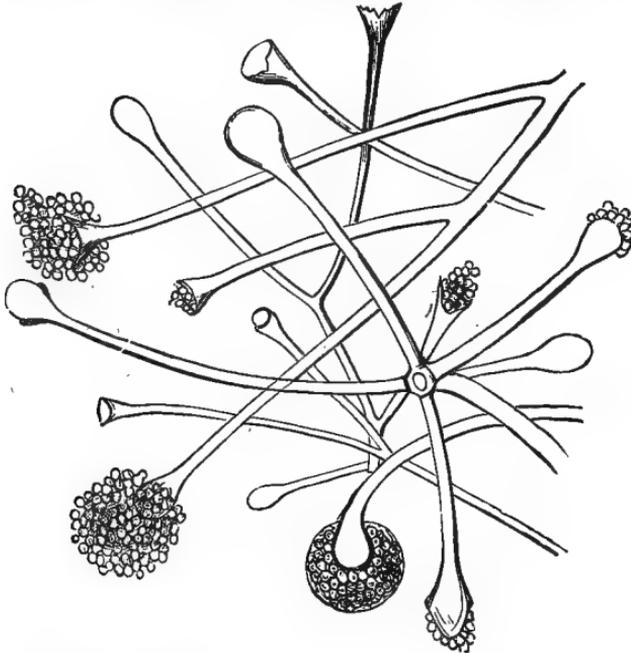


FIG. 92.—*Mucor cor.*

**Aspergillus Glaucus.**—This is very widely distributed in nature, occurring on fruits, moist bread and other food substances and very frequently as a contamination in laboratory cultures. The aerial spore-bearing hypha (conidiophore) is erect, about 1 mm. long, swollen at the end to a diameter of 20 to 40 $\mu$ . On the surface of this spherical head are numerous closely packed spore-bearing sterigmæ, each of which bears at its tip a chain of spherical spores (conidia) which are budded off from it. The conidia are gray to olive green in color. Ascospores are also produced, grouped together as yellow masses, called perithecia, on the surface of the medium. The mold is not pathogenic. Probably a considerable number of different species have been included under this name.



FIG. 93.—*Aspergillus fumigatus* from the lung of a parrot. (After Plaut.)

**Aspergillus Fumigatus.**—The growth of this mold is at first bluish and later grayish-green. It is widely distributed. The sterigmæ are unbranched, thickly set on the swollen end of the spore-bearing hypha. The conidia measure 2.5 to 3 $\mu$ . The formation of ascospores has also been observed. *Aspergillus fumigatus* plays a part in the heating of hay and sprouting barley, and is the most common of the pathogenic aspergilli. It infects doves and other birds naturally, sometimes causing veritable epidemics, and the disease has been observed in bird fanciers, in whom it runs a clinical course very similar to that of pulmonary tuberculosis. Fragments of the mycelium are found in the sputum. Doubtless the human disease is contracted from the birds in these cases. This mold has been found as the apparent cause of inflammation in the auditory canal in a large number of cases and in the nasal fossæ in a few instances. Various other mammals

are susceptible to inoculation and natural infection has been observed in horses, cattle, sheep and dogs.

Many other species of pathogenic aspergilli have been described, of less frequent occurrence than *A. fumigatus*.

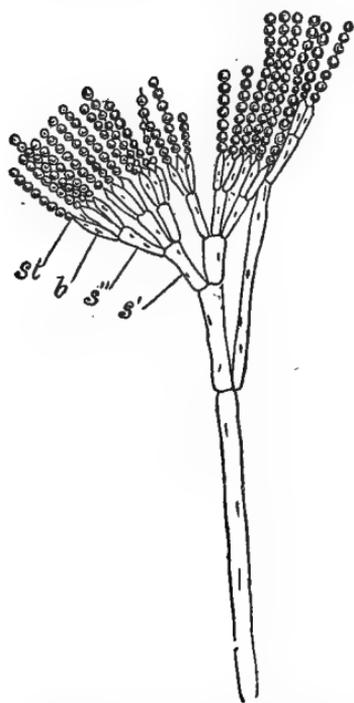


FIG. 94.—*Penicillium crustaceum*. Conidiophore with verticillate branches, *s'*, *s''*; *b*, *st*, sterigmata abstricting chains of conidia.  $\times 540$ . (From Jordan after Strasburger.)

***Penicillium crustaceum* (glaucum)** is the commonest contaminating micro-organism met with in the laboratory, and is probably the most widely distributed mold. Ascospores, similar to those of *Aspergillus glaucus* have been observed, but they are rarely produced. The aërial fruiting hypha (conidiophore) is erect, septate and branched at the upper end like a brush. At the end of these branches are bottle-shaped sterigmæ from which the conidia are constricted off. The growth is at first white and then it becomes blue-green, the development of color being at the center. It is not pathogenic, but the extracts from cultures of some varieties are poisonous when injected into animals. It is possible that several distinct species have been included under this one name of *Penicillium crustaceum*. One nearly related organism, *Penicillium*

*rocqueforti*, Thom., is an important agent in the ripening of Rocquefort cheese.

***Claviceps Purpurea*.**—This is a fungus parasitic upon rye and a few other plants. The spores gain access to the flower of rye and develop a mycelial mass which grows in the utricle, displacing the grain, the rudiment of which lies above the mass of the mold. Closely packed conidiophores produce oval conidia and at the same time secrete a sweet milky fluid which attracts

insects and thus furthers the distribution of the parasite. Later the mycelial mass produces sclerotia, which are masses of thick-walled cells containing starch and oil together with specific poisonous substances, and the whole becomes dry and hard with black outer covering, forming the ergot grain, which is considerably larger than the normal rye grain. In autumn this falls to the ground and remains until spring, when numerous red stalks grow out of it. Upon the swollen ends of these stalks, abundant ascospores are produced, and these serve to infect again the flowers of the new crop of rye.

This fungus is of great importance as the source of the drug, ergot, and as a cause of food poisoning, ergotism, in certain countries. It is one example of a mold parasitic upon higher plants. There are very many different species of such parasitic fungi, and they are probably the best known microbic agents causing diseases of plants.<sup>1</sup>

**Saccharomyces Cerevisiæ.**—This organism is the type of the true yeasts. The cell is spherical or ovoid and multiplies by budding. Ascospores are produced, usually four to eight in a single cell. *Saccharomyces cerevisiæ* is found widely distributed, especially on fruits and in sugar-containing substances. It has been used for centuries in the leavening of bread and in the alcoholic fermentation. Varieties of the species are distinguished by differences in physiological activity and especially in respect to the amounts of alcohol which they produce.

Material for study may be obtained from commercial compressed yeast, which contains vegetating cells of *Saccharomyces* mixed with other organisms including as a rule *Oidium lactis* and various bacteria, or from commercial dried yeast in which the ascospores are present. Pure cultures may be obtained by plating<sup>\*</sup> this material on nutrient gelatin. *Saccharomyces* is found in the gastric juice at times and is evidently capable of multiplying within the stomach when the acidity of the gastric juice is diminished.

<sup>1</sup> For a consideration of molds in relation to plant pathology, see Masseur, Diseases of cultivated plants and trees, New York, 1910.

**Coccidioides Immitis.**—Posadas<sup>1</sup> and Wernicke<sup>2</sup> first observed in human lesions the doubly contoured spherical forms of this organism, which multiplies in the tissues by endogenous spore formation. They regarded the parasite as a protozoon. The organism was named *Coccidioides immitis* by Rixford and Gilchrist in 1896 and it was recognized as a mold by Ophüls and Moffitt in 1900. Wolbach, in 1904, made an extensive study

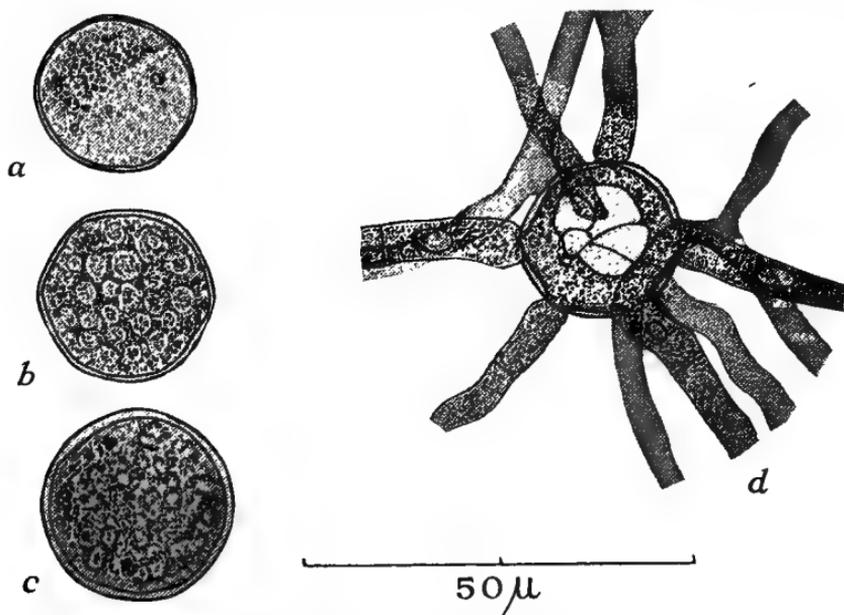


FIG. 95.—*Coccidioides immitis*: *a*, *b* and *c* represent the doubly contoured spheres seen in fresh pus; *d* represents the same organism as *a* after incubation for 24 hours at 33° C. in a hanging-block culture. (After MacNeal and Taylor.)

of the organism in cultures and by inoculation of animals. In 1914, MacNeal<sup>3</sup> and Taylor followed the transformation of the

<sup>1</sup> Posadas, Infectiose generalisierte Psorospermiosis. Buenos Aires, 1897; Ref. in Monatshefte f. prakt. Dermatol., 1898, 27, p. 593; Psorospermiose infectante généralisée. Revue de Chirurgie, 1900, 21, p. 276.

<sup>2</sup> Wernicke, Ueber einen Protozoenbefund bei Mycosis fungoides? Centralbl. f. Bakt., 1892, 21, p. 859.

<sup>3</sup> MacNeal and Taylor, *Coccidioides immitis* and coccidioidal granuloma. Journal of Med. Rsch., 1914, 30, p. 261. References to previous literature are given in this paper.

doubly contoured spherical parasitic form into mycelial growth in the agar hanging block inoculated with pus. The reverse transformation of the mycelium into spherical forms was also followed in the inflammatory exudate of animals inoculated with the mycelial growth and finally it was shown that, by the exclusion of air, the parasitic spherical form could be made to continue for a time its multiplication by endogenous spore formation in artificial culture.

*Coccidioides immitis* causes a highly fatal disease of man, coccidioidal granuloma, protean in its clinical manifestations, usually chronic in course and often presenting in the histology of its lesions the most perfect mimicry of tuberculosis. The parasite in such lesions is a spherical body 20 to 35  $\mu$  in diameter, with a doubly contoured wall, filled with a granular protoplasm, sometimes vacuolated, sometimes segmented. Guinea pigs are susceptible to inoculation. The disease seems to be confined to the western hemisphere and a large proportion of the reported cases have developed in California. The mode of transmission of the disease and the possible existence of the parasite in the external world under natural conditions have not been ascertained.

**Botrytis Bassiana.**—This mold was shown to be the cause of muscardine, a disease of silkworms, by Bassis and Audouin in 1837, a discovery following closely the recognition of the itch mite, *Sarcoptes scabiei*, as the cause of scabies in 1834. The infected silkworm becomes sluggish and dies, and the aërial hyphæ of the fungus grow out from its surface and pinch off round or pear-shaped conidia. These spores gain the surface of other silkworms or butterflies by contact or by air transmission, and germinate, sending threads into their bodies. Sickle-shaped spores are produced from these inside the body, and these grow out into threads, forming a mycelial network throughout the body of the victim and causing its death. It is possible that several different species of molds may be concerned in the causation of muscardine.

The fungus is of interest because it was probably the first

mold to be recognized as a cause of disease, and also because it is an example of a large group of fungi which attack various insects. The disease muscardine is, moreover, one of considerable importance to the silk industry.

**Oidium Lactis.**—*Oidium lactis* is very widely distributed and is almost always present in milk and milk products, and in brewer's and baker's yeast, and it is an especially prominent organism in

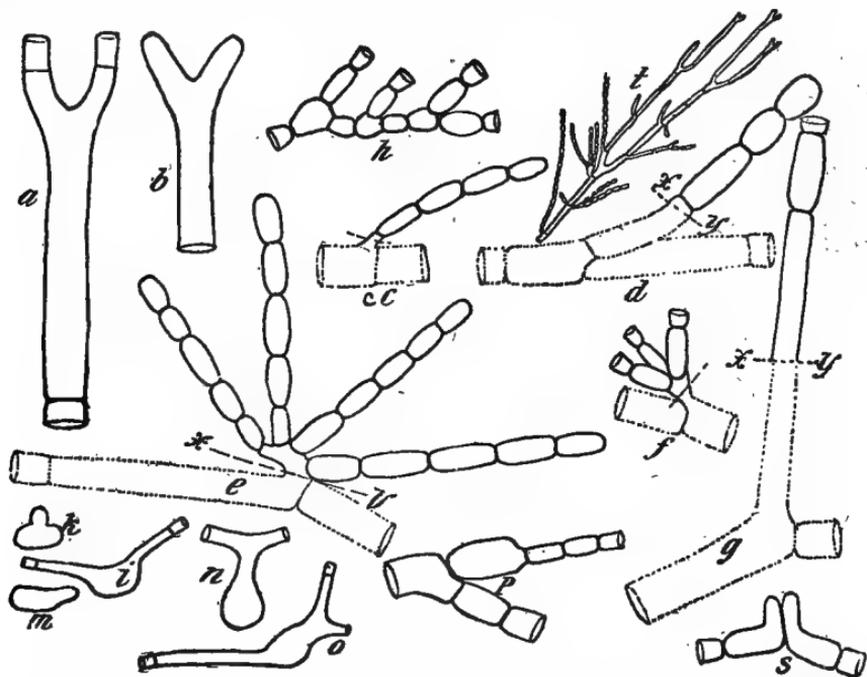


FIG. 96.—*Oidium lactis*. *a, b*, Dichotomous branching of growing hyphæ; *c, d, g*, simple chains of oidia breaking through substratum at dotted line *x-y*, dotted portions submerged; *e, f*, chains of oidia from a branching outgrowth of a submerged cell; *h*, branching chain of oidia; *k, l, m, n, o, p, s*, types of germination of oidia under varying conditions; *t*, diagram of a portion of a colony showing habit of *Oidium lactis* as seen in culture media. (From Bull. 82, Bur. Animal Industry, U. S. Dept. Agr.)

the further fermentation of acid substances, such as sauerkraut, sour milk and cheese. The organism is especially important in the ripening of Camembert cheese. It grows well on ordinary nutrient gelatin. The colony consists of a loosely woven, white network of septate, branched and anastomosing threads, chiefly

in the substratum but also extending into the air. The peripheral threads are divided by septa to form chains of oval or spherical conidia.

This mold may be readily obtained for study by making plate cultures from compressed yeast.

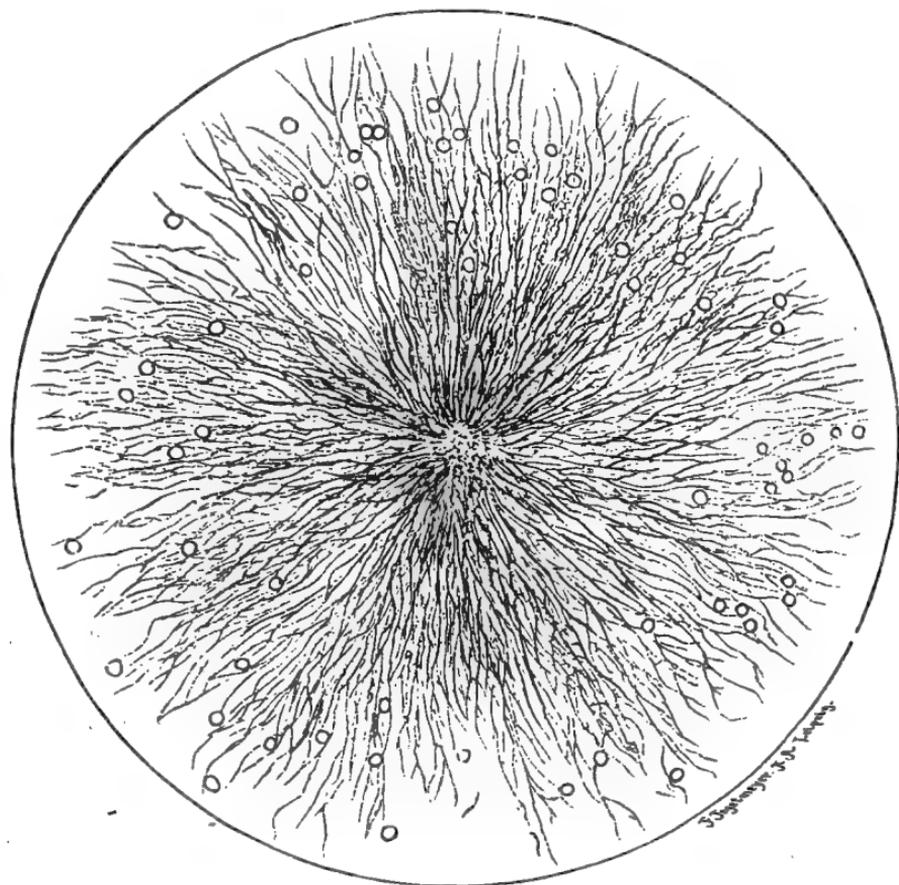


FIG. 97.—*Oidium albicans*. A deep colony on a plate culture of the liquefying variety, showing chlamydozoospores. (After Plaut.)

**Oidium Albicans (Monilia Candida).**—The thrush fungus was discovered by von Langenbeck in 1839 and by Berg in 1841, but the popular recognition of a relation between this disease and a mold seems to have preceded this discovery by many years. Berg (1841) transferred the fungus from cases of thrush to healthy

children with positive results. His work was confirmed by numerous other investigators (1842-43). Robin (1847) accurately described the parasite, with illustrations, classed it as an oidium, and gave it the name *Oidium albicans* (1853). Grawitz (1877) obtained the first pure cultures and successfully inoculated rabbits and puppies with them.

In the throat lesion as well as in cultures the organism consists of mycelial threads and oval yeast-like cells. It grows readily on various artificial media and the appearance of the growth is quite variable, not only because of the proportional relation between the oval cells and the threads, but also in pigmentation and in density of growth. Two varieties, one liquefying gelatin



FIG. 98.—*Oidium albicans*. Mycelial thread with four ripe chlamydospores; and conidia in the middle of the picture. (After Plaut.)

and producing large ( $5\mu$ ) oval conidia, and the other failing to liquefy gelatin and producing small ( $2.5\mu$ ) spherical conidia are distinguished.

Thrush is most common on the buccal mucous membrane of young infants, but it also occurs on the vaginal mucosa of pregnant women, and it may attack others when weakened by disease, especially diabetics. The disease also occurs naturally in birds, calves and foals. The threads of the mold penetrate the squamous epithelium and even enter the subepithelial tissue, sometimes penetrating blood-vessels and giving rise to metastases. It results in death in about 20 per cent of the cases in infants. The predisposing digestive disorder or other primary disease is however, usually more important than the thrush, and demands first consideration in treatment. The thrush lesion may be carefully removed with a soft swab and the eroded area treated with silver nitrate, 0.1 per cent. Generalization of the disease

is rare, but several cases have been observed. Inoculation of animals (mice, guinea-pigs, puppies, rabbits) is sometimes successful, and generalized thrush has followed intravenous injection of young rabbits. The fungus seems to exert some poisonous action; in addition to the mechanical effect upon the tissues.

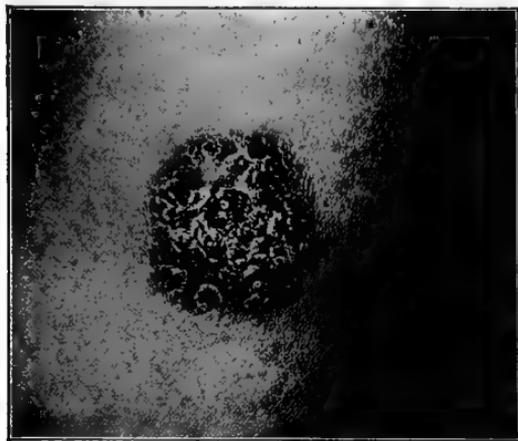


FIG. 99.—Scutulum of favus on the arm of a man. (After Plaut.)

**Monilia Psilosis.**—Ashford<sup>1</sup> has found a yeast-like organism on the tongue and in the feces of persons suffering from sprue and in collaboration with Michel has demonstrated the presence of a complement-fixation reaction between the blood of sprue patients and an antigen prepared from cultures of this organism. The cultures are made on acid glucose agar or on Sabouraud's medium. Sprue is a chronic disease characterized by recurrent attacks of diarrhea, with foamy, whitish and bulky stools, progressive emaciation and weakness. The subject requires further investigation before the causal relation of *Monilia psilosis* to the disease can be accepted as established.

**Achorion Schoenleinii.**—The fungus of favus was discovered by Schoenlein in the skin lesions of this disease in 1839, two years after the recognition of *Botrytis bassiana* as the cause of muscardine. Remak in 1845 grew the mold on slices of apple and

<sup>1</sup> Ashford, Amer. Journ. Med. Sciences, 1916, 151, p. 520; *ibid.*, 1917, 154, p. 157

successfully inoculated his skin with these cultures. He named the organism *Achorion schoenleinii*. In the lesion of favus the threads of the fungus are found growing in the horny layer of the epidermis, usually about a hair, and giving rise to a dry, circular, yellow crust with depressed center, the "*Scutulum*." By macerating this crust in 50 per cent antiformin the elements of the mold are made clearly visible under the microscope. In the center of the lesion are doubly contoured oval or rectangular conidia, 3



FIG. 100.—Typical scutulum of favus in a mouse. (After Plaut.)

to  $8\mu$  by 3 to  $4\mu$ , single and in chains. The mycelial threads are indistinguishable in the center, but are seen at the periphery as tubes of very irregular width, refractive with granular protoplasm, often branched or knobbed at the end. The *scutulum* in its interior is a pure culture of the mold, entirely free from other organisms. The mold also grows in the interior of the hair shaft, and by macerating the hair in alkali the fungus may be demonstrated microscopically.

Cultures may be obtained upon various media. Plaut recommends a medium containing pepton 1 to 2 per cent, glycerin



specifically identical with, *Achorion schoenleinii*. The exact relationships of the parasites are not very fully settled as yet.

**Microsporon Audouini.**—This mold is found growing in the hair-shaft in alopecia areata. If the hair be pulled out it breaks near the lower end and the oval conidia and jointed threads of the parasite may be demonstrated by macerating this broken end. The disease is very contagious, chronic and resistant to treatment, but proceeds without inflammation or subjective symptoms, the conspicuous sign being loss of the hair. Cultures grow slowly and are snow white. Animal inoculation is rarely successful.

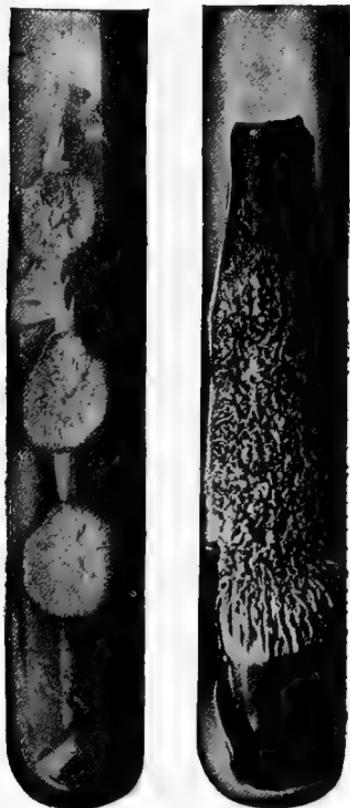


FIG. 102.—*Sporotrichum schenckii*. Cultures on the glucose-pepton agar of Sabouraud. (After Gougerot.)

**Microsporon Furfur.**—This mold is found in the superficial layer of the skin in pityriasis versicolor, as short thick hyphæ 3 to 4 $\mu$  wide by 7 to 13 $\mu$  long, together with abundant doubly contoured single conidia. Pityriasis versicolor occurs most frequently on the skin of the chest and is one of the commonest affections of the skin.

**Tricophyton Acuminatum.**—The mold invades the hair shaft and causes it to break off close to the surface of the skin. In such a hair long chains of oval cells of the parasite may be seen. The parasite also attacks the skin and produces ringworm. Several other species of tricophyton are distinguished. These parasites are concerned in the causation of barber's itch, eczema marginatum, tinea cruris, and other skin affections of this type.

**Sporotrichum Schencki.**—Schenck, at Baltimore in 1898, described this parasitic mold which he found in the lesions of a

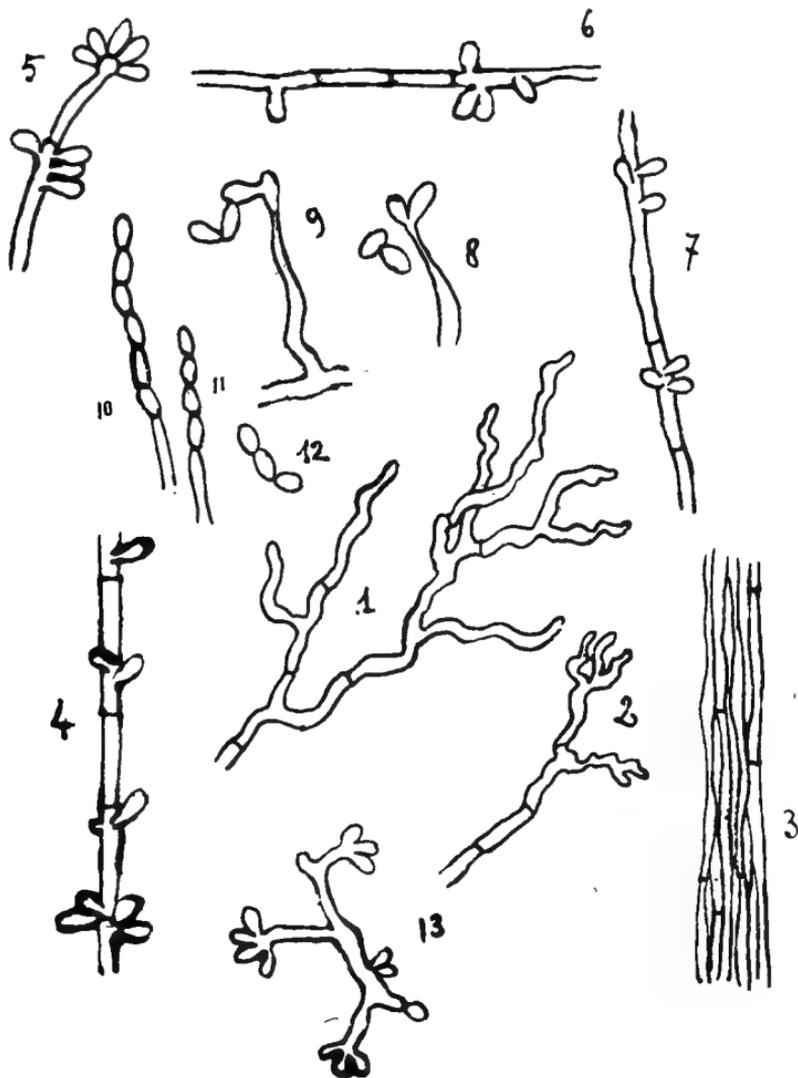


FIG. 103.—*Sporotrichum schencki*. Various forms of mycelium with and without conidia. (After Gougerot.)

peculiar disease, beginning as a localized ulcer, with later involvement of the neighboring lymph glands, in which cold abscesses

formed and opened to the exterior. A second similar case was described by Hektoen and Perkins. Ruediger<sup>1</sup> has reported a large series of cases of sporotrichosis and the references to American literature will be found in his paper. The organisms are not readily found in the pus by microscopic examination and seem to exist there only as conidia. In cultures a branching mycelium with clusters of conidia is produced. Dogs are susceptible to inoculation.

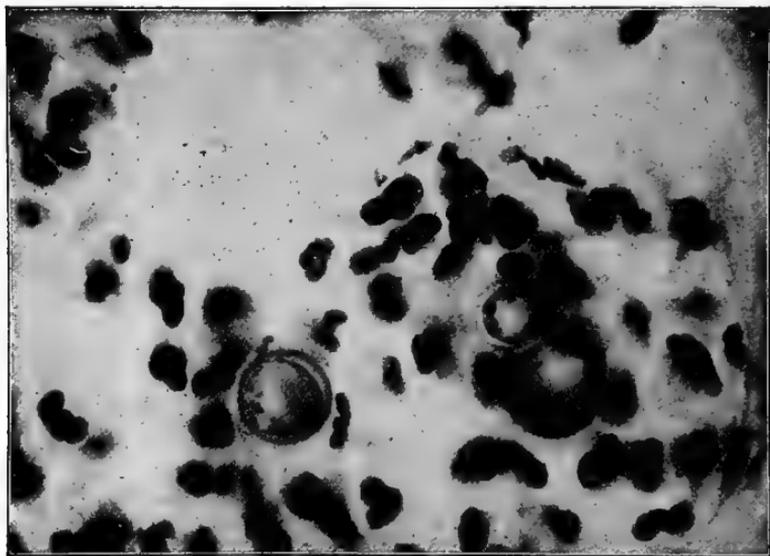


FIG. 104.—Doubly contoured organisms found in oidiumycosis (blastomycosis).  
(From Buschke after Hyde and Montgomery.)

**Sporotrichium Beurmanii.**—De Beurmann and Ramond at Paris in 1903 found this parasite in a case of lymphangitis. It seems to be different from the organism described by Schenck but may ultimately prove to be the same species.

**Cryptococcus Gilchristi.**—Doubly contoured yeast-like cells which multiply by budding in human tissues, were first discovered by Busse and Buschke<sup>2</sup> in 1894, in a case presenting abscesses in

<sup>1</sup> *Journ. Infect. Diseases*, 1912, Vol. XI, pp. 193-206.

<sup>2</sup> *Deutsch. med. Wochenschr.*, 1895, Nr. 3.

the bones and internal organs together with lesions of the skin. They obtained cultures of the organism and classed it as a yeast. About the same time Gilchrist<sup>1</sup> independently observed similar organisms in cases of dermatitis at Baltimore. The organisms have been most thoroughly studied by Ricketts.<sup>2</sup> Most of the cases have been observed in the United States, particularly at Baltimore and at Chicago. The disease is designated as oidiomycosis, blastomycosis and blastomycetic dermatitis. It exists most commonly as a chronic purulent dermatitis but infection of the pericardium and of the meninges with these organisms and even generalized blastomycosis has been reported.<sup>3</sup> In the earlier literature this organism was often confused with *Coccidioides immitis*. On glucose agar, the parasites usually grow without difficulty and the growth resembles that of an oidium, often with abundant aërial hyphæ. Inoculation of guinea-pigs with pus or with cultures is usually followed by formation of abscesses in which the typical spherical or ovoid parasites may be found. Further investigations are required to determine the specific relationships of the parasites found in different cases.

<sup>1</sup> Gilchrist: *Johns Hopkins Hosp. Rept.*, Vol. I, p. 209, 1896.

<sup>2</sup> *Journ. Med. Research*, Vol. VI, No. 3.

<sup>3</sup> Sihler, Peppard and Cox, Case of systemic blastomycosis, *Journal-Lancet* (Minneapolis) 1917, 37, p. 253.

## CHAPTER XV

### TRICHOMYCETES

The trichomycetes or higher bacteria are intermediate in morphological characters between the molds and the lower bacteria. They resemble the molds in the formation of long threads, sometimes branching and interlacing to produce a network, and in the formation of oval or spherical conidia constricted off from the ends of the threads. They resemble the lower bacteria in their small transverse diameter, the delicacy of their structure and their mode of life. Petruschy<sup>1</sup> recognizes four genera, *Actinomyces*, *Streptothrix* (*Nocardia*), *Cladothrix* and *Leptothrix*.

**Actinomyces Bovis.**—Bollinger in 1877 studied the lumpy-jaw disease of cattle and described this parasite which occurs in the lesions. Israel, in the following year found the organism in granulomatous lesions in man. The infection also occurs in horses, sheep, swine and dogs. In the tissues and in the purulent discharge from the lesions, the organism occurs in small yellowish masses, sometimes visible to the naked eye but usually smaller (10 to 200 $\mu$  in diameter). Such a mass is a single colony of the parasite or a conglomerate of several colonies. The colony is a dense network of threads in the center, with radially arranged threads about the periphery, most of the latter being swollen, club-shaped, at their free ends. Spherical bodies may also be present, but whether these are conidia or degeneration forms of the parasite is uncertain. The organism is Gram-positive.

Inoculation of pus or bits of tissue containing the parasite from one animal into another usually fails to transmit the disease, although positive results have been obtained in a few instances. Attempts at culture have failed also in many instances, and the

<sup>1</sup> Kolle and Wassermann: Handbuch, 1912, Vol. V, p. 270.

difficulty here seems to depend in part upon the oxygen requirements of the organism. The material for culture should be obtained from uncontaminated tissue containing the fungus. If this is impossible, the granule of actinomyces should be washed in several changes of sterile salt solution, then crushed between sterile glass slides or, better, ground up in a sterile mortar with a small amount of sterile sand. A series of dilution cultures should then be made in tall tubes of melted glucose agar cooled to 45° C., the tubes chilled in cold water and incubated at 37° C.

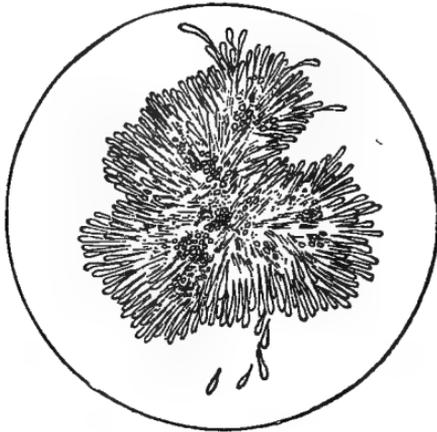


FIG. 105.—*Actinomyces bovis*. The ray-fungus from cow. (Diagrammatic.)

Colonies of the fungus may be expected to develop some distance below the surface of the agar. Wolf and Israel were able to reproduce the disease in animals (rabbits and guinea-pigs) by the inoculation of pure cultures. More recently Harbitz and Gron-dahl<sup>1</sup> isolated twenty-seven strains of actinomyces, but their inoculation experiments were wholly negative. It would appear that other factors are essential to the development of actinomy-cosis in addition to the inoculation of the specific parasite. Many authors regard the presence of bits of straw or sharp grains in wounds of the mucous membrane of the mouth or pharynx as important elements in predisposing to infection with actinomyces.

<sup>1</sup> *Amer. Journ. Med. Sciences*, 1911, Vol. CXLII, pp. 386-395.

The disease shows little or no tendency to be transmitted from animal to animal in a herd. Several varieties of actinomyces have been described, and possibly more than one species will eventually be recognized.

**Streptothrix Maduræ.**—Kanthack (1892) and Gemy and Vincent (1892) discovered the fine mycelial threads in pus from cases of Madura foot. Granules about the size of a pin-head occur in the pus, and under the microscope these are found to consist of a network of threads 1 to  $1.5\mu$  in thickness, arranged radially at the periphery and presenting somewhat swollen ends. These granules are white in some cases, yellow, red and black in others. The nature of the disease seems to be the same in all cases, but the micro-organisms are apparently not the same, that found in the black variety probably representing a distinct species. Cultures may be obtained by inoculating the pus, collected without contamination, into several flasks of sterilized hay infusion, and shaking daily to insure abundant oxygen supply. It also grows upon other media. Gelatin is not liquefied. The growth is made up of interwoven, slender branching threads about  $1\mu$  in thickness. Spores (conidia) capable of resisting a temperature of  $75^{\circ}$  C. for five minutes are produced at the surface of the culture. Inoculation of animals usually gives negative results, but Musgrave and Klegg<sup>1</sup> have succeeded in infecting monkeys.

The disease, Mycetoma or Madura foot, is a localized chronic inflammation, almost painless, and usually involving the foot, the hand or some exposed portion of the body. The disease involves the tissues by direct extension, attacking the bones as well as the soft tissues. It usually remains localized to one extremity.

The black variety of Madura foot is due to a different organism, the threads of which are 3 to  $8\mu$  in thickness.<sup>2</sup> This organism seems to be an aspergillus, and has been named *Madurella mycetori*.

<sup>1</sup> *Philippine Journ. of Science*, 1907, Vol. II, pp. 477-512; A complete bibliography by Polk is included.

<sup>2</sup> Wright: *Journ. of Exp. Medicine*, Vol. III, pp. 421-433.

**Streptothrix putorii** (*Nocardia putorii*).—Dick and Tunnickliff<sup>1</sup> have found this organism in a case of fever following the bite of a weasel. Somewhat similar organisms have been found in fever following rat bites. The usual cause of rat bite fever is, however, evidently a spirochete, *Spirochæta (morsus) muris*.

Streptothrices have also been found in abscesses<sup>7</sup> of the brain and in chronic disease of the lung clinically resembling tuberculosis in man. Many of them are Gram-positive and some are relatively acid-proof when stained with carbol-fuchsin. Such acid-proof forms are common in the feces of cattle where short segments of them may be mistaken for tubercle bacilli. Organisms of this type are very abundant in the soil, which is doubtless their natural habitat.

**Cladothrix**.—The cladothrix forms resemble the streptothrices very closely but the cells of the threads do not branch. The apparent branching of the threads is explained as due to a transverse division of the thread with continuing growth of the one free end which pushes out beyond the other, giving rise to the appearance of branching or so-called "false branching." Organisms of this type have been described as occurring in abscesses of the brain and in other parts of the body. The distinction from streptothrix has not always been clearly made.

**Leptothrix Buccalis**.—This is a normal inhabitant of the mouth cavity. It consists of slender filaments which do not branch. The organism has been found in abundance in small white patches on the tonsils, where it sometimes causes a very chronic but mild inflammation. Artificial culture of the organism ordinarily results in failure. Arustamoff<sup>2</sup> appears to have obtained it on a neutral or acid agar inoculated with leptothrix from urine.

<sup>1</sup> Dick and Tunnickliff: *Journ. Infectious Diseases*, 1918, 23, p. 183.

<sup>2</sup> Kolle and Wassermann: *Handbuch*, 1912, Bd. V, S. 290.

## CHAPTER XVI

### THE COCCACEÆ AND THEIR PARASITIC RELATIONSHIPS

**Diplococcus Gonorrhææ (Neisseria Gonorrhææ).**—The gonococcus was discovered by Neisser<sup>1</sup> in 1879 in the discharge of acute urethritis and he recognized its probable causal relationship to the disease. Cultures were first obtained by Bumm<sup>2</sup> in 1885 and he proved the relationship by inoculating the human urethra with his cultures. The organism naturally lives and multiplies only in the human body and is the cause of gonorrhœa and many of its complicating inflammations.

The gonococcus is found in both the serum and the polynuclear cells of the purulent discharge, usually in pairs with the adjacent surfaces flattened. The long diameter of the pair is about  $1.25\mu$ . It stains readily, best perhaps with Löffler's methylene-blue. It is decolorized when stained by Grams' method, a fact of great importance in the quick recognition of the organism. The staining procedure has to be carefully carried out and a beginner should practice upon cultures of the gonococcus and upon samples of gonorrhœal pus and staphylococcus pus before placing too much reliance upon the appearance of his Gram-stained preparation. The reaction to the Gram stain, together with the remarkably characteristic appearance of the pus cell full of diplococci are usually sufficient for the recognition of the organism in acute urethritis.

Cultures of the gonococcus were obtained by Bumm on coagulated human blood serum. Wertheim<sup>3</sup> employed serum agar

<sup>1</sup> Neisser: *Centralbl. f. d. med. Wissenschaft*, 1879, Bd. XVII, S. 497-500.

<sup>2</sup> Bumm: *Deutsche med. Wochenschr.*, 1885, Bd. II, S. 910 and 911.

<sup>3</sup> *Deutsche med. Wochenschr.*, 1891, Bd. XVII, S. 958; S. 1351 and 1352.

made by mixing human blood serum at  $40^{\circ}$  C., one part, with ordinary nutrient agar melted and cooled to  $40^{\circ}$  C., two parts. The medium may be inclined in tubes or may be employed for plating. Human ascitic fluid or hydrocele fluid is just as good as blood serum. A large drop of pus from an acute urethritis should be mixed with 2 to 3 c.c. of serum or ascitic fluid in a test-tube and, from this, dilutions made to a second and a third tube. The contents of a tube of agar (5 to 6 c.c.), previously melted and cooled to about  $40^{\circ}$  C., is then added to each tube of



FIG. 106.—Gonococci and pus-cells.  $\times 1000$ .

serum, mixed thoroughly and poured into Petri dishes to solidify. At  $37^{\circ}$  C., colonies appear within 24 hours and at the end of this time measure about 1 mm. in diameter. The colony is circular, grayish-blue and transparent and of a mucoid consistency. The individual cocci disintegrate rapidly, even within the first 24 hours at the center of the colony, and for microscopic study simple staining and staining by Gram's method, cultures 5 to 10 hours old are recommended. Even under favorable conditions the gonococcus ordinarily dies out in the culture tube in about a week, although exceptionally it may survive for three weeks.

It should be transplanted every few days and a large quantity of growth must be transferred. When transplanted from vigorous cultures to plain agar the gonococcus grows for a few days, but it cannot be successfully propagated for any length of time on ordinary media.

The gonococcus is very sensitive to drying and to temperatures above  $40^{\circ}$  C. It is usually impossible to recover it from dried pus, but in moist material it may live for 1 to 24 hours. The organism is easily killed by chemicals germicides, of which silver nitrate is probably the most effective.

Inoculation of animals in the urethra or on the conjunctiva is without result. Intraperitoneal injection of cultures into white mice or guinea-pigs usually kills the animals in 24 hours and the gonococci can be recovered from the peritoneal fluid and the heart's blood. These effects seem to be due to toxins of the injected material rather than actual infection. The specific poisons seem to be intracellular and set free upon disintegration of the organism. The poison withstands heating to  $100^{\circ}$  C. for hours. Inoculation of the human urethra with cultures of the gonococcus has been repeatedly done and has resulted nearly always in the production of typical gonorrhoea.

Gonorrhoea has been recognized as a contagious disease since the dawn of history. The most important forms are (1) urethritis with tendency to extension in the female to the cervix uteri, oviducts and peritoneum, and in the male to the prostate, seminal vesicles and epididymis, and in both sexes to the blood stream, heart valves and joints; (2) conjunctivitis and keratitis leading to scarring of the cornea and permanent blindness; (3) vulvo-vaginitis in girl babies, an exceedingly contagious disease, especially in hospital wards. The disease tends to become chronic and eventually latent, that is, the symptoms subside but the micro-organisms remain alive in certain locations, such as the prostate in the male and the cervix uteri in the female. The acute inflammation may be followed by scars resulting in strictures of the urethra or occlusion of the epididymis. In the female, pyosalpinx is a not unusual

complication. Secondary infection with staphylococci is common in chronic gonorrhœa.

Specific diagnosis by finding gonococci usually presents no difficulties in acute inflammations of the genital tract, in which the characteristic groups of Gram-negative intracellular diplococci are practically diagnostic. In chronic cases and in extra-genital inflammations the diagnosis presents greater difficulty. Both microscopic and cultural examinations should be made and if negative they should be repeated many times. Even repeated failure to find the gonococcus by these methods does not justify the positive assertion that it is absent. Specific diagnosis by the method of complement fixation has been developed by Schwartz and McNeill.<sup>1</sup> The antigen is prepared from several culture strains of the gonococcus and in all other respects the test is similar to the Wassermann test for syphilis. Irons<sup>2</sup> has employed a cutaneous test, using a glycerin extract of gonococci. The technic is similar to that of the von Pirquet test for tuberculosis.

The prevalence of gonorrhœa throughout the civilized world is much greater than has been popularly supposed. Erb, in a study of 2000 males among private patients of the middle and better classes, found a history of gonorrhœa in 50 per cent. Many other students of the disease disagree with Erb, regarding his figures as much too low. The large mass of statistics obtained by examination of recruits for war service in 1917 and 1918 indicates that approximately 2 per cent of men in the age period 21 to 31 years in the United States are afflicted with recognizable gonorrhœa at any one time. Among women in German obstetrical hospitals, largely from the poorer class, gonorrhœa is present in 10 to 30 per cent. The danger to the eyes of the new-born infant is now overcome by the use of silver nitrate in the eyes when they are first cleansed. The general prevention and restriction of gonorrhœal infection is engaging more and more the serious attention of

<sup>1</sup> *Amer. Journ. med. Sciences*, 1912, Vol. CXLIV, pp. 815-826.

<sup>2</sup> *Journ. Infec. Diseases*, 1912, Vol. XI, pp. 77-93.

thoughtful citizens, and it is already recognized as a sanitary problem of the first magnitude.

**Diplococcus Meningitidis (Neisseria Intracellularis).**—Weichselbaum in 1887 examined the cerebrospinal fluid in six sporadic cases of meningitis and found in all of them a very definite Gram-negative intracellular diplococcus, the meningococcus. He obtained cultures but his animal inoculations all gave negative results. Jaeger in 1895 seems to have found a similar organism in fourteen cases of epidemic meningitis and Huebner in 1896 apparently found it in five cases. The cultural work of these authors seems to be unreliable as their cultures were Gram-positive. More conclusive confirmation of the relation of this organism to epidemic meningitis was furnished by Councilman, Mallory and Wright<sup>1</sup> in 1918.

The meningococcus is found in the bodies of patients suffering from meningitis, occasionally on the nasal mucous membrane of healthy persons and of cases of rhinitis, and very rarely in other situations. In cerebrospinal meningitis the organism is present in the cerebrospinal fluid, in the meninges, often on the nasal and pharyngeal mucous membrane, sometimes in the blood and on the conjunctivæ, and rarely in the urethra, where it may be mistaken for the gonococcus. It is usually found without difficulty in the cerebrospinal fluid in the first few days of the disease, but may be very difficult to find at a later stage.

The organism is found for the most part inside polynuclear leukocytes and in its form, size, arrangement and behavior to the Gram-stain resembles very closely the gonococcus. The outline of the cocci is often somewhat hazy, suggesting possible disintegration, and this sometimes makes their recognition somewhat difficult in microscopic preparations of cerebrospinal fluid. Cultures may be made on ascitic-fluid agar or blood agar, upon which small dew-drop colonies appear in 24 hours at 37° C. A better medium is obtained by laking human blood or rabbit's

<sup>1</sup> Report of the Mass. Bd. of Health on Epidemic Cerebrospinal Meningitis, etc., Boston, 1898.

blood with distilled water and adding this to melted glucose agar in a quantity sufficient to give it a pink tint. The color of blood is unaltered by the growth. Cultures may be obtained on Löffler's blood serum, although this medium is not very satisfactory for this purpose. The meningococcus grows more luxuriantly than the gonococcus, as a rule, and adapts itself more readily to growth on ordinary media, but its cells disintegrate rapidly in the colony, which is viscid. In nearly every respect it resembles very closely the gonococcus.

Intraperitoneal inoculation of white mice and of guinea-pigs usually results in fatal peritonitis and the organism can be recovered from the heart's blood. Intraspinial inoculation of monkeys with large doses causes typical meningitis with symptoms similar to those of the disease in man. In man the disease is undoubtedly transmitted very largely by coccus-carriers, healthy people or people with slight pharyngitis or rhinitis, who carry the virus on their mucous membranes and distribute it.

Several serologically different types of meningococci are recognized and for the specific recognition of the meningococcus by the agglutination test, it is advisable to employ polyvalent serum as well as the various mono-valent sera in dilutions of 1 to 100 and 1 to 200. Suspensions of living cultures grown on plain agar or on serum agar, if necessary, are mixed with the serum and the tubes are incubated 16 hours at 55° C. A control tube of normal serum should be included in the test.

Ordinarily, specific agglutination and type determination may be neglected in the recognition of meningococci found in the cerebro-spinal fluid. When isolated from the pharynx, bronchi or lungs, confusion with other organisms is more probable and, in these instances, agglutination tests are required. Bacteriologists undertaking this work should consult the paper of Flexner<sup>1</sup> and the literature there cited, especially the Gordon report.

*Antimeningococcus serum* is prepared by immunizing horses

<sup>1</sup> Flexner, S.: Mode of infection, means of prevention and specific treatment of epidemic meningitis, *Journ. Amer. Med. Assn.*, 1917, **69**, p. 639, p. 721, p. 817.

with a mixture of many typical and atypical meningococcus cultures injected subcutaneously. At first the cultures are killed by heat before injection, and only one or two loopfuls are given. The dose is increased and repeated every 8 to 10 days until the growth on two Petri dishes is being injected. Living cultures are then given, and finally old cultures which have disintegrated are also used. The serum is used after the horse has been treated for 8 to 10 months. Jochmann showed that the subcutaneous injection of the serum is without effect upon meningitis in monkeys but that when introduced into the spinal canal is specifically curative. Flexner<sup>1</sup> and his co-workers have studied this very fully and there can no longer be any question of the value of the serum in the treatment of meningococcus meningitis.

*Cerebrospinal fluid* is obtained by Quinke's puncture. For children a needle 4 cm. long and with a lumen of 1 mm. is introduced in the medium line directly forward so as to enter the spinal canal between the second and third or the third and fourth lumbar vertebræ. From 20 to 50 c.c. of fluid may be withdrawn if it comes away under pressure, and then the curative serum is injected through the same needle. The fluid withdrawn should be examined to establish the presence of meningitis and its variety. In general the examination includes a macroscopic examination and description of the appearance of the sample, a microscopical numerical count of the cells present, chemical examination of the cell-free fluid for excessive protein<sup>2</sup> content, microscopic and cultural examination of the sediment for bacteria and of the

<sup>1</sup> Flexner: Harbin lectures. *Journ. State Medicine*, 1912, Vol. XX, pp. 257-270.

<sup>2</sup> Noguchi's test: To 0.5 c.c. of blood-free fluid add 1 c.c. 10 per cent butyric acid, boil; add 0.2 c.c. normal NaOH and boil again. Set aside to cool. A flocculent precipitate indicates an increase in the globulin content.

Pandy's test: Add 1 drop of the spinal fluid to 1 c.c. of a saturated aqueous solution of carbolic acid. The immediate formation of a bluish-white ring or cloud indicates an increased protein content.

Ross-Jones test: Layer equal quantities of spinal fluid and ammonium sulphate solution, saturated by boiling. A white ring indicates abnormal globulin content.

filmy clot which may form after standing an hour or so for tubercle bacilli, and sometimes it includes the Wassermann reaction. In meningococcus meningitis the cell count is generally above 100 per cu. mm., and most of the cells are polynuclear leukocytes. Within these cells the meningococci may or may not be found. In case of doubt, plate cultures on blood-agar and ascitic-fluid agar should be made. The recognition of a Gram-negative intracellular

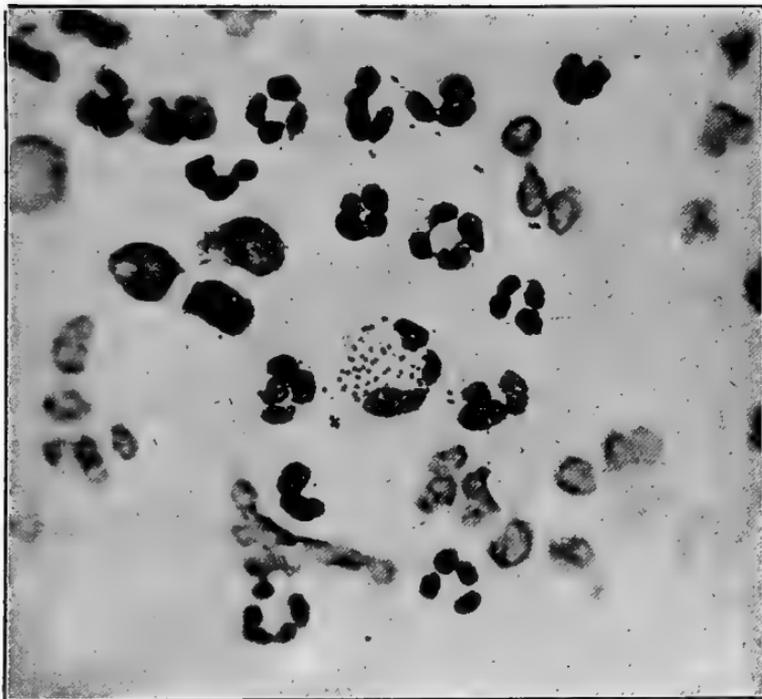


FIG. 107.—Meningococcus in spinal fluid. (After Hiss and Zinsser.)

diplococcus in the fluid is sufficient for a tentative diagnosis, and the appearance of characteristic colonies on the plates may be considered conclusive.

**Diplococcus (Micrococcus) Catarrhalis.**—This organism is commonly present on the mucous membrane of the upper air passages, especially in catarrhal inflammations. It is usually seen as a Gram-negative intracellular diplococcus not to be

distinguished microscopically from the meningococcus or gonococcus. In examining material from the air passages this organism has to be considered. It is readily distinguished by cultural methods. On ascitic-fluid agar the colony is dry and brittle, quite different from the meningococcus or gonococcus. Furthermore, it grows readily at once on ordinary agar.

**Diplococcus Pneumoniæ.**—Sternberg in 1880 injected the saliva of healthy persons into rabbits and produced a rapidly fatal bacteremia with abundant lance-shaped diplococci in the blood and internal organs of the animal. Pasteur, independently and at about the same time, injected the saliva of a boy suffering from rabies into rabbits with a similar result. The organism was spoken of as the diplococcus of sputum septicemia or the septicemic microbe of saliva. Koch in 1881 demonstrated the organism microscopically in sections of lung. Friedlaender (1882-1884) found the organism microscopically in a large number of cases of pneumonia and accurately described its form, the capsules and staining properties. His cultures, however, which were made on gelatin at room temperature, brought to development not the pneumococcus but a wholly different organism which he believed to be identical with it, Friedlaender's pneumobacillus. A. Fraenkel obtained the first undoubted pure cultures on solidified blood serum, proved the identity of the organism in pneumonia with that of normal saliva seen by Sternberg and Pasteur, and distinguished it absolutely from the pneumobacillus of Friedlaender. He also succeeded in producing typical pneumonia by injecting cultures of moderate virulence intravenously into rabbits.

The pneumococcus is somewhat variable in form. In the animal body it occurs in pairs of lance-shaped individuals with the points directed away from each other, and the pair is surrounded by a thick gelatinous capsule.<sup>1</sup> The organism is always Gram-

<sup>1</sup> In demonstrating the capsules, the method of Hiss gives excellent results. Spread some blood or tissue juice on a cover-glass and as soon as the film of moisture has disappeared, fix the preparation by heat. Then stain with hot aqueous gentian violet and wash off the dye with a 20 per cent solution of copper sulphate. Examine in the copper solution. Blot the preparation, dry it in air and mount in balsam.

positive. In cultures the capsules are less well developed and often cannot be demonstrated at all. The individuals are often less pointed and frequently resemble short bacilli in form. They may remain attached together in chains of six to eight cells.

Cultures may be obtained on ordinary media but they are prone to die out quickly. Blood-agar, serum agar or ascitic-fluid agar are the best solid media, but even with these weekly transplantation is usually necessary. Broth to which serum or ascitic fluid has been added forms an excellent medium. There is prac-

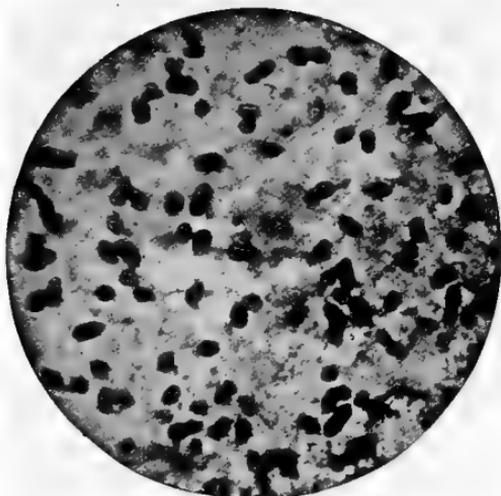


FIG. 108.—Pneumococcus, showing capsule, from pleuritic fluid of infected rabbit, stained by second method of Hiss.

tically no growth below  $25^{\circ}$  C. On blood agar, the colony is surrounded by a zone of greenish discoloration, a character of great value in the early recognition of the pneumococcus isolated from the body. The virulence of the microbe diminishes very rapidly in artificial culture. Virulent material is best kept in stock by preserving in a desiccator dried blood taken from a rabbit dead of pneumococcus infection. The fluid blood may also be kept in sealed capillaries in the refrigerator. By these methods the virulence may be preserved for months. Rabbits, mice and young rats are the most susceptible animals.

The pneumococcus is the microbic agent in from 80 to 95 per cent of cases of acute lobar pneumonia. It also occurs in otitis media, mastoiditis, meningitis, peritonitis and arthritis. Its presence is usually associated with a fibrino-purulent exudate, In severe pneumonia it is often present in the circulating blood.

Pneumonia, or inflammation of the lungs, may be caused by a great variety of organisms, the tubercle bacillus, the pneumobacillus of Friedlaender, the streptococcus, the typhoid bacillus and many others. Typical lobar pneumonia, however, a disease characterized by a definite sequence of pathological changes in the lung and by a rather typical clinical course, is rarely caused by any organism other than *Diplococcus pneumoniae*. This is a very frequent disease in adults and doubtless the most frequent cause of death in persons over 50 years of age.

The nature of the poisons produced by the pneumococcus is not definitely known. When killed by heat, the dead germ substance is not very toxic. One very remarkable property of the organism is its susceptibility to the action of bile and solutions of bile salts. These cause a complete and prompt solution of suspensions of pneumococci. Cole<sup>1</sup> has shown that a powerful poison is set free by this disintegration of pneumococci, the toxic action of which resembles that seen in the phenomenon of anaphylaxis.

It has been possible to induce a high degree of immunity in horses, and the serum of these animals is protective and curative in animal experiments. By use of such serum a large number of serologically different strains of pneumococci have been recognized. Cole and his associates have found that a considerable proportion of lobar pneumonia in New York City is caused by one of these serological types, which they have designated as Group I, and they have been able to produce an effective anti-serum against these strains. A second, somewhat less homogeneous collection of strains is characterized by specific reaction with another immune

<sup>1</sup> Cole: *Journ. Exp. Med.*, 1912, Vol. XVI, pp. 644-664; Harvey Lectures, 1913-14, Lippincott, p. 85. This paper gives references to literature.

serum and these types are designated as Group II. Group III includes the morphologically different *Pneumococcus mucosus*, characterized by its large capsules, the viscid nature of its colonies on culture media and of the peritoneal exudate which it produces in the mouse. The remaining strains of pneumococci are placed in a heterogeneous Group IV. Infections with pneumococci of Group I are favorably influenced by the injection of Type I serum. The Type II serum appears to be of some value in treating infections with pneumococci of Group II. No therapeutic serum has been produced for Group III and the sera obtained in Group IV are potent against only the particular strain employed for immunization of the animal. Determination of the Group of the infecting pneumococcus should, therefore, precede the therapeutic use of the serum.

For type determination<sup>1</sup> the sputum should be obtained from the deeper air passages under immediate supervision of the bacteriologist and should be examined immediately. Microscopic examination of preparations stained by (1) Gram's method, (2) Ziehl-Neelsen method and (3) Hiss capsule stain should be done. *Pneumococcus mucosus* shows wide capsules in both the Gram and the Hiss preparations. A piece of sputum, volume about 0.2 c.c., is washed through four changes of salt solution in Petri dishes, placed in a mortar, ground up and emulsified in about 1 c.c. of salt solution, which is added drop by drop. About 0.5 c.c. of this suspension is injected intraperitoneally into a white mouse. The common sputum organisms which grow in the peritoneal cavity of the mouse are the pneumococcus, the influenza bacillus, *Micrococcus catarrhalis*, staphylococci and streptococci. The former two also invade the blood stream. After 5 to 24 hours the mouse is killed and the peritoneal fluid and heart's blood streaked on blood agar plates. Gram stain and Hiss capsule stain of the peritoneal exudate are examined. Then the peritoneal fluid is washed out with 5 c.c. of salt solution and the suspension is transferred to a centrifuge tube, whirled at low speed to throw

<sup>1</sup> Blake, F. G.: *Journ. Exp. Med.*, 1917, 26, p. 67.

down the leukocytes and fibrin. The supernatant fluid is then transferred to a clean tube and whirled at high speed to sediment the bacteria. The supernatant fluid is discarded and the bacterial sediment is suspended in salt solution sufficient to make a translucent mixture. Five tubes are then set up as follows:

*Tube 1*—0.5 c.c. of 1 : 20 dilution Type I serum, 0.5 c.c. of the bacterial suspension.

*Tube 2*—0.5 c.c. of undiluted Type II serum, 0.5 c.c. of the bacterial suspension.

*Tube 3*—0.5 c.c. of 1 : 20 dilution Type II serum, 0.5 c.c. of the bacterial suspension.

*Tube 4*—0.5 c.c. of 1 : 5 dilution of Type III serum, 0.5 c.c. of the bacterial suspension.

*Tube 5*—0.1 c.c. sterile ox bile, 0.3 c.c. of the bacterial suspension.

The tubes are immersed in water bath at 37° C. for one hour. Agglutination in the homologous type serum is usually prompt. If no agglutination occurs after one hour and the organism is a bile soluble encapsulated diplococcus, it is classed as a pneumococcus of Group IV. If agglutination is present in Tube 2 but absent in Tube 3, the organism is classed in Subgroup II.

Various simpler methods<sup>1</sup> of typing pneumococci have been devised, but they are less reliable even in expert hands.

Kyes<sup>2</sup> has injected chickens with massive doses of pneumococci and has obtained a powerful anti-serum which seems to be potent against various strains and types of pneumococci. Further confirmation of his results should be awaited.

**Streptococcus Viridans.**—Schottmueller<sup>3</sup> has found a streptococcus, resembling in some respects the pneumococcus, in the blood of cases of subacute endocarditis or endocarditis lenta. On the blood-agar plates the colonies appear after two to five

<sup>1</sup> Mitchell and Muns; *Journ. Med. Rsch.*, 1917, 37, p. 339.

Avery, O. T.; *Journ. Amer. Med. Assn.*, 1918, 70, p. 17.

<sup>2</sup> Kyes, P. *Journ. Amer. Med. Assn.*, 1911, 56, p. 1878; *Journ. Med. Rsch.*, 1918, 38, p. 495.

<sup>3</sup> *Muenchener med. Wochenschr.*, 1903 (I), No. 20, p. 849.

days as opaque granules surrounded by a cloudy but distinctly greenish zone. The organism is being found very frequently in cases of subacute endocarditis,<sup>1</sup> and is apparently the specific cause of this particular fairly well-defined type of endocarditis. The same organism is found normally in the mouth and pharynx and has been designated also as *streptococcus salivarius*. It is also commonly present in abscesses at the roots of the teeth.

**Streptococcus Pyogenes.**—Bacteria were observed in pyemic abscesses by Rindfleisch in 1866 and in the following years this observation was confirmed by numerous pathologists. Klebs (1870-71) recognized the "*Microsporion septicum*" as the cause of wound infections and the accompanying fever, as well as the resulting pyemia and septicemia. Ogston (1882) first clearly distinguished between the chain-form, streptococcus, and the grape-form, staphylococcus, of the pus cocci, not only on the basis of their grouping but also in respect to the types of inflammation with which they are associated. Pure cultures were first obtained by Fehleisen (1883) from erysipelas (*Streptococcus erysipelatos*) and by Rosenbach (1884) from the pus of wounds (*Streptococcus pyogenes*). The former produced typical erysipelas by inoculating the human skin with his cultures. There is no specific distinction between the streptococci found in erysipelas and those found in other lesions. The difference in the pathological process depends rather upon the portal of entry of the infection, the virulence of the microbe and the resistance of the host.

*Streptococcus pyogenes* lives naturally upon the mucous membranes, especially in the pharynx, nose and mouth, the intestine and on the vaginal mucosa. Such streptococci found in normal individuals are relatively non-virulent. Virulent streptococci occur in erysipelatos lesions of the skin, in infected wounds on the inflamed pharyngeal mucosa, and in the lochia, uterine wall and in the circulating blood in puerperal fever. Streptococci are frequently found in pyemic abscesses, bacteremia, meningitis

<sup>1</sup> Major, *Johns Hopkins Hosp. Bull.*, 1912, Vol. XXIII, pp. 326-332.

and pneumonia. It seems probable that these virulent races originate from the ordinary relatively harmless parasitic forms in some instances, when an opportunity is presented for successful invasion of tissues by a lowered resistance of the host, and that by successive transfer from one susceptible individual to another the virulence is still further enhanced.

The individual cells of a chain vary in size from 0.6 to 1.5 $\mu$  and in form from flattened disks to long ovals. The chains are variable in length and in general the more virulent types form longer chains in broth cultures. In old cultures the cells are very irregular in size, and it was once supposed that the larger spheres were special resistant forms, "arthrospores." They are now regarded as involution or disintegrating forms. The streptococcus stains readily and is Gram-positive.

Cultures on ordinary media are relatively poorly developed and of short life. Broth or glucose broth serves very well, and a few cultures in series may be obtained on glycerin agar or glucose agar. Löffler's blood serum is better than these. Serum agar, ascitic-fluid agar and blood agar are the best solid media and ascitic-fluid broth is an excellent fluid medium for cultivation of streptococci. Blood agar is especially valuable in plating pus or exudates because of the rather characteristic appearance of the small colony surrounded by a very clear zone of hemolysis which the streptococcus produces on this medium. In making cultures from the blood in bacteremia, plain agar previously melted and cooled to 45° C. is mixed with freshly drawn blood of the patient and allowed to solidify in a Petri dish. In other cases naturally sterile defibrinated rabbit's blood may be used, the technic of plating being analogous to that described for the gonococcus. The streptococcus grows very slowly below 20° C. and poorly in ordinary gelatin, which it does not liquefy. On solid media, agar or serum-agar, at 37° C., small round elevated colonies develop, 0.5 to 1.0 mm. in diameter, and they tend to remain discrete. In broth only a slight cloud develops, but considerable granular deposit made up of streptococci is found at

the bottom of the tube. Various carbohydrates are fermented with the production of acid and without formation of gas, but the behavior of streptococci toward these substances seems so variable that the attempts to utilize the fermentative power as a basis for classifying the streptococci has not led to wholly satisfactory results. The differences in fermentative power seem to depend more upon vigor of growth than upon essential qualitative differences between the streptococci tested.<sup>1</sup>

The streptococcus is relatively very resistant to heat, at times requiring one to two hours heating at 65° C. or one hour at 70° C. in order to insure sterility, according to V. Lingelsheim. Most investigators have found 60° C. for twenty minutes sufficient. Its poisons seem to be chiefly intracellular and set free upon disintegration of the organisms. Soluble poisons have nevertheless been found in some cultures.

Laboratory animals are not very susceptible to inoculation with streptococci. White mice and rabbits are most useful, and they ordinarily succumb to intraperitoneal injection of virulent strains.

The enormous importance of the streptococcus as a cause of sickness and death before the aseptic era is difficult to realize at the present time. Veritable epidemics of streptococcus infection in the surgical and obstetrical wards of hospitals made this one of the most dreaded of diseases. Even to-day the virulent streptococcus is held in great respect by many surgeons, and cases of erysipelas and other recognizable active streptococcus infections are commonly excluded from surgical wards.

In war wounds the streptococcus is a common and serious infectious agent. Its presence may be rapidly detected by inoculating the wound exudate into broth containing a bit of liver or other tissue.

Erysipelas is an acute febrile disease characterized by a local redness and edema of the skin which tends to spread to contiguous areas. In the lymph spaces beneath the epithelium there is a

<sup>1</sup> V. Lingelsheim in Kolle und Wassermann, Handbuch, 1912, Bd. IV, S. 462.

collection of leukocytes and serum, and the streptococci are also found here, especially at the periphery of the reddened area. In follicular tonsillitis and many cases of pseudo-membranous angina as well as in the pharyngitis of scarlet fever, streptococci occur in large numbers, and doubtless bear a causal relation to at least a part of the pathological process. In true diphtheria, streptococci seem to play rather frequently the rôle of important secondary invaders. From the pharynx the streptococcus may gain access to the middle ear and the mastoid cells, to the meninges, to the trachea, bronchi and lungs, setting up purulent inflammations in any of these locations. It is an important secondary invader in pulmonary tuberculosis. The streptococcus seems also to cause enteritis, particularly in infants. In the puerperium, streptococci are practically always present in the lochia. In spite of many attempts to differentiate between virulent and non-virulent types in this situation, it is still impossible to distinguish them. Probably local conditions in the uterus as well as the general condition of the patient have much to do in determining her resistance to infection of the uterine wall with these normal streptococci. Undoubtedly the frightful epidemics of puerperal fever in some hospitals previous to 1875 were due to the transference of virulent organisms from patient to patient by the attending physicians and nurses. This was first suggested by Holmes (1843) and more definitely proven by Semmelweiss (1861), but their ideas received little credence until the last quarter of the nineteenth century. Streptococcus bacteremia is commonly a terminal phenomenon, but it may occur without immediate fatal issue, and may result in endocarditis and streptococcus arthritis.

Immunity to streptococcus infection is slight in degree and very temporary. Koch showed that erysipelas could be repeatedly produced on the same area of the skin by inoculation at intervals of 10 to 12 days. Rabbits and horses acquire a high degree of immunity when treated with gradually increasing doses of many different strains of streptococci. The serum of such

animals has a marked protective influence when injected into animals and has been employed in treating human infections, in some cases with success, while in others the serum has apparently exerted no influence on the course of the disease. In localized chronic streptococcus infections, treatment with autogenous bacterial vaccines (bacteria suspended in salt solution and killed by heat) seems to produce favorable effects in some cases.

**Streptococcus Lacticus (Micrococcus Ovalis).**—This is a variety of streptococcus growing normally in the intestine and of special importance as the cause of the normal souring of milk.

The classification of the streptococci has been studied extensively in recent years. The fermentative effects upon blood and upon various sugars, especially lactose and mannite, are important criteria. The advanced student should consult the article of Blake<sup>1</sup> and the monograph of Brown,<sup>2</sup> both of which give references to the literature.

**Staphylococcus (Micrococcus) Aureus.**—By the early observers (Rindfleisch, Klebs) this organism was not distinguished from the streptococcus. Pasteur in 1880 obtained it in broth cultures from pus. Ogston in 1882 clearly distinguished it from the streptococcus. Rosenbach (1884) by his extensive investigations established the position of the staphylococcus as a cause of wound infection and of osteomyelitis.

Staphylococci have their natural habitat on the skin, in the mouth, in the nasal cavities and in the intestine, without the presence of inflammation. More virulent forms occur in infected wounds, furuncles, carbuncles, various localized purulent inflammations, bacteremia (staphylococcemia), endocarditis, osteomyelitis, meningitis and pneumonia.

The cell is spherical, 0.7 to 0.9 $\mu$  in diameter. Division takes place in various planes, giving rise to irregular bunches of cocci. The organism stains readily and is Gram-positive. Cultures

<sup>1</sup> Blake, F. G., *Journ. Med. Resch.*, 1917, 36, p. 99.

<sup>2</sup> Brown, J. H., *Monographs of the Rockefeller Institute for Medical Research*, No. 9, 1918.

are readily obtained on all the common media and growth occurs between 9° and 42°, best at 37° C. Broth is diffusely clouded with abundant sediment. In gelatin stab-culture, growth occurs all along the line of inoculation with funnel-shaped liquefaction (Figure 109). On agar slant the growth is confluent and yellowish after 24 hours. There is similar growth on Löffler's serum, often with liquefaction of the medium.



FIG. 109.—Gelatine culture *Staphylococcus aureus* one week old.

The staphylococcus is relatively resistant to heat and chemical germicides. It is killed at 62° C. in ten minutes and at 70° C. in five minutes. V. Lingelsheim<sup>1</sup> found it more resistant, requiring ten minutes at 80° C. and an hour at 70° C. to kill his strains, but his figures cannot be accepted without further confirmation.<sup>2</sup> It is about as resistant to chemical poisons as any of the sporeless bacteria, and is commonly employed as a test object in the investigation of germicides. Mercuric chloride 1-1000 requires three to five hours to kill staphylococcus cultures and much longer if the organisms are present in pus. Carbolic acid, 3 per cent, kills them in two to ten minutes.

The pigment is a lipochrome and is produced only in the presence of oxygen. The tryptic ferment diffuses out of the cells and is capable of liquefying gelatin, albumen and fibrin. The staphylococcus produces a soluble poison which kills leukocytes (leukocidin) and others which dissolve red blood cells (staphylolysin) and cause clumping of red blood cells (agglomerin). These substances are true soluble toxins and they are destroyed by heating to 80° C. Other soluble poisons seem also to be present. The bacterial cells killed by heat are only slightly toxic,

<sup>1</sup> Neisser: *Kolle und Wassermann, Handbuch*, 1912, Bd. IV, S. 361.

<sup>2</sup> Compare with similar tests on streptococci by v. Lingelsheim, p. 273.

yet, it is very probable that in the disintegration of the cocci in an inflammatory process more poisonous substances may be derived from their cell protein.

Rabbits are the animals of choice for inoculation with staphylococci. Intravenous injection with virulent cultures usually causes multiple abscesses in the internal organs with death in 4 to 8 days. Typical endocarditis has been produced by injected organisms from potato cultures, and with greater certainty when the heart valves are injured mechanically, especially in young rabbits. Osteomyelitis sometimes follows intravenous injection in growing rabbits, especially if the bone be slightly injured at the time of inoculation. In man, typical furuncles and carbuncles have been produced by rubbing pure cultures on the skin (Garrè, 1885) and by subcutaneous injection.

In man this organism is a frequent cause of local purulent inflammations, and it sometimes gives rise to pyemic abscesses and general bacteremia. Recurrent furuncles and carbuncles are ordinarily due to staphylococci.

Animals have been immunized to staphylococci but the serum obtained from them has relatively slight value in treatment. Specific treatment by means of dead bacterial cells, bacterial vaccines, has been developed by A. E. Wright and has proved its value in the treatment of chronic furunculosis. A suspension in salt solution of bacterial cells from an agar slant, sterilized by heating to 60-65° C. for 30 minutes and standardized by microscopic count of the bacterial cells, is employed. Doses from 50 million to 1000 million bacterial cells are injected two or three times a week for a long period of time, the size and frequency of dosage being governed by the clinical condition of the patient. Determination of the opsonic index is probably unnecessary and is now quite generally neglected. Autogenous vaccines (made with the staphylococcus isolated from the patient) are usually superior to stock vaccines.

**Staphylococcus Albus.**—This is quite similar to *Staphylococcus aureus* in all respects except pigment production. Usually,

but not always it is less virulent. *Staph. epidermidis* (Welch) is an avirulent variety of *Staph. albus*, very abundant on the normal skin. Many other varieties of staphylococci have been described.

**Micrococcus Tetragenus.**—This organism occurs in lung cavities in phthisis, and in the sputum, usually in groups of four cells, tetrads, enclosed in a transparent capsule. It is Gram-positive, grows on ordinary media and does not liquefy gelatin. White mice and guinea-pigs are susceptible and ordinarily die of general bacteremia in two to six days after inoculation. The pathogenic rôle of the organism in man is doubtful.

**Sarcina Ventriculi.**—Goodsir in 1842 observed sarcines in vomitus. The coccus is large,  $2.5\mu$  in diameter, and occurs in cubes of eight cells or as large conglomerates of these. It grows on ordinary media, usually producing a yellow pigment. It is found in the stomach in some conditions in which the acidity of the gastric juice is diminished. It is apparently non-pathogenic.

**Sarcina Aurantiaca.**—This is a common saprophytic coccus found in fermenting liquids and occasionally in the air. It grows well on ordinary media and liquefies gelatin. An orange pigment is produced. Typical packets are produced in liquid media, especially in hay infusions.

**Micrococcus (Planococcus) Agilis.**—This organism occurs in surface waters. It liquefies gelatin and produces a rose-red pigment on agar and potato. Its remarkable feature is the possession of a flagellum and active motility. It is Gram-positive.

## CHAPTER XVII

### BACILLACEÆ: THE SPOROGENIC AËROBES

The aërobic spore-forming bacilli are essentially inhabitants of the soil and the fermenting organic material likely to occur there. Along with a few species of this group we shall consider one pathogenic sporogenous bacterium, the anthrax bacillus, which resembles them very closely except in its virulence for animals and its lack of active motion, both of which may perhaps justly be regarded as variations from the group type due to its parasitic mode of life.

**Bacillus Mycoides.**—This organism is universally distributed in fertile soils and also occurs in surface waters and in the air. It is a large rod with rounded ends, usually growing in threads. Large median spores are formed without distorting the cell. It is motile but rather sluggish. Growth occurs on all ordinary media. In gelatin stab-culture, thread-like processes extend out on all sides from the line of puncture giving the appearance of an inverted pine tree. Later the gelatin becomes entirely liquefied. The organism is an important agent in the decomposition of plant residues in the soil. It is without pathogenic properties.

**Bacillus (Mesentericus) Vulgatus.**—This is another widely distributed soil bacterium. It is commonly called the potato bacillus. The cell is short and relatively thick with rounded ends, actively motile, often in pairs or threads. Large spherical median spores are produced without distortion of the cell. These spores are very resistant to heat and germicides, sometimes surviving the temperature of boiling water for several hours. *B. vulgatus* grows well on all ordinary media. Gelatin is liquefied. Milk is coagulated and then digested. On potato a wrinkled

membrane is produced, so characteristic that the name "mesentericus" was applied to this species. It is not pathogenic.

**Bacillus Subtilis.**—*Bacillus subtilis*, or the hay bacillus, is abundant in the soil and on the surface of plants, and common in surface waters and in the air. It is readily obtained by boiling hay in water and then setting the infusion aside for a few days. The cell is relatively large, about  $1.2\mu$  wide by  $5\mu$  long, with ends somewhat rounded. Long threads are commonly formed. It is motile with peritrichous flagella. Large oval median spores

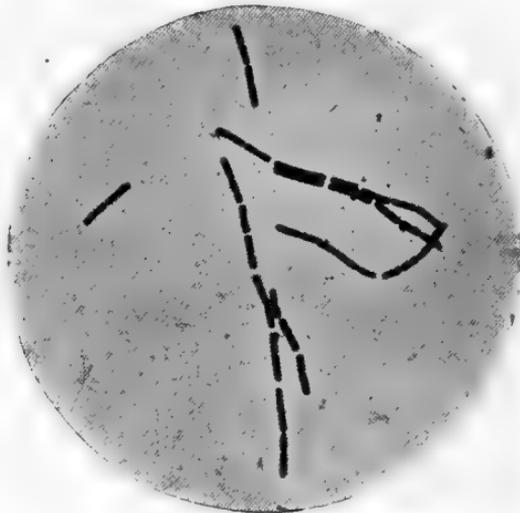


FIG. 110.—*Bacillus subtilis*.  $\times 1000$ .

are formed without distortion of the cell and these are almost as resistant as the spores of the potato bacillus. *B. subtilis* grows rapidly on ordinary media in the presence of air, best at about  $30^{\circ}$  C. Gelatin is liquefied and milk is digested. The organism is typically saprophytic, but it has been found growing in the intestine by some investigators, and has been found in a few instances in infections of the human eye, cases of panophthalmitis following injury.<sup>1</sup>

<sup>1</sup> Silberschmidt, *Annales de l'Institut Pasteur*, 1903, Vol. XVII, pp. 268-287; Also see Kneass and Sailer, *Univ. Penn. Med. Bull.*, June, 1903, Vol. XVI, pp. 131-133.

**Bacillus (Bacterium) Anthracis.**—Pollender in 1849 and Davaine and Rayer in 1850. observed thread-like bodies in the blood of animals dying of anthrax. Robert Koch in 1876 obtained pure cultures of the organism, using the aqueous humor of the ox's eye as culture medium. He saw the small rod-shaped bodies found in the anthrax blood elongate into threads in this medium, and observed the formation of the bright refractive bodies in these threads, which he correctly recognized as spores. Finally by inoculating healthy animals with his cultures he pro-

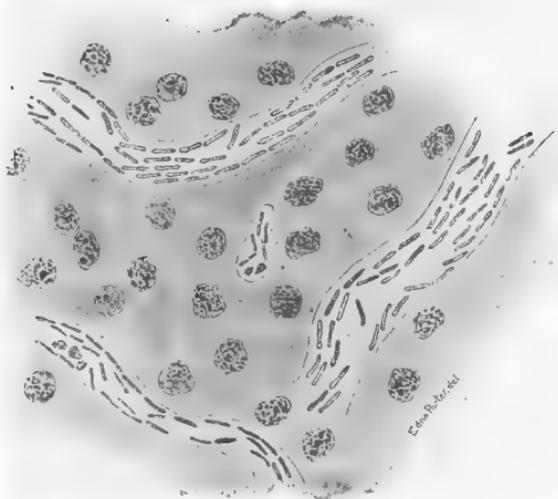


FIG. 111.—Anthrax bacilli in the capillaries of the liver of a mouse.

duced typical anthrax in them; thus proving conclusively for the first time the causal relation of a bacterium to a disease.

The anthrax bacillus occurs in the blood and throughout the tissues of animals suffering from anthrax, and in the excretions of such animals. Its spores occur on hides and in wool derived from anthrax animals. Furthermore, the soil of fields where anthrax animals have grazed harbors these organisms for many years. It seems probable that the bacilli multiply in the soil during the warm wet seasons and it is certain that the spores may lie dormant for as long as ten years in dry places.

The cell is about  $1.25\mu$  wide and 3 to  $10\mu$  long, with rounded ends when single, but in the threads the contiguous ends are

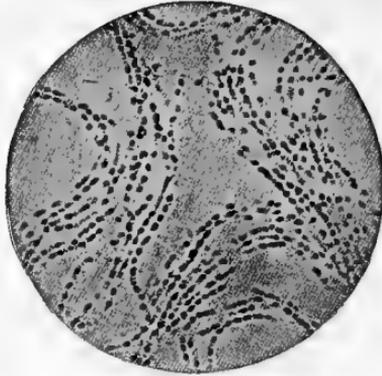


FIG. 112.—*Bact. anthracis*. Spore production. (From Marshall after Migula.)

square-cut. In the circulating blood the bacilli are single or in pairs and spores are never formed in the animal body (Fig. 111). In cultures long threads are produced and spores are usually

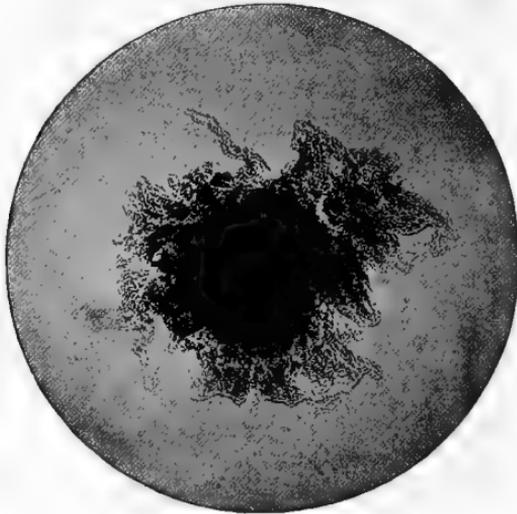


FIG. 113.—*Bact. anthracis*. Colony upon a gelatin plate.  $\times 100$ . (After Fraenkel and Pfeiffer.)

formed after 24 to 48 hours (Fig. 112). The anthrax bacillus is aërobic and grows readily on all ordinary media, best at  $37^{\circ}$  C.

Gelatin is slowly liquefied. The colony presents a very characteristic appearance, especially as it grows on gelatin, which is due to the large coils of long parallel threads, of which the colony is composed. The vegetative bacillus is rather easily killed but the spores may survive boiling in water for 5 minutes and in some instances as long as half an hour when afforded some mechanical protection. Chemical germicides cannot be relied upon to destroy the spores. Sterilization in the autoclave is the safest method of disposing of anthrax material.

Anthrax is a disease which occurs spontaneously in cattle and



FIG. 114.—*Bact. anthracis*. Showing the thread formation of colony. (After Kolle and Wassermann.)

sheep and rarely in horses, swine and in man. The disease is produced by inoculation in many other animals. Mice, guinea-pigs and rabbits are susceptible in the order named. The disease is common in European and Asiatic stock-raising districts and in Argentine Republic. Several local epizootics have occurred in the United States and a few cases of human anthrax. Experimental anthrax is readily produced in susceptible animals by subcutaneous inoculation, less certainly by feeding the spores. In the acute form the bacilli are found in large numbers everywhere in the blood, and this is the common picture in cattle, sheep, rabbits, guinea-pigs and mice. Chronic forms occur,

however, either because of lowered virulence of the germ or of increased resistance of the host, and in these cases the bacteria may be very scarce and difficult to find microscopically, even after death of the animal. Cultures from the spleen will usually show the presence of the bacillus there. The mechanism by which the bacillus causes death is unknown. In the acute cases, as in the mouse, the bacilli are so abundant in the blood that mechanical interference with the circulation seems a plausible explanation, but this certainly does not suffice for other types of the disease in which chemical poisoning must play the chief rôle. So far it has not been possible to demonstrate any powerful poisons in cultures of the anthrax bacillus. It is probable that the essential poisons are produced by a reaction between the substance of the bacillus and the fluids of the host, particularly the enzymes of the latter, which cause disintegration of the bacterial bodies.

The infection is acquired by grazing animals through the alimentary tract primarily, but also to some extent by inoculation (contact, flies, intermediate objects). In man there are three recognized types (*a*) malignant pustule, (*b*) pulmonary anthrax, and (*c*) intestinal anthrax. Malignant pustule results from inoculation of the skin, especially in those who handle hides or care for anthrax animals. It is at first a local pustular and necrotic lesion tending to involve contiguous tissue by extension, but soon invading the lymph vessels and walls of the veins. The bacteria thus gain the blood stream and a rapidly fatal general bacteremia supervenes. Recovery sometimes occurs before the disease becomes generalized. Several instances of malignant pustule of the face were observed in soldiers in 1918, the infection being derived from shaving brushes, in some of which anthrax bacilli were found. Pulmonary anthrax is caused by inhalation of anthrax spores (wool sorter's disease). Intestinal anthrax is uncommon in man but has occurred. Both are very fatal forms of the disease.

Immunity to anthrax was first successfully produced by Pas-

teur through vaccination with attenuated living cultures. Broth cultures inoculated with bacilli taken directly from the animal body were grown at 42° C. to 43° C. At this temperature spores are not produced and the bacillus gradually loses its virulence. When it will no longer kill guinea-pigs but will still kill mice the strain is again grown at 37° C. and injected into cattle and sheep as the first vaccine. Twelve days later a second vaccine is injected, which is a somewhat more virulent culture, still capable of killing guinea-pigs but not powerful enough to cause fatal infection of rabbits. As a result of these two treatments, nearly all animals become immune to the natural disease or to inoculation with fully virulent cultures. Sobernheim<sup>1</sup> and Sclavo<sup>2</sup> have induced a high degree of immunity in sheep and in asses by repeated injection of the bacilli, and have found the serum of such hyper-immune animals to be protective and curative upon injection into other animals. The injection of this serum along with a dose of living culture of about the strength of Pasteur's second vaccine has been employed in immunizing cattle and sheep. All the necessary treatment is thus given at one time. The serum has also been successfully employed in conjunction with the appropriate medical and surgical measures in the treatment of malignant pustule in man.<sup>3</sup>

<sup>1</sup> Sobernheim: *Zeitsch. f. Hyg.*, 1897, XXV, pp. 301-356; *Centralbl. f. Bakt.*, 1899, XXV, p. 840.

<sup>2</sup> Sclavo: *Centralbl. f. Bakt.*, 1899, XXVI, p. 425.

<sup>3</sup> For a discussion of treatment of human anthrax consult Boidin, Vignaud and Fortineau, *Presse Médicale*, Aug. 14, 1912; also Becker, *Munch. med. Wochenschr.*, Jan. 23, 1912.

## CHAPTER XVIII

### BACILLACEÆ: THE SPOROGENIC ANAËROBES

The bacteria of this group are hindered in their development by the presence of free oxygen and their artificial culture is ordinarily successful only when they are protected from oxygen, at least in the early stages of development. Like the sporogenic aërobes, they live in the soil, but they are associated here more especially with decomposing materials of animal origin, and are less frequently found in soils which have not received fertilizers from animal sources. There is good reason to believe that their essential habitat is the intestinal canal of animals, especially the mammals, and that their life in the soil does not represent the most active stage of their existence, but that they reach the soil with animal excreta and the bodies of dead animals and continue to live in the soil for a considerable period. For this group of bacteria the Committee of the Society of American Bacteriologists has suggested the generic name, *Clostridium* Prazmowski 1880.

**Clostridium Edematis (Vibrion septique).**—Pasteur in 1877 injected infusions of putrid flesh into laboratory animals and produced a fatal subcutaneous edema with penetration of the bacteria into the blood in some instances. The organism which he called “Vibrion septique” was found to be an obligate anaërobe, the first anaërobic organism ever recognized. Koch (1881) studied the organism in pure culture on solid media and named it *Bacillus edematis maligni*.

The bacillus is very widely distributed in soil and dust, and is very common in the feces of herbivorous animals. It is especially abundant in putrefying animal matter. The cell is about  $1\mu$  thick by 3 in length, although considerable variation in size and shape occurs. It is usually slightly motile and possesses

peritrichous flagella, stains readily, is only relatively Gram-positive, some of the cells being decolorized by prolonged treatment with alcohol. The spores are central, or intermediate in position, with bulging of the cell.

In cultures *Cl. edematis* is a strict anaërobe. It liquefies gelatin. Milk is slowly coagulated and the coagulum digested, the reaction remaining alkaline to litmus. The cultures have a foul odor. The spores withstand boiling sometimes for 2 to 3 hours. The morphological and physiological properties of this organism are quite variable and the many intermediate types between it and *B. feseri* makes distinction between the two species somewhat difficult.

In animals and man, malignant edema occurs spontaneously as a wound infection, but it is not very common. It has been observed most frequently in horses and in new-born calves. The guinea-pig is susceptible. In general a mere injection of the bacilli fails to produce serious disease. The presence of foreign bodies or extensive tissue destruction favors the infection.

In war wounds the Vibriion septique is an important cause of gaseous edema, usually in association with other anaërobic bacteria.

**Clostridium Feseri.**—Feser and Bollinger (1875–1878) observed the large narrow rods in the diseased tissues and exudates of symptomatic anthrax or black leg, a fatal disease of cattle and sheep. Man is not affected. Arloing, Cornevin and Thomas (1884) obtained the organism in culture. The organism is a strict anaërobe and resembles *B. edematis* very closely. Black leg is a local emphysematous inflammation usually beginning in one leg of cattle or sheep, rapidly extending and resulting in death as a rule. Immunity is obtained by injecting small doses of the virulent bacteria or by injecting attenuated organisms, and also by injecting the virus together with an immune serum.<sup>1</sup>

**Clostridium Perfringens (Bacillus Welchii).**—Welch and Nuttall in 1892 discovered this organism at autopsy in a body

<sup>1</sup> Kitt, Kolle and Wassermann, Handbuch, 1912, Bd. IV, S. 819–836.



FIG. 115.—*Cl. perfringens* in agar culture, showing gas formation.

showing general emphysema of the tissues and gas bubbles in the blood-vessels. They obtained cultures by anaërobic methods and caused similar *post-mortem* emphysema in the bodies of rabbits. The organism lives and multiplies in the intestine of man and other mammals, is widely distributed in the soil and is commonly present in milk and other animal food products. The cell is a large rod surrounded by a capsule when grown on media rich in protein or in the animal body. The width of the cell (without capsule) varies<sup>1</sup> from 1.1 to 1.7 $\mu$  with a mean of 1.3 $\mu$  and the length from 2.6 to 7.6 $\mu$ , with an average of 4.6 $\mu$ , the measurements being made on organisms grown in an agar stab-culture 24 hours at 37° C. When grown in blood broth the germ is capsulated and the measurements, including the capsule are as follows: width 1.9 to 2.5 $\mu$  with average of 2.1 $\mu$  and length, 2.8 to 6.6 $\mu$  with average of 4.7 $\mu$ . Usually the organism is non-motile, but flagella can sometimes be demonstrated. In the intestine and in protein media the organism forms spores, usually median without bulging of the cell, but these are not commonly observed

<sup>1</sup> The measurements are taken from Kerr, The *Bacillus welchii*, Thesis, Univ. of Illinois, 1909.

in cultures. The organism is a strict anaërobo. Its most striking property is the enormously rapid production of gas in media containing dextrose or lactose. Cultures are obtained most readily by heating a suspension of feces to 80° C. for 15 minutes and inoculating it into glucose broth mixed with blood in a Smith fermentation tube. After 24 to 48 hours incubation its presence will usually be revealed by abundant production of gas. Milk is coagulated and rendered acid with an abundant production of gas (stormy fermentation). On blood-agar plates incubated in hydrogen, the colony is round with regular outline and surrounded by a clear zone of hemolysis.

Emphysematous gangrene occurs in man as a rapidly extending, very fatal disease, due to the infection of wounds with this organism. The presence of necrotic tissue seems to be necessary in order that the organism may gain a foothold, but when once begun the inflammation may extend with great rapidity. The gas found in bodies at autopsy is usually the result of an agonal or a *post-mortem* invasion by the bacilli from the intestine.

There are several other types of sporogenic anaërobes of the same general nature as *Cl. edematis*, *Cl. fesceri*, and *Cl. welchii*, which live normally in fertilized soil and in the intestines of animals. The organisms of this group have assumed great importance in modern warfare as the causes of anaërobic wound infection, variously termed gaseous gangrene, gaseous edema or toxic edema. The development of the disease depends to some extent upon the presence of foreign bodies or devitalized or partly disorganized tissue in a wound. It may appear early and run a rapid course to death. Such cases are usually infected with *Cl. perfringens*. Late, more slowly progressing gaseous edema is often due to a mixture of bacteria, including the *Vibrion septique* or *Cl. œdematiens* of Weinberg and Seguin or both of these. The monograph<sup>1</sup> of these authors should be consulted by those students who are interested in the anaërobic infection of wounds. These authors have made substantial progress in the production of

<sup>1</sup> Weinberg et Seguin: La gangrene gaseuse, Masson et Cie, Paris, 1918.

anti-sera for the prophylaxis and treatment of these wound infections.

**Clostridium Tetani.**—Tetanus has been recognized as a complication of wounds since the time of Hippocrates. Forscher, Carle and Rattone, in 1884, first proved it to be inoculable by injecting pus from a human case into 12 rabbits, of which 11 died of tetanus. Nicolaier in 1884 produced tetanus by injecting soil into mice, guinea-pigs and rabbits, and found a slender bacillus in the animals at the point of inoculation. He was able to propagate the bacillus in mixed culture on coagulated sheep's serum. Kitasato obtained the first pure cultures by subjecting the mixed culture to a temperature of 80° C. for an hour, inoculating agar plates and incubating them in an atmosphere of hydrogen. With his pure cultures, he caused typical tetanus in animals.

The organism occurs in the soil which has received animal fertilizers and in the intestine of herbivorous mammals. The bacterial cell is 0.3 to 0.5 $\mu$  wide and 2 to 4 $\mu$  long, single in young cultures, but often joined end to end to form long threads in older cultures. It is motile and possesses abundant peritrichous flagella. The spore is very characteristic. It is usually spherical, 1 to 1.5 $\mu$  in diameter, situated at the extremity of the cell, giving it the appearance of a drumstick. The bacillus stains readily and is Gram-positive.

Isolation of *Cl. tetani* from mixed material or from wounds known to contain it is not always easy. The material should be planted in glucose broth and incubated in hydrogen at 37° C. for 2 to 3 days. Microscopic examination of the sediment may then reveal the drumsticks. Kitasato's procedure should then be followed, employing agar distinctly alkaline to litmus and containing 2 per cent of glucose. If many other spore-forming bacteria are present in the mixture, special procedures are necessary, such as preliminary culture for 8 days at 37° C. in a deep stab in coagulated rabbit's blood with subsequent heating to 80° C. to get rid of *Cl. edematis*, or culture for 8 days at 37° C. in milk with subsequent heating to get rid of *Cl. perfringens*. Aërobic

spore-formers may be eliminated by successive transfers in animals.

The spores of *Cl. tetani* resist the temperature of boiling water for 5 to 30 minutes. Biological products to be introduced into the human body need to be sterilized in the autoclave or else carefully examined by anaërobic culture methods to insure their freedom from tetanus spores. The danger of infection from this source has been emphasized by Smith.<sup>1</sup>

The colony in glucose gelatin or glucose agar consists of a compact center with slender, radiating, straight or irregularly curved threads about the periphery. Liquefaction of gelatin becomes evident in stab-culture after about two weeks at 20° C. Milk is sometimes but not always coagulated and the casein is eventually digested.

The cultures of the tetanus bacillus are extremely poisonous, especially so when they are developed under very strict anaërobic conditions. A nerve poison, tetanospasmin, and a hemolytic poison, tetanolysin, are present. The former is the more important constituent of the tetanus toxin. Neutral or slightly alkaline plain nutrient broth, incubated in an atmosphere of hydrogen for ten days after inoculation gives the most powerful toxin. The bacteria-free fluid from such a culture has been found to kill a mouse of 10-grams weight in a dose of 0.000 005 c.c. The toxin is unstable in solution but very stable when dried. Dry material of which 0.000 000 1 gram is the fatal dose for a mouse is readily obtained. The watery solution loses its toxicity when heated to 60° C. for 20 minutes, but when dry the toxin withstands heating at 120° C. for an hour.

Tetanus presents essentially the same picture in inoculated animals as in the natural disease, which is indeed, as a general rule, merely an accidental inoculation. The presence of insoluble material and of other bacteria mixed with them in a wound favors the development of tetanus bacilli. The tetanus bacilli always remain localized near the point of inoculation and may be hard

<sup>1</sup> *Journ. A. M. A.*, Mar. 21, 1908, Vol. L., pp. 929-934.

to find. The poison produced by the organisms is probably absorbed by the nerve endings<sup>1</sup> and transmitted to the central nervous system through the axis cylinders or in the perineural lymph spaces of the motor neurones rather than through the blood stream. The symptoms arise after the poison reaches the central nervous system in sufficient concentration to stimulate the nerve cells. In guinea-pigs and mice the spasm always begins near the point of inoculation, but in man and the large mammals it often begins in the muscles of the jaw and neck regardless of the location of the wound. Wassermann and Takaki have shown that 0.1

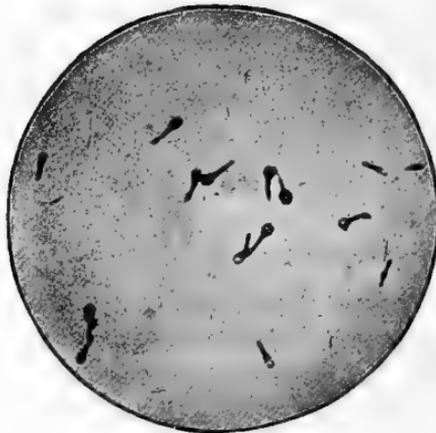


FIG. 116.—Tetanus bacilli showing terminal spores. (After Kolle and Wassermann.)

gram of brain substance suspended in salt solution is able to neutralize 10 fatal doses of tetanus toxin, forming a loose combination from which the toxin may be set free by drying. Most mammals are very susceptible, although cats and dogs are only slightly so. Birds are relatively resistant and some reptiles are wholly refractory to the tetanus toxin.

Von Behring and Kitasato in 1890 produced immunity in rabbits, and later in horses, by injecting into them toxin to which iodine trichloride had been added, and subsequently unaltered toxin. The immunized animal was able to survive an injection

<sup>1</sup> Von Lingelsheim, Kolle and Wassermann, Handbuch, 1912, Bd. IV, S. 766.

many times greater than the amount necessary to kill a normal animal. Moreover, the cell-free blood serum of the immunized animal was found to neutralize the poison in a test-tube and to protect a normal animal against fatal doses of it. The new substance of the blood capable of rendering the toxin harmless was called antitoxin. One antitoxic unit of tetanus antitoxin, according to Von Behring is the amount which will neutralize 40 million times the amount of fresh tetanus toxin necessary to kill a mouse weighing 15 grams ( $40 \text{ million} \times \text{the } 15 + \text{Ms dose}$ ) so completely that only a slight local contraction, indicated by a folding of the skin, results from subcutaneous injection of the mixture into a mouse (the  $L_0$  effect). This amount of toxin ( $40 \text{ million} \times \text{the } 15 + \text{Ms dose}$ ) is generally measured in practice against a standard antitoxin and is designated as a toxic unit. The toxin is preserved in a dry state. To test a new antitoxin one employs  $\frac{1}{1000}$  of a

toxic unit ( $40,000 \times \text{the } 15 + \text{Ms dose}$ ) and ascertains the amount of serum which must be added so as to neutralize it to the  $L_0$  end point. Each trial mixture is diluted to 1 c.c. with salt solution and 0.25 c.c. per 10 grams of body weight is injected into a mouse. When the typical  $L_0$  effect is produced in the mouse, the amount of antitoxic serum employed in the preparation of this particular mixture is said to represent  $\frac{1}{1000}$  antitoxic unit. Ordinarily the mixture of toxin and anti-

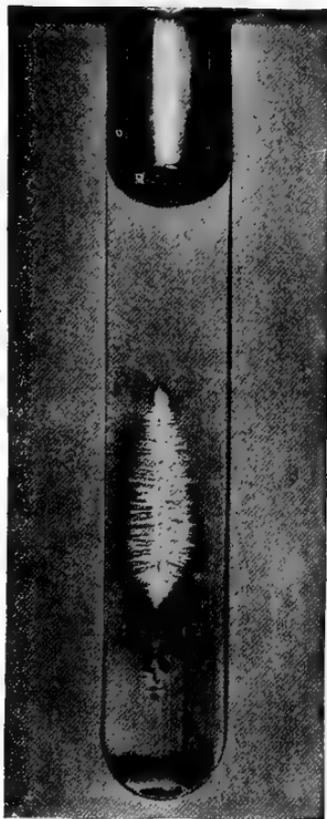


FIG. 117.—*Clostridium tetani*. Stab culture in glucose gelatin, six days old. (From McFarland after Fraenkel and Pfeiffer.)

toxin is allowed to stand 30 minutes before injection. Comparable results are obtained only by following a definite procedure and it is especially necessary to use the conventional dose of  $\frac{1}{1000}$  antitoxic unit and  $\frac{1}{1000}$  toxic unit in the standardization of sera.

The standard unit employed in the United States is somewhat different from the Von Behring antitoxic unit. The American immunity unit of tetanus antitoxin is ten times the least amount of antitetanic serum necessary to preserve the life of a



FIG. 118.—*Clostridium botulinum*. Some individuals containing spores. (After van Ermengem.)

guinea-pig weighing 350 grams for 96 hours against the official test dose of standard tetanus toxin furnished by the Hygienic Laboratory of the U. S. Public Health Service.<sup>1</sup> Tetanus antitoxin deteriorates with moderate rapidity. The reaction between tetanus toxin and antitoxin seems to take place in two stages, first a reversible absorption and following this a specific chemical union.

<sup>1</sup> Rosenau and Anderson: U. S. Hygienic Laboratory, *Bulletin* No. 43, 1908, p. 59. The official test dose of toxin is 100 times the amount of a dry tetanus toxin required to kill a 350 gram guinea-pig in four days.

Tetanus antitoxin seems to be a reliable preventive of tetanus if given soon after the wound is inflicted in a dose of 20 antitoxic units (German) or 1500 immunity units (U. S. Standard). After symptoms of tetanus have appeared, antitoxin is of less use. At this time the poison is present not only in the vicinity of the wound and in the blood but also in the peripheral nerves and in the central nervous system. The toxin in the last two situations is only slightly or not at all influenced by subcutaneous injection of antitoxin. That in the peripheral nerves may be reached by intraneural injection, and in subacute or chronic cases recovery may sometimes take place. Acute cases in which symptoms appear in a few days after infliction of the wound offer no hope. Prophylactic use of tetanus antitoxin in all punctured and lacerated wounds, especially those caused by gunpowder (Fourth of July) is an essential feature of the effective treatment for tetanus. Surgical cleansing and antiseptic open treatment of such wounds is to be recommended.<sup>1</sup>

Late tetanus may appear even after the antitoxin has been given early, but in such cases the disease is usually milder in character and may be successfully treated by further antitoxin and surgical attention to the wound.

**Clostridium Botulinum.**—Van Ermengem in 1895 discovered the spores of this organism in the intermuscular connective tissue of a ham which had given rise to 30 cases of food poisoning with 3 deaths. Other anaërobic as well as aërobic bacteria were also present in the meat. Its natural habitat is unknown but it seems to occur in the feces of swine. The bacillus 0.9 to 1.2 $\mu$  wide by 4 to 6 $\mu$  long and occurs single or in pairs. It is slightly motile and has 4 to 8 peritrichous flagella. It is Gram-positive. The spores are oval and usually nearer one end of the cell. They are quite variable in resistance. Van Ermengem found that they were killed at 80° C. in 30 minutes and by boiling for 5 minutes. More recent careful tests by Burke<sup>2</sup> have shown that the spores may

<sup>1</sup> Editorial, *Jour. A. M. A.*, 1909, Vol. LIII, p. 955.

<sup>2</sup> Burke, *Journ. Amer. Med. Assn.*, 1919, 72, p. 88.

sometimes resist boiling in water for 2 hours and autoclave heat at 5 pounds pressure for 10 minutes.

Strict anaërobiosis is necessary for successful culture, except when *B. botulinum* grows in symbiosis with aërobes. Growth is best at 25–30° C., very slight at 37°–38.5° C., and best in a medium slightly alkaline to litmus. Gelatin is quickly liquefied and abundant gas is produced in glucose media. The organism appears to be incapable of growth in the animal body. Cultures are very poisonous when injected into or fed to animals.

The poison "Botulin" resembles in some of its properties the tetanus toxin. It is destroyed rapidly at 70°–80° C., and preserves its toxicity for years when dried. It is neutralized by mixing with brain substance. It differs from the other powerful toxins, however, in its ability to resist the gastric juice and to poison by absorption through the alimentary canal. Forssman has immunized guinea-pigs, rabbits and goats, and has obtained an antitoxic serum from these animals.

Botulism is a form of food poisoning definitely recognized as such as early as 1820. The symptoms are very characteristic, appearing in 18 to 48 hours after ingestion of the poisonous food. There is vomiting, dryness of the mouth and constipation, motor paralysis, especially early in the external ocular muscles. The involvement of the central nervous system may progress to complete motor paralysis and death. The mind is usually clear even in the fatal cases. The early outbreaks of the disease followed the consumption of sausage, hams, fish and other cured or preserved meats. More recently outbreaks of botulism have been recognized in the United States with increasing frequency and their causation has been traced not only to meat foods but to various canned vegetables and even fruits. Dickson<sup>1</sup> and his associates have shown that commercial canners processes as well as home canning methods cannot be relied upon to kill the spores of *Cl. botulinum* if this organism happens to be present in the raw

<sup>1</sup> Dickson, Burke and Ward: Archives of Internal Med., 1919, 24, p. 581. References to literature are given in this paper.

material. All canned food, which shows the slightest evidence of spoilage, should either be discarded or else boiled before it is eaten, as botulin is destroyed by boiling.

Forage poisoning in domestic animals has been shown to be due to *Cl. botulinum* by Graham<sup>1</sup> and his associates.

<sup>1</sup> Graham and Brueckner: Studies in forage poisoning, *Journ. of Bact.*, 1919, 4 p. 1. References to literature are given in this paper.

## CHAPTER XIX

### MYCOBACTERIACEÆ: THE BACILLUS OF DIPHTHERIA AND OTHER SPECIFIC BACILLI PARASITIC ON SUPERFICIAL MUCOUS MEMBRANES

**Bacillus (Corynebacterium) Diphtheriæ.**—Klebs in 1883 discovered this organism in the microscopic study of pseudomembranes from fatal cases of epidemic diphtheria. Löffler in 1884 obtained pure cultures of the bacillus and by inoculating the abraded mucous membrane of susceptible animals with his cultures, he produced local lesions similar to those observed in human diphtheria, in some instances followed by death or paralysis.

*B. diphtheriæ* occurs in the exudate (false membrane) which occurs in the pharynx, larynx and adjacent mucous membranes in epidemic diphtheria, on the mucous membranes of those who have recovered from the disease and, much less commonly, on the mucous membranes of healthy throats. It is a rod-shaped organism extremely variable in size, shape and staining properties. The width is ordinarily between  $0.3$  and  $0.8\mu$  and the length varies from  $1$  to  $6\mu$ . The cell is straight or slightly curved and very frequently of uneven diameter, with swelling at one end or in the middle portion. The cell contents stain unevenly in many of the cells. Many different morphological types are thus presented which may be designated roughly as regular cylinders, clubs, spindles and wedges according to form, and as uniformly pale, uniformly dark, regularly or irregularly banded or granular according to internal structure of the stained cell. These variations in form and internal structure are best seen after staining the bacillus with Löffler's methylene blue and are especially valuable in the quick recognition of *B. diphtheriæ* as it grows in the diphtheritic membrane or in culture on Löffler's blood serum.

On other media, such as glycerin agar, the morphological irregularities are less marked as a rule. The organism in young cultures

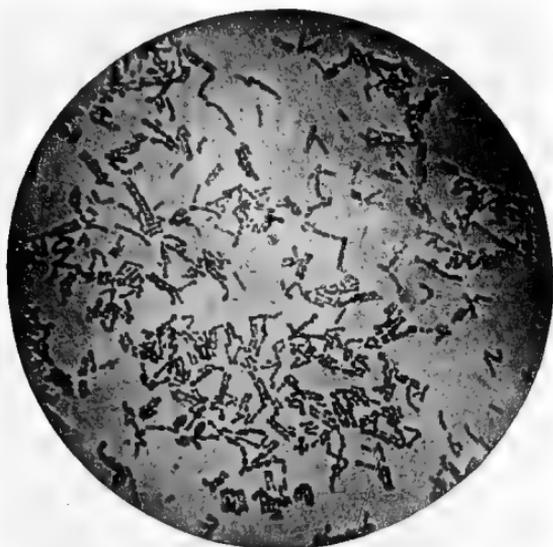


FIG. 119.—Bacillus of diphtheria.  $\times 1000$ .

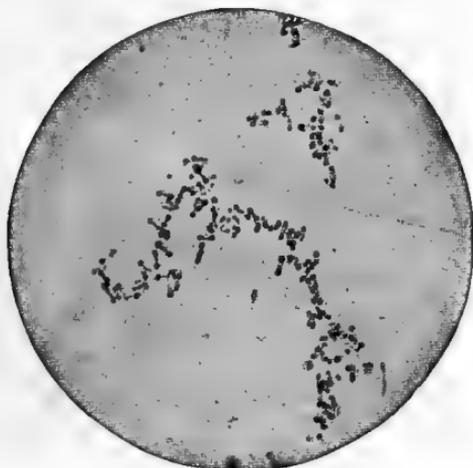


FIG. 120.—*B. diphtheria* stained by Neisser's method.

stains readily, best perhaps with Löffler's methylene blue in the cold. It is Gram-positive. Old cultures stain with great difficulty.

Löffler's blood serum is the medium of choice. The colonies develop at 37° C. in 8 to 12 hours as grayish, slightly elevated points and become 2 to 3 mm. in diameter in the course of 48

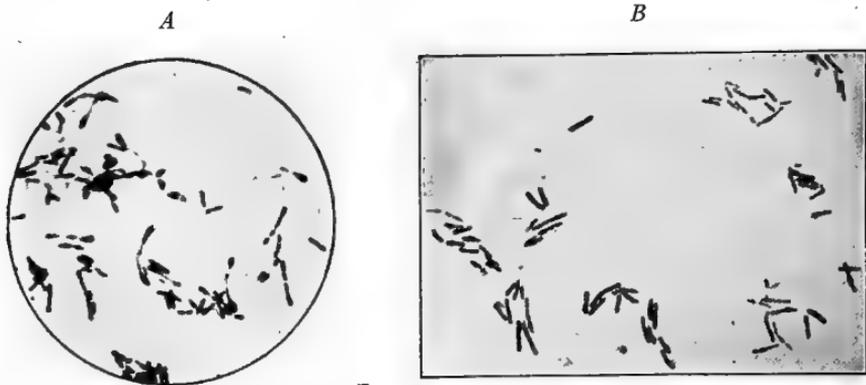


FIG. 121.—Forms of *B. diphtheriae* in cultures on Löffler's serum. A, Characteristic clubbed and irregular shapes with irregular staining of the cell contents.  $\times 1100$ . B, Irregular shapes with even staining.  $\times 1000$ . (After Park and Williams.)

hours. Contiguous colonies become confluent. On glycerin agar after 25 hours at 37° C., the colony is coarsely granular with somewhat jagged outline. Many variations from this typical

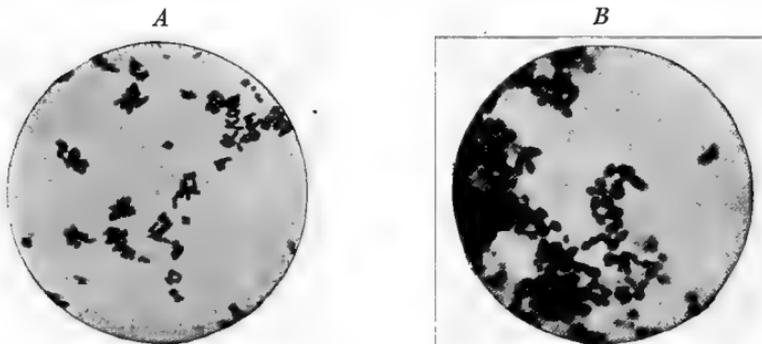


FIG. 122.—Forms of *B. diphtheriae* in cultures on agar. A, Bacilli small and uniform.  $\times 1000$ . B, Spherical forms in culture 24 hours old. On Löffler's serum this same organism produced granular forms.  $\times 1410$ . (After Park and Williams.)

appearance occur. Growth in gelatin is slow and ceases below 20° C. The medium is not liquefied. The bacillus grows in milk without producing coagulation. In broth the growth

may occur as a granular sediment, as a diffuse cloudiness or as a pellicle on the surface, depending upon the reaction and pepton content of the medium and the vigor of growth of the culture. The growth on the surface produces the best yield of toxin. Acid is produced in dextrose broth. The organism is killed when moist by heating to 60° C. for 20 minutes. It is fairly resistant to drying and has been found alive in bits of dry diphtheritic membrane after four months.

Roux and Yersin in 1888 filtered broth cultures of the diphtheria bacillus through porcelain filters and found the filtrate

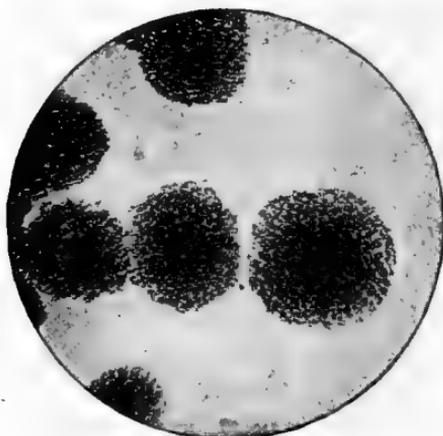


FIG. 123.—Colonies of *B. diphtheria* on agar.  $\times 200$ . (After Park and Williams.)

extremely poisonous. By injecting it into animals they were able to produce the signs of local and general intoxication which are observed in the natural disease. A favorable medium for toxin production is a veal broth containing 2 per cent pepton and having a titre of 9 c.c.<sup>1</sup> of normal sodium hydroxide above the neutral point to litmus. It should be placed in flasks in a thin layer to allow abundant air supply. Incubation for from 5 to 10 days gives the maximum toxicity. The filtrate from such a culture may kill a 250 gram guinea-pig in a dose of 0.002 c.c. Less powerful toxin is frequently obtained, so that sometimes

<sup>1</sup> Per 1000 c.c. of the medium.

even 0.5 c.c. or more may be required to kill a guinea-pig, and some strains of bacilli morphologically indistinguishable from *B. diphtheriæ* seem to produce no toxin at all. The toxin is



FIG. 124.—*B. diphtheriæ*, culture on glycerine agar.

quickly destroyed by boiling and loses 95 per cent of its strength in five minutes at 75° C. It gradually deteriorates even at low temperatures. Its chemical nature is unknown. Ehrlich has shown that old toxin which has lost much of its poisonous property is still able to combine with as much antitoxin as before. This deteriorated toxin is called toxoid. He explains the phenomenon by assuming the existence of two distinct chemical groups in the toxin molecule, one serving to combine with antitoxin and being relatively stable, the other bearing the poisonous properties and readily undergoing disintegration. The former he has called the haptophorous group and the latter the toxophorous group. In toxoid the toxophorous group has degenerated.

Diphtheria was recognized as a distinct disease by Bretonneau in 1821. It is characterized by a local inflammation, usually on the mucous membrane of the throat, the nose, more rarely the genital mucous

membrane, or the surface of a wound, and by an accompanying general intoxication giving rise to focal necrosis in various parenchymatous organs and affecting more particularly the heart and the nervous system. The local inflammation may

be only a mild reddening or it may be a widespread area of necrosis. Most frequently there is an exudate of plasma containing leukocytes, epithelial cells and bacteria, and this coagulates on the mucous surface. The epithelium underneath also undergoes necrosis in moderately severe cases and is firmly attached to the exudate by the fibrin threads. In severer forms there is an escape of blood into the exudate giving it a dark color. The local lesion is largely due to soluble toxin formed by the bacilli. The general disturbance is, as a rule, due solely to the absorbed toxin. The bacilli remain at the site of the lesion and do not appear in the blood or internal organs in any appreciable numbers. They are occasionally found in the spleen or kidney of fatal cases, but not more frequently than the streptococcus is found in these organs in apparently uncomplicated fatal cases of diphtheria.

The local lesion in the throat may be simulated very closely by inflammation due to streptococci, but the general manifestations are not duplicated in such conditions. Mixed infection with diphtheria bacilli and virulent streptococci may present a clinical picture of great severity. Bacteriological examination is often a great help in diagnosis even to the expert clinician, and is quite generally employed.

*Bacteriological Diagnosis of Diphtheria.*—In many large cities the bacteriological diagnosis of diphtheria is undertaken by boards of health. The methods used differ somewhat in detail, but are similar in the main, and are based upon the procedure devised by Biggs and Park for the Board of Health of New York City. Two tubes are furnished in a box. The tubes are like ordinary test-tubes, about three inches in length, rather heavy and without a flange. Both are plugged with cotton. One contains slanted and sterilized Löffler's blood-serum mixture (Fig. 125);

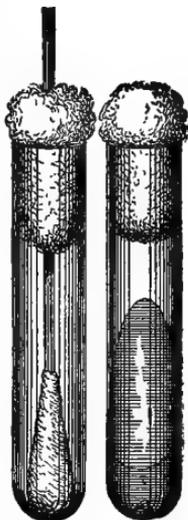


FIG. 125.—Swab and culture-tube used in the diagnosis of diphtheria. The pledget of cotton on the wire shown is much too bulky.

the other contains a steel rod, around the lower end of which a pledget of absorbent cotton has been wound. These tubes containing the swabs are sterilized. The swab is wiped over the suspected region in the throat, taking care that it touches nothing else, and is then rubbed over the surface of the blood-serum mixture. The swab is returned to its test-tube and the cotton plugs are returned to their respective tubes. The plugs, of course, are held in the fingers during the operation, and care must be taken that the portion of the plug that goes into the tube touches neither the finger nor any other object. The principles, in fact, are the same as those laid down in general for the inoculation of culture-tubes with bacteria (see page 111). In board-of-health work these tubes are returned to the office. When it is desirable, a second tube may be inoculated from the swab. The tubes are placed in the incubator, where they remain for from 6 to 15 hours and a microscopic examination is then made of smear preparations stained with Löffler's methylene blue. After use the tubes and swabs should be most carefully and thoroughly sterilized.

On Löffler's blood-serum kept in the incubator the bacillus of diphtheria grows more rapidly than most other organisms which are ordinarily encountered in the throat, a property which to a certain extent sifts it out, as it were, from them, and makes its recognition with the microscope easy in most cases. The appearance of the bacilli under the microscope is quite characteristic. The diagnosis of the diphtheria bacillus in practice is made from the character of the growth upon the blood-serum and the microscopical examination, taking into account the size and shape of the bacilli, with the frequent occurrence of irregular forms and the peculiar irregularities in staining, and this usually suffices; but in doubtful cases a second culture should be made from the throat, and at the same time another tube of Löffler's serum should be inoculated from the first culture. On the next day plate cultures on glycerin agar may be made, from which typical colonies should be transplanted to broth. After 48 hours

at 37° C. the broth is injected into two guinea-pigs in doses of 0.5 c.c. and one of the guinea-pigs should receive at the same time diphtheria antitoxin. In this way *virulent* diphtheria bacilli may be accurately detected.

The very large number of examinations that have been made by various boards of health have shown that the diphtheria bacillus may persist in the throat for a long time—occasionally several weeks after the patient has apparently recovered; also that diphtheria bacilli are occasionally found in the throat, when there is an inflammatory condition without any pseudo-membrane and that they not only appear in an apparently healthy throat, especially in hospital nurses and in children who have been associated with cases of diphtheria, but also in those who have had no traceable contact with diphtheria cases.<sup>1</sup> It has been found that bacilli sometimes occur in the throat, which have all the morphological and cultural properties of the diphtheria bacillus, but which are devoid of virulence when tested upon animals. Such diphtheria bacilli have frequently been called *pseudodiphtheria bacilli*. A bacillus closely resembling the diphtheria bacillus, but without virulence, has been found in xerosis of the conjunctiva. It is called the *xerosis bacillus*. If not a transformed diphtheria bacillus, it is at least closely related. The diphtheria bacillus is subject to wide variations in morphology, so that, in dealing with unknown cultures where the forms are not characteristic and injection into animals is without result, it may be difficult to decide whether or not the organisms are diphtheria bacilli.

The disease is undoubtedly transmitted very largely by immediate contact, especially with persons harboring the bacilli but not seriously ill, and by fomites. Children in school or at play readily transfer secretions of the mouth, and a cough or sneeze may distribute such material over a wide area.

Immunity to diphtheria was produced by Von Behring in 1890 by injecting the toxin into animals, the general method of procedure being quite similar to that followed in the production

<sup>1</sup> Sholly: *Journ. Infect. Dis.*, Vol. IV, 1907, pp. 337-346.

of tetanus antitoxin. The blood serum of the immunized animal was found to be capable of neutralizing the poisonous property of diphtheria toxin. The brilliant success of Roux (1884) in treating diphtheria with antitoxic serum caused the rapid adoption of antitoxin as a therapeutic agent throughout the world. Park and his co-workers, Atkinson, Gibson and Banzhaf, have developed a method of concentrating diphtheria antitoxin which is now generally employed.

For the production of antitoxin<sup>1</sup> young healthy horses are selected with great care. They are specifically tested for tuberculosis and glanders. A powerful diphtheria toxin is then injected into the horses, in an amount of sufficient to kill 5000 guinea-pigs, together with 10,000 units of antitoxic serum. The toxin is subsequently injected at intervals of three days and each succeeding dose is increased by about 20 per cent as long as the condition of the horse is satisfactory. At the end of two months the dose is about fifty times as large as the initial dose. Antitoxin is given only at the start. The serum of the horse is tested from time to time and, when the desired antitoxic strength has developed, the blood is drawn once a week for the preparation of antitoxin. A dose of toxin is given after each weekly bleeding. The blood is drawn from the jugular vein into jars containing a 10 per cent solution of sodium citrate, nine parts of blood to one of the citrate solution. The material is mixed and allowed to sediment in a refrigerator. The plasma is then siphoned off into large bottles and heated to 57° C. for 18 hours to change part of the soluble globulins<sup>2</sup> to euglobulins, insoluble in a saturated solution of sodium chloride. An equal volume of saturated aqueous solution of ammonium sulphate is then added. The precipitate which forms consists of the globulins and nucleoproteins of the plasma. This precipitate is collected on a filter

<sup>1</sup> For details of the method see Park and Williams, *Pathogenic Bacteria and Protozoa*, Phila.

<sup>2</sup> Banzhaf: *The Preparation of Antitoxin; Johns Hopkins Hosp. Bull.*, 1911, Vol. XXII, pp. 106-109.

and extracted with a saturated solution of sodium chloride, in which the pseudoglobulin fraction, carrying with it the antitoxic property, is dissolved. This is precipitated by the addition of dilute acetic acid, filtered out and again taken up in salt solution. It is carefully neutralized with sodium carbonate and dialyzed for several hours against water to remove the inorganic salts. - The residue in the dialyzer is then passed through a Berkefeld filter to sterilize it, a preservative is added, and it is ready to be tested and put up in containers for distribution. The final product contains 75 to 90 per cent of the original antitoxic strength and is only about one-third as bulky. The serum albumin, euglobulin and nucleoprotein have also been to a large extent eliminated in the process of concentration.

The antitoxic strength of anti-diphtheritic serum is expressed in immunity units and is ascertained by animal experimentation. The von Behring unit is contained in ten times the amount of serum required to protect a 250 gram guinea-pig perfectly from the effects of ten times the dose of fresh diphtheria toxin which kills a similar guinea-pig in four days. The dose of toxin is first ascertained by trial on guinea-pigs and the dose necessary to kill in four days (minimum lethal dose) determined. Ten times this quantity is then injected along with varying doses of antitoxic serum into a series of guinea-pigs until the quantity of serum, which not only saves the animal but prevents loss of weight and local induration at the site of injection, has been ascertained. Ten times this amount contains one immunity unit.

Ehrlich has carefully standardized an antitoxic serum and has preserved it as a dry powder, of which one gram contains 1700 immunity units. This standard is now employed as the official standard for comparison in the United States. In standardizing an antitoxin by the Ehrlich method, one unit of the standard antitoxin is injected along with various quantities of a toxin to ascertain how much of the latter is required so that the animal dies after four days. This dose of toxin, which when combined with one unit of the standard antitoxin, kills a 250

gram guinea-pig in four days is called the  $L_+$  dose. One next injects this  $L_+$  dose along with varying quantities of the new antitoxin, and the amount of the latter which keeps the guinea-pig alive for just four days, or, in other words, produces the same effect as the standard unit, is known to contain one immunity unit. In the United States, the Hygienic Laboratory at Washington furnishes standard antitoxin to manufacturers for this official test and all marketed sera are tested by this method.

Diphtheria antitoxin not only prevents the development of diphtheria when injected in doses of 1000 units, but it also exerts a marked influence as a therapeutic agent in diphtheria, neutralizing the poison produced by the bacilli in the body of the patient. It does not kill the bacilli but it nullifies their chief offensive weapon, the soluble diphtheria toxin. Its value in treatment of diphtheria is everywhere attested by clinical evidence. The inflammation in the throat subsides and the membrane disappears. The bacilli, however, may remain for a considerable time. Local antiseptics may assist the natural agencies of the body in their destruction. In some cases they persist for months in spite of vigorous treatment.

Certain untoward effects have followed the injection of anti-diphtheritic serum. Sudden death has occurred in very rare instances and skin rashes are rather common. These effects are probably due to toxic substances set free in the parenteral digestion of the foreign protein and are doubtless of the same general nature as the phenomenon of anaphylaxis. Since the introduction of the concentrated antitoxin fatalities have become exceedingly rare or have been entirely eliminated. The serum rashes and cases of nervous shock do occur, especially in asthmatic individuals and in those who have received a previous injection of horse serum. In these persons it is well to give a minute quantity, 0.2 c.c., of the serum as a preliminary injection, wait two or three hours and then give the full dose. The danger of serious reactions due to anaphylaxis may thus be avoided.<sup>1</sup>

<sup>1</sup> Vaughan: *Amer. Journ. Med. Sciences*, 1913, Vol. CXLV, pp. 161-177.

*The Schick Reaction.*—In an outbreak of diphtheria it is often helpful to ascertain which of the exposed persons may be susceptible to the disease. For this purpose the diphtheria *toxin* is diluted with salt solution so that 0.2 c-c. of the solution contains  $\frac{1}{50}$  M. L. D. (minimum lethal dose for a 250-gram guinea pig). This amount is injected through a fine needle *into* the person's skin so that a white swelling is produced. A control injection may be made in another place with the same toxin previously rendered inert by heating at  $75^{\circ}$  C. for 5 minutes. A positive reaction is manifested by a red, swollen and infiltrated area 7 to 20 mm. in diameter after 48 to 96 hours at the test area without a similar reaction at the control site and this result indicates that the individual is susceptible to diphtheria.

**Bacillus (Corynebacterium) Xerosis.**—This organism occurs on the normal mucous membranes, particularly the conjunctiva. It resembles *B. diphtheriæ* very closely, simulating the granular morphological type. Its cultures are not poisonous.

**Bacillus Hofmanni.**—This organism is also called the pseudo-diphtheria bacillus. It occurs frequently in cultures from the nose and pharynx, and resembles the short solid-staining morphological types of *B. diphtheriæ*. It does not produce toxin, nor does it produce acid from dextrose.

**The Morax-Axenfeld Bacillus.**—This is a small non-motile diplo-bacillus, the individuals measuring about  $1 \times 2\mu$ , which occurs in one form of epidemic conjunctivitis. It can be cultured on Löffler's serum, which it liquefies, and the disease has been produced in man by inoculation with pure cultures.

**The Koch-Weeks Bacillus.**—This a non-motile rod  $0.25\mu$  wide and 1 to  $2\mu$  long, which occurs in epidemic conjunctivitis. It is cultivated with difficulty and abundant moisture is essential to success. Inoculation with pure cultures causes conjunctivitis.

**Bacillus (Hemophilus) Pertussis (Bordet-Gengou Bacillus).**—Bordet and Gengou in 1906 described a minute, non-motile bacillus  $0.3 \times 1.5\mu$  which occurs in the sputum and on the mucous membrane of the trachea and bronchi in whooping cough. They

obtained cultures of the organism on blood agar and, employing these cultures as an antigen, they demonstrated an antibody in the blood of patients by means of the complement-fixation



FIG. 126.—The Morax-Axenfeld bacillus in the exudate of conjunctivitis. (From McFarland after Rymowitsch and Matschinsky.)

est. Klimenko<sup>1</sup> has further succeeded in producing a chronic catarrh of the respiratory passages in monkeys and puppies by



FIG. 127.—Koch-Weeks bacillus in muco-pus from conjunctivitis.  $\times 1000$ . (From Park and Williams after Weeks.)

applying pure cultures to the tracheal mucosa. The bacillus is a minute rod, motionless, stained with moderate difficulty,

<sup>1</sup>*Centralbl. f. Bakt. Orig.*, 1909, Bd. XLVIII, S. 64-76.

and Gram-negative. It occurs in large numbers between the cilia of the epithelial cells lining the trachea and bronchi in cases of whooping cough where it mechanically<sup>1</sup> interferes with the action of the cilia and gives rise to irritation. It is an obligate aërobe and at first grows well only on media containing blood, ascitic fluid or other protein. Later it adapts itself to artificial culture on ordinary media. Gelatin is not liquefied.

**Bacillus (Hemophilus) Influenzæ.**—Pfeiffer in 1892 isolated a small bacillus  $0.25\mu$  wide by  $0.5$  to  $2.0\mu$  long from the bronchial secretion in cases of epidemic influenza. The bacillus occurs in enormous numbers in acute uncomplicated cases of influenza in the nasal and bronchial mucus. It is non-motile, aërobic, rather difficult to stain and Gram-negative. Cultures are obtained on ordinary agar smeared with fresh human or rabbit's blood or upon a mixture of blood and agar. Hemoglobin seems essential to growth. The bacillus is very sensitive to drying, and its transmission would seem to occur largely through close association, and the scattering of moist droplets of material from the nose and mouth in sneezing, coughing and talking. The cultures are toxic for rabbits and monkeys. The causal relation of *B. influenzae* to influenza is not as yet fully established. Conditions resembling influenza very closely seem to be caused by other organisms, such as the cocci.

The influenza pandemic of 1918 has stimulated numerous investigations of the disease but the bacteriology of it has not been fully elucidated.<sup>2</sup>

<sup>1</sup> Mallory: Pertussis: The Histological Lesion in the Respiratory Tract, *Journ. Med. Rsch.*, 1912, Vol. XXVII, pp. 115-124; Mallory, Hornor and Henderson, *Journ. Med. Rsch.*, 1913, Vol. XXVII, pp. 391-397.

<sup>2</sup> Park: Bacteriology of recent pandemic of influenza and complicating infections, *Journ. Amer. Med. Assn.*, 1919, 73, p. 318; Huntoon and Hannum: Rôle of *Bacillus influenzae* in clinical influenza, *Journ. of Immunology*, 1919, 4, p. 167; MacNeal: The influenza epidemic of 1918 in the American Expeditionary Forces in France and England, *Archives Int. Med.*, 1919, 23, p. 657; Blake and Cecil: The production of an acute respiratory disease in monkeys by inoculation with *Bacillus influenzae*, *Journ. A. M. A.*, Jan. 17, 1920, 74, p. 170.

**Bacillus (Bacterium) Chancrici (Bacillus of Ducrey).**—Ducrey in 1889 found a short bacillus in the soft venereal sore known as chancroid, obtained it in pure culture and produced typical lesions by inoculation in man. The organism is about  $0.5 \times 1.5\mu$ , often growing in threads. It grows on a blood-agar mixture at  $37^{\circ}$  C. Material for culture should be obtained from an unbroken pustule or from a chancroidal bubo, so as to avoid contaminating organisms. The bacillus possesses very little resistance to drying or to germicides. Successful inoculation experiments have been carried out on man, on monkeys and on cats. Other organisms<sup>1</sup> appear to produce soft chancre in the absence of the Ducrey bacillus in some cases.

<sup>1</sup> Herbst and Gatewood: *Journ. A. M. A.*, 1912, Vol. LVIII, pp. 189-191.

## CHAPTER XX

### MYCOBACTERIACEÆ: THE TUBERCLE BACILLUS AND OTHER ACID-PROOF BACTERIA

**Bacillus (Mycobacterium) Tuberculosis.**—Robert Koch in 1882 discovered the minute rods in tuberculous tissue, planted the tissue on slanted inspissated blood serum and obtained pure cultures of the tubercle bacillus, inoculated these cultures into animals and produced typical tuberculosis. He succeeded in doing this with natural tuberculosis of man and many other mammals and also with the tuberculosis of birds. Silbey in 1889 observed with the microscope morphologically similar bacilli in a snake. Rivolta and Mafucci in 1889 pointed out the differences between the tubercle bacillus of birds and that of mammals and their work, together with subsequent confirmatory investigations, has established a distinct avian type of tubercle bacillus, *B. tuberculosis* var. *gallinaceus*. In 1897 Bataillon, Dubard and Terre found acid-proof bacilli in definite histological tubercles in a fish (carp), obtained cultures and recognized it as distinct from the mammalian form, and it was subsequently designated as *B. tuberculosis* var. *piscium*. Theobald Smith in 1898 published the results of a careful and extensive comparative study of tubercle bacilli from human sputum and tubercle bacilli from tuberculous tissue of the bovine pearl disease (tuberculosis), and pointed out distinct differences in morphology, cultural characters and virulence between the organisms derived from the two sources. The mammalian tubercle bacilli were thus divided into two types, and subsequent investigation has fully justified the recognition of *B. tuberculosis*

var. *humanus* and *B. tuberculosis* var. *bovinus*. Some, or perhaps all four of these types may be eventually recognized as distinct species. At present the designation as types or varieties seems more appropriate.

**Bacillus Tuberculosis var. Humanus.**—This organism occurs in the infiltrated lung in human phthisis and also in the great

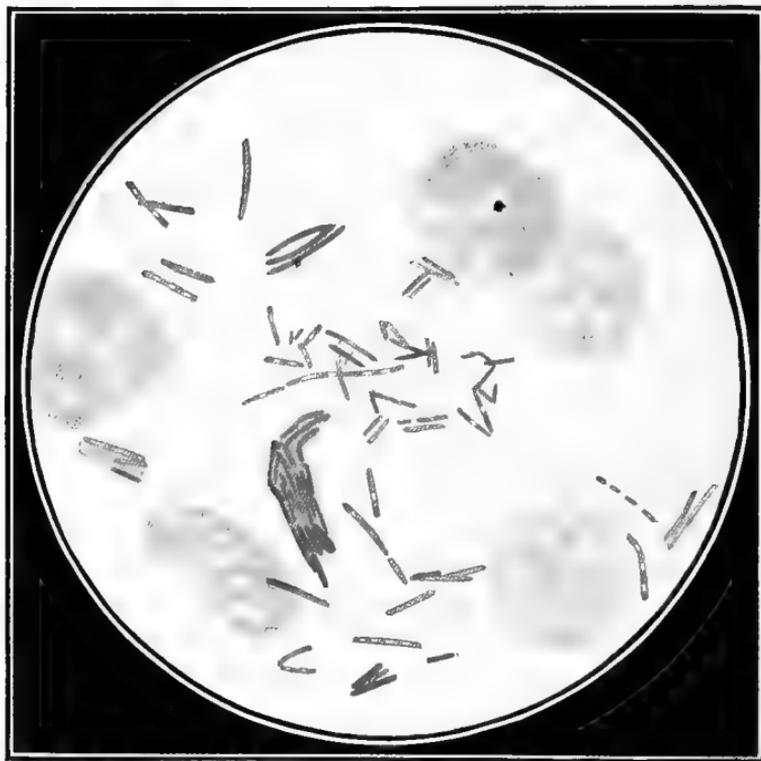


FIG. 128.—*Bacillus tuberculosis* in the sputum of a consumptive; stained by Ziehl method.  $\times 2100$ . (After Kossel.)

majority of the other tuberculous lesions in man. In the external world it does not grow naturally and passes there a more or less temporary existence in discharges from the body, of which the most important is the sputum. The cell is about  $0.4\mu$  in width and quite variable in length,  $0.5$  to  $8.0\mu$ . The longer

forms are often somewhat bent, and they frequently contain refractile granules. When stained these forms have a beaded or banded appearance. Spores have not been observed. Branching forms occur sometimes in cultures, suggesting a close relation to actinomyces and streptothrix. There is a considerable amount of a waxy substance in the body of the bacillus, which makes it difficult to stain and also difficult to decolorize after it has been stained. Hot carbol-fuchsin is generally employed, applying it for one to two minutes. The preparation is then washed and decolorized in dilute mineral acid (2 to 20 per cent) and in alcohol.

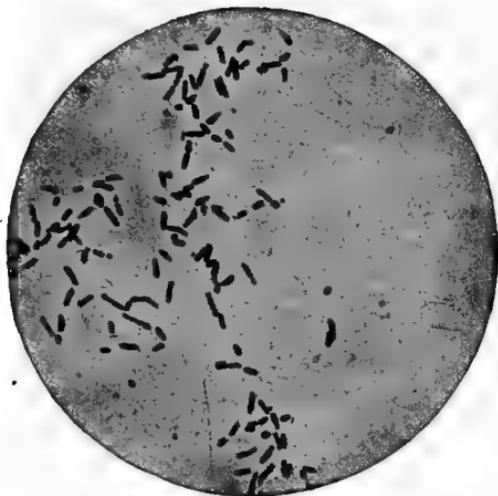


FIG. 129.—*Bacillus tuberculosis*, from a pure culture.  $\times 1000$ .

Tissue elements and most other materials may be completely bleached by this treatment, leaving the tubercle bacilli still colored. *B. tuberculosis* is Gram-positive.

Cultures are most readily obtained by transferring bits of tuberculous tissue, free from other micro-organisms, to moist slants of inspissated blood serum or Dorset's egg medium. If the available material is already contaminated, the extraneous organisms may usually be eliminated by inoculating it into guinea-pigs and making the cultures from the tuberculous guinea-

pig tissue, about four weeks later.<sup>1</sup> The tubes may be sealed with rubber caps or paraffin and incubated at 37° C. Better results are obtained by leaving the tubes unsealed and incubating at 37° C. in an atmosphere saturated with moisture, as the bacillus is a strict aërobe, but this requires special care and is not absolutely essential to success. After two or three weeks a dry, white growth is developed which may later become folded. Transplants from the primary culture to glycerin agar, glycerin broth or



FIG. 130.—Tubercle bacillus showing branching and involution forms. (After Migula.)

glycerin potato are usually successful. Old cultures on potato and agar often become yellowish or even pink in color.

The chemical composition of tubercle bacilli has been extensively studied. The moisture content varies from 83 to 89 per cent. The ash (inorganic salts) amounts to about 2.6 per cent of the dry substance, and about half of this is phosphoric acid ( $\text{PO}_4$ ). The waxy constituent of the bacterial cells is of

<sup>1</sup> It is possible to cultivate tubercle bacilli directly from contaminated material such as sputum, by careful technic, although the results are somewhat uncertain. A method is described in detail by Williams, W. W. and Burdick, *Journal of Bact.*, 1916, I, p. 411-414.

particular interest. This makes up from 8 to 40 per cent of the dry substance, less in young and more in old cultures. The acid-proof staining property depends upon this waxy substance, for the bacilli from which it has been extracted by ether-alcohol are no longer acid-proof while the wax itself exhibits this peculiarity of staining. It is also known that the bacilli in young cultures are on the whole less acid-proof than those from old cultures in which chemical analysis shows a greater concentration of the waxy substance. The protein substances, largely nuclein, make up about 25 per cent of the dry cell substance. Several other constituents of the cell have been identified. As in the case of other bacteria the chemical composition varies within rather wide limits according to the nutritive medium, conditions of growth and especially the age of the culture.

The poisons of the tubercle bacillus exist to a large extent in an inactive form in the culture fluid and more particularly as an undissolved constituent of the bacterial cell bodies. Culture filtrates exert little or no effect upon injection into normal animals. The dead bacilli, however, give rise to local inflammation and in many instances stimulate the formation of typical tubercles at the point where they lodge. Evidently the poison is set free from some substance in the dead cells by the action of the tissue cells or



FIG. 131.—*Bacillus tuberculosis*. Culture on glycerin agar several months old. (From McFarland after Curtis.)

body fluids upon them, and it is quite certain that the bacteria-free culture fluid (old tuberculin) becomes toxic as a result of such an action.

Tubercle bacilli outside the body are moderately resistant to harmful influences. In dried sputum, they have been found alive after eight months. Direct sunlight kills the bacilli in sputum in a few minutes if this be exposed in a thin transparent layer. In thicker masses the effect of light is uncertain. In buried cadavers the bacilli remain alive and virulent for 2 to 6 months. In watery suspensions the bacilli are killed by heating to  $60^{\circ}$  C. for 15 minutes. In milk, heating at  $60^{\circ}$  C. for 20 minutes or at  $65^{\circ}$  C. for 15 minutes kills the tubercle bacilli, provided all the fluid is heated to this temperature for the full period. The bottle should be tightly stoppered and completely immersed in the hot water. Dry heat at  $100^{\circ}$  C. for 30 minutes is effective. Against chemical disinfectants *B. tuberculosis* is rather resistant, doubtless because of the waxy constituent of the cells. Absolute alcohol and mercuric chloride 1 to 500 fail to disinfect sputum in 24 hours. Five per cent carbolic acid is effective in this time. Formalin, 5 per cent solution, requires about 12 hours. *B. tuberculosis* remains alive in strong antiformin solutions (a proprietary preparation of chlorinated caustic alkali) for 30 to 60 minutes, whereas ordinary bacteria are rapidly disintegrated by this chemical agent.

*Tuberculin* is a name applied to various chemical products of the tubercle bacillus. The oldest and most important tuberculin was described by Koch in 1890. It is made by growing the bacillus on the surface of 4 per cent glycerin broth in shallow flasks at  $37^{\circ}$  C. for eight to ten weeks, steaming the cultures for one hour and filtering through porcelain, or often merely through paper, to remove the dead bacilli. The filtrate is then concentrated to one-tenth its original volume by evaporation at  $90^{\circ}$  on the water-bath. The product keeps indefinitely in sealed containers and is known as Koch's old tuberculin ("alt tuberculin"). Chemical study of tuberculin has shown that the

specific active substance is a thermostable, dialyzable substance, insoluble in alcohol, which gives most of the protein reactions but not the biuret test. It is digested by pepsin and by trypsin.<sup>1</sup> Koch's new tuberculin, better known as tuberculin B. E. ("Bacillen-emulsion") is made from the solid bacterial growth on glycerin broth. The growth is pressed between filter papers, dried and then pulverized in a ball mill for about three months, then suspended in 50 per cent aqueous solution of glycerin, 0.002 gram of the powder to each cubic centimeter. Finally it should be sterilized by heating to 60° C. for 20 minutes. This tuberculin is a suspension, not a solution, and must be thoroughly mixed each time before use. Numerous other tuberculins have been prepared, of which perhaps the "Bouillon filtré" of Denys is the most important. It is the porcelain filtrate of the unheated glycerin-broth culture of the tubercle bacillus. It resembles Koch's old tuberculin except that it is not heated and is not concentrated.

Inoculation of animals with *B. tuberculosis* gives rise to typical tuberculous lesions and death in most mammalian species. The guinea-pig is very susceptible to subcutaneous injection but not readily infected by the alimentary route. The lesions are usually well developed four or five weeks after subcutaneous inoculation and death occurs as a rule in 6 to 12 weeks. Rabbits are less susceptible to inoculation with the human type and they usually recover when injected with small doses of a culture, 0.001 gram intravenously. Cattle are quite immune to this organism. Large doses of cultures or of sputum have been injected into calves and older bovines without producing tuberculosis, and quarts of tuberculous sputum have been fed to bovine animals with negative results.

Tuberculosis is, economically, the most important human disease. Approximately one death in every three between the age of 20 and 45, the active period of life, is due to it. It was recognized as a contagious disease by the ancients. Laennec,<sup>2</sup>

<sup>1</sup> Löwenstein in Kolle und Wassermann, Handbuch, 1912, Bd. V, S. 554-555.

<sup>2</sup> For a history of tuberculosis see Landouzy: *Cent ans de phisiologie*, 1808-1908, Sixth Internat. Cong. on Tuberculosis, Special Volume, pp. 145-189.

in 1805, by extensive *post-mortem* studies recognized the essential pathological unity of tuberculous processes. Villemin, in 1865, conclusively demonstrated its transmissibility by successful inoculation of animals with tuberculous tissue from man and from cattle.

The response of the infected tissue to the presence of the tubercle bacillus results in a localized mass of granulation tissue, the tubercle, of which the histological structure is so characteristic that the presence of tuberculosis may be recognized by it alone. From the point of introduction the bacilli may be distributed by the lymph or blood stream or may be carried by wandering cells. Eventually a bacillus comes to rest and grows slowly in the intercellular spaces of connective tissue. Very soon, the neighboring fixed tissue elements, connective-tissue cells and endothelial cells, begin to multiply by karyokinesis and at the same time the cells become swollen with nuclei large and bladder-like, forming the so-called epithelioid cells. The bacilli are found in and between these cells. As the pathological process continues the nucleus of an occasional epithelioid cell divides many times without division of the cytoplasm, giving rise to a multi-nucleated giant cell. Very early in its development the peripheral portion of the tubercle becomes infiltrated with lymphocytes and later, as the giant cells are formed, numerous polynuclear leukocytes are also present. Newly formed blood vessels are absent. With further extension, the center of the tubercle undergoes a caseous necrosis and liquefaction, and eventually this necrotic center enlarges so as to break through an epithelial surface to a passage to the exterior. This gives rise to open tuberculosis and tubercle bacilli may usually be found in the discharge from the lesion at this stage.

The tubercle is the essential histological unit of tuberculosis. An infiltrated tissue may contain myriads of these tubercles in all stages of evolution. At any stage in its evolution the development of the tubercle may become arrested and it may retrogress and heal if the infected tissue is able to overcome the bacilli.

If this occurs early the bacilli may be entirely destroyed and the abnormal tissue may disappear completely or remain only as a little hyaline or fibrous tissue. After caseation has occurred, healing results in the formation of a dense fibrous nodule, usually with calcareous material in the center, in which living tubercle bacilli can usually be demonstrated.

The mode of infection in human tuberculosis has been a matter of some controversy and much of the evidence concerning it has been derived from animal experimentation. Unquestionably tubercle bacilli may pass through epithelial surfaces, especially of mucous membranes, without production of any demonstrable lesion. Ingested bacilli readily pass through the intestinal mucosa, especially during the digestion of fat, and they may first produce lesions in the mesenteric lymph glands, the liver or in the lungs. In the latter instance, they doubtless pass with the absorbed fat through the thoracic duct, superior vena cava and right heart to the pulmonary arteries. In man, the most important mode of infection is through inhaling the dust of dry powdered sputum, as a result of which lesions develop in the lungs. Tuberculosis may occur in any tissue of the body, reaching it through the blood and lymph. A massive infection of the blood stream often leads to generalized miliary tuberculosis with minute tubercles in all the organs.

The bacteriological diagnosis of the disease depends upon finding the tubercle bacilli in discharges from the suspected lesion. In sputum an acid-proof bacillus of the proper size and shape is almost invariably a tubercle bacillus and a diagnosis based upon such a finding by an experienced microscopist is justly regarded as very accurate. Inoculation of guinea-pigs will clinch the proof. The latter procedure will also sometimes detect tubercle bacilli when careful microscopic search has failed. In discharges from the intestine or urinary organs one may meet with other acid-proof organisms (*B. smegmatis*), and more care is necessary in arriving at a diagnosis. In tuberculous meningitis, the tubercle bacillus may be detected by microscopic

examination of the cerebrospinal fluid<sup>1</sup> in nearly every case. The filmy clot which usually forms in such a fluid in a half hour after drawing it is the most favorable material for examination.

When a considerable amount of purulent or mucoid material is available for examination and one has failed to find the tubercle bacilli by the usual method of microscopic examination, it is often advisable to try some method of concentration. One of the common methods of general applicability is that of Uhlenhuth, in which antiformin is employed to dissolve the tissue elements, leaving the bacilli unchanged. Löffler's modification<sup>2</sup> of the Uhlenbuth method is a convenient one. The material to be examined is mixed with an equal amount of 50 per cent anti-formin and brought to a boil. This dissolves the sputum or other material and serves to kill the bacilli. It is then cooled and, for each 10 c.c., 1.5 c.c. of chloroform-alcohol (1 : 9) is added. The mixture is next violently shaken to form a fine emulsion, and is then centrifugalized at high speed for 15 minutes. The solid matter collects as a tough mass on top of the drop of chloroform and beneath the watery liquid. This mass is rinsed in water, crushed between slides mixed with a little egg albumen or with some of the original untreated exudate, spread, fixed, stained and examined in the usual way. The albuminous material is necessary to make the preparation adhere to the slide.

Allergic reactions are extensively employed in the diagnosis of tuberculosis. Tuberculin is without particular effect upon normal individuals but in the tuberculous individual it gives rise to irritation and intoxication. The phenomenon is analogous to that of anaphylaxis, the irritant or toxic substance being set free from the tuberculin by the action of specific ferments produced and present in the body as a result of previous contact with the tubercle bacillus and its products. The tuberculous

<sup>1</sup> Holt: *Amer. Journ. Dis. Children*, Jan., 1911, Vol. I, pp. 26-36. Hemenway: *ibid.*, 1911, Vol. I, pp. 37-41. Koplik: *Johns Hopkins Hosp. Bull.*, 1912, Vol. XXIII, pp. 113-120.

<sup>2</sup> Williamson: *Journ. A. M. A.*, 1912, Vol. LVIII, pp. 1005-7.

individual is therefore sensitized to tuberculin. The sensitization may be local and confined to the tissue immediately surrounding a solitary tubercle, or it may be general as a result of more extensive lesions. Tuberculin is applied to the skin mixed with an equal amount of lanolin (Moro test), or applied to a scarified point undiluted (Von Pirquet test), or injected into the substance of the skin in a dose of 0.1 c.c. of 1 to 1000 dilution (Hamburger intracutaneous test), or applied to the conjunctiva in a dose of one drop of a freshly prepared 1 per cent solution of old tuberculin (Wolff-Eisner or Calmette test), or finally it may be introduced into the circulation by subcutaneous injection of a dilution representing 0.00001 gram of old tuberculin, with subsequent progressive increase of the dose up to 0.010 gram if reaction is not obtained. The local reaction is that of irritation, evidenced by redness and edema, sometimes by vesiculation. The general reaction is evidenced by malaise, irritation at site of the lesion (increased cough in pulmonary tuberculosis) and a rise in body temperature. The reaction depends upon the tuberculin coming into contact with the specific ferment, and the location, extent and activity of the tuberculous process are important elements influencing the outcome of the various tests. Tuberculosis in the eye causes such a violent reaction to the conjunctival test that this method should never be employed without first excluding ocular tuberculosis. The subcutaneous test will often detect tuberculosis not revealed by the other methods. It is, however, a more serious procedure than the skin tests, which are indeed practically harmless.

The various tuberculins are now extensively employed in the treatment of tuberculosis, largely because of the favorable results obtained by Trudeau. It is given subcutaneously every 5 to 7 days beginning first with a blank dose of salt solution and next with 0.00001 gram of tuberculin. The dose is kept at the point at which the least general reaction possibly recognizable occurs, or just below this amount, the general purpose being to induce an immunity to tuberculin. It is often possible to begin with a

case which reacts to 0.0001 gram of tuberculin and after treatment for 6 months so change the sensitiveness that 0.5 gram may be injected without reaction. Some cases do remarkably well when treated with tuberculin together with the usual careful hygienic-dietetic treatment<sup>1</sup> given in sanatoria, but the value of tuberculin for treatment of the average case, is, perhaps, not yet fully established.<sup>2</sup> In general the tuberculin treatment stimulates the production of a thicker capsule about the healing tuberculous lesion and thus tends to insure against renewed activity of the process at a subsequent time.

**Bacillus Tuberculosis var. Bovinus.**—The bovine type of tubercle bacillus is found in the lesions of tuberculous cattle (*perlsucht*), frequently in hogs, in a considerable percentage of tuberculous lesions in children, and very rarely in the tuberculous lungs of adult human beings. In artificial culture on solid media, the cell is about  $1\mu$  long, shorter than that of the human type, and is easily stained. In glycerin broth the length of the cell and the staining is more irregular. On all media the growth is at first much less abundant than that of the human type. Smith has shown that the bovine type produces alkali in glycerin broth during the first two months, whereas the human type tends rather to produce acid. The virulence of the bovine bacillus is greater than that of the human type for all mammals, and it also infects birds. Intravenous injection of 0.0001 gram of culture in thin emulsion kills rabbits with generalized tuberculosis in about three weeks, while a similar dose of the human variety is without such effect. Subcutaneous injection of rabbits shows a similar difference. Calves are very susceptible to the bovine type, not to the human.

Tuberculosis of cattle is widely distributed and is very prevalent in the older European dairy regions. The lesions are most common in the bronchial and retropharyngeal lymph glands, but they may occur anywhere in the body of the animal. The

<sup>1</sup> Brown: *Journ. A. M. A.*, 1912, Vol. LVIII, pp. 1678-81.

<sup>2</sup> Brown: *Amer. Journ. Med. Sciences*, 1912, Vol. CXLIV, pp. 469-624.

disease may remain localized for years in a single lymph gland or it may extend rapidly causing marked emaciation and death of the animal. The bacilli escape from the living bovine animal most commonly in the feces,<sup>1</sup> sometimes in the mucus and spray from the nose and mouth, in the uterine discharge and in the milk, and of great importance is the fact that animals may be excreting the bacilli without showing any gross evidence of the presence of the disease. Tuberculin is extensively employed in the detection of tuberculosis in cattle. A dose of 0.2 to 0.5 gram diluted with 9 volumes of 0.5 per cent carbolic acid is injected subcutaneously at the side of the neck. The typical positive reaction includes a rise in temperature of 2° or 3° F. over that of the previous day. The test is very accurate when positive but not so reliable when negative. Tuberculous animals should be segregated from healthy animals and food products from them used only after effective disinfection, or they should be slaughtered under inspection.

Great interest has been manifested in the question of susceptibility of man to the bovine tubercle bacilli and the solution has been reached by isolating bacilli from human tissue and identifying them. Park and Krumwiede<sup>2</sup> have summarized the results of 1511 such examinations, and conclude that somewhat less than 10 per cent of the deaths from tuberculosis in young children are due to the bovine tubercle bacillus, while in adults infection with this bacillus is much less frequent.

**Bacillus Tuberculosis var. Gallinaceus (Avium).**—This variety occurs particularly in the tuberculous lesions of barnyard fowls, but also in many other birds. The form of the bacillus is not specially characteristic except that in old cultures there is a marked tendency to the production of branching threads. In glycerin broth the growth is more delicate, and development takes place at the bottom of the flask as well as on the surface

<sup>1</sup> Briscoe and MacNeal: Ill. Agr. Exp. Sta. *Bull.* 149, 1911; Assn. for Tuberculosis, Transactions, 1912, pp. 460-465.

<sup>2</sup> *Journ. Med. Resch.*, 1912, Vol. XXVII, pp. 109-114.

of the liquid. Chickens are very susceptible to intravenous inoculation with this type of bacilli but quite refractory to the mammalian types. Mice and rabbits are also susceptible, while guinea-pigs are relatively resistant. The avian tubercle bacillus has been found in human tuberculous lesions in a very few instances.

**Bacillus Tuberculosis var. Piscium.**—This variety occurs in natural tuberculous lesions of snakes, fish, turtles and frogs. The bacillus is quite different from the preceding varieties, as it grows rapidly on ordinary media at temperatures ranging from 12° to 36° C., and the bacilli developed on the poorer media are often not at all acid-proof. When grown in bouillon with frequent shaking the culture becomes diffusely cloudy, and the organisms of such cultures are said to be motile. Most warm-blooded animals are wholly refractory to inoculation, but, in the guinea-pig, inoculation has sometimes been followed by the production of typical tubercles with epithelioid and giant cells, usually encapsulated and tending to heal.

**Bacillus (Mycobacterium) Lepræ.**—Hansen in 1873 and Neisser in 1879 discovered this organism in the nodular lesions of leprosy. Successful artificial culture has been reported by many authors but the identity of the organisms in these cultures has not been established with certainty.

*B. lepræ* is a slender rod 0.2 to 0.45 $\mu$  wide by 1.5 to 6 $\mu$  long as it occurs in tissues. In its staining properties it closely resembles the tubercle bacillus. The organism occurs in enormous numbers in most of the nodular lesions of leprosy and is often abundant in the nasal mucus of these cases. When less numerous the antiformin method of Uhlenhuth may assist in finding them. For diagnosis a small piece should be excised from one of the nodules or fragments may be obtained from lesions in the nose or pharynx by means of a curette. From these pieces smears on slides are stained at once for acid-fast bacilli. Pieces of the tissue are embedded in paraffin, sectioned and stained to demonstrate the bacilli.

Leprosy has been known since the dawn of history and has

been considered to be transmissible for a long time. It is widely distributed over the earth, especially in Norway, Russia, Iceland and in Turkey. In the United States there are leper colonies in Louisiana, Minnesota and in Hawaii. Lepers are occasionally seen in the clinics of all the larger cities.

Leprosy is universally considered to be due to the leprosy bacillus, but as to mode of transmission, whether direct from man to man, or from the external world, or how, little or nothing is really known. It seems certain that the disease is always contracted in some way from a previous case, but it is certainly not very readily transmitted. Segregation without absolute isolation is the common method of handling lepers. The disease is not ordinarily inherited.

**Bacillus Smegmatis.**—This organism occurs in the smegma on the genitals of man and other mammals and also in moist folds of the skin where there are collections of moist desquamated epithelium. It resembles the tubercle bacillus in form and staining properties, but is, on the average, more readily decolorized in alcohol. This property cannot be relied upon to differentiate the two organisms in any given case. Proper care in collecting specimens for examination usually suffices to exclude this organism. Urines to be examined for tubercle bacilli should be obtained by catheter. In doubtful cases inoculation of a guinea-pig is necessary. *B. smegmatis* has been grown in artificial culture and after a time adapts itself to ordinary media.

**Bacillus Moelleri.**—Acid-proof organisms resembling the tubercle bacillus in form and staining properties were found on timothy hay by Moeller. The bacillus is likely to be found in milk and other dairy products. Probably the "butter bacillus" of Rabinowitsch is identical with it or a near relative. When introduced into guinea-pigs these organisms sometimes produce lesions resembling tubercles, but these do not progress and kill the animal and a second animal inoculated from the lesions of the first gives a negative result. Cultures are easily obtained on ordinary media, and the organisms grow rapidly at 25° to 30° C.

**Other Acid-proof Organisms.**—Many of the streptothrices which grow in the soil and upon plants are to some extent similar in their staining properties to the tubercle bacillus and when broken up into short segments may be a source of confusion. These are most likely to be met with in examining agricultural products and especially in the feces of cattle. Mere microscopic examination of such materials for tubercle bacilli has, as a rule, little value, as both positive and negative findings are questionable. Brem,<sup>1</sup> in the Canal Zone, has made the important observation that acid-proof bacilli may grow in distilled water stored in bottles in the laboratory and that, when such water is used in preparing the microscopic objects for examination, these extraneous bacilli may be mistaken for tubercle bacilli. Burvill-Holmes<sup>2</sup> has made similar observations at Philadelphia. Pseudo-bacilli, microscopic bodies somewhat resembling tubercle bacilli, some times occur in microscopic preparations stained with carbol-fuchsin. These deceptive pictures seem to be common in preparations of laked or digested blood.<sup>3</sup>

<sup>1</sup> *Journ. A. M. A.*, 1909, Vol. LIII, pp. 909-911.

<sup>2</sup> *Proc. Path. Soc. Phila.*, 1910, N. S. Vol. XIII, pp. 154-160.

<sup>3</sup> Calmette, Sixth Internat. Cong. on Tuberculosis, 1908, Spec. Vol., p. 70; see also Bacmeister, Kahn and Kessler, *Münch. med. Wochenschr.*, Feb. 18, 1913.

## CHAPTER XXI

### BACTERIACEÆ: THE BACTERIA OF THE HEMORRHAGIC SEPTICEMIAS, PLAGUE AND MALTA FEVER

For the bacteria of the hemorrhagic septicaemias the Committee of the Society of American Bacteriologists has adopted the generic name, *Pasteurella* Trevisan 1887.

**Bacillus Avisepticus (Pasteurella Cholerae-gallinarum).** Moritz<sup>1</sup> in 1869 observed this minute rod in the blood of chickens with chicken cholera. Toussaint (1879) and Pasteur (1880) obtained pure cultures in liquid media and Pasteur (1880) made the far-reaching discovery of the method of immunization by means of attenuated bacterial cultures while working with this organism. *B. avisepticus* occurs in enormous numbers in the blood, internal organs, urine and feces of the acutely affected birds, in far smaller numbers in those having the chronic form of the disease and has also been found in the intestinal contents of apparently healthy birds. It is 0.3 $\mu$  wide and 0.2 to 1 $\mu$  in length, the shorter ones being joined together. It is non-motile and Gram-negative. Cultures are readily obtained on ordinary media by inoculation with heart's blood. Gelatin is not liquefied. Minute quantities of a virulent culture suffice to produce a fatal infection in chickens and many other birds, either by feeding or by subcutaneous injection. Rabbits are also extremely susceptible, guinea-pigs almost immune. Artificial cultures kept for three to ten months in contact with air are no longer capable of causing a fatal infection in chickens and their injection is followed by recovery and a state of immunity to the fully virulent organism. Acute chicken cholera is the typical hemorrhagic septicemia of birds, with abundant bacteria in the blood, and hemorrhages on the serous membranes and into the stomach and intestine.

<sup>1</sup> Vallery-Radot: Life of Pasteur, 1911, Vol. II, p. 75.

**Bacillus (Pasteurella) Plurisepticus.**—This name is applied to an organism occurring in the hemorrhagic septicemias of various mammals and birds. The virulence is variable and seems to be especially developed for the species of animal in which the organism is found. It does not differ essentially from *B. avisepticus*. Other minute bacteria exhibiting the same general characteristics and occurring as a generalized infection in diseases of animals are *Bacillus murisepticus* in mice and *Bacillus (Bacterium) rhusiopathiæ suis* in swine.

**Bacillus (Pasteurella) Pestis.**—This organism was discovered simultaneously by Kitasato and Yersin in 1894 in the bodies of

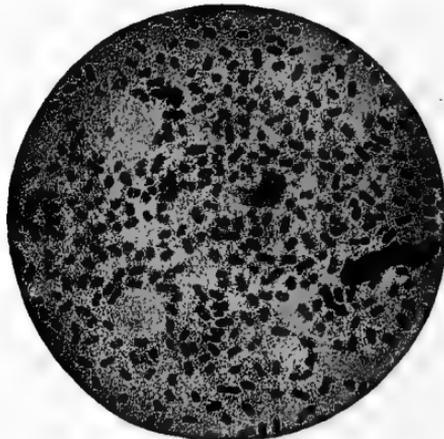


FIG. 132.—Bacillus of bubonic plague. (Yersin.)

persons dying of bubonic plague in the epidemic at Hongkong. The description of Yersin has proven to be the more accurate. The organism is unquestionably the cause of plague, as in addition to the evidence of animal experimentation there are several instances of fatal infection of men working with the organism in laboratories far removed from any focus of the disease, and finally the very unfortunate accident at Manila<sup>1</sup> where cholera vaccine mixed with a culture of *B. pestis* by mistake was injected into men and caused fatal bubonic plague.

<sup>1</sup> Freer: *Journ. A. M. A.*, 1907, Vol. XLVIII, pp. 1264-65.

*B. pestis* in the body of the patient is a short plump rod, 0.5 to 0.7 $\mu$  wide by 1.5 to 1.8 $\mu$  long, and rounded at the ends. When stained the ends become deeply colored while the equator remains pale (bipolar staining). Alongside this typical form many irregular organisms are usually found, especially longer and shorter bacilli, some pale, some irregularly outlined, and some swollen and poorly stained. The last-mentioned types of bacilli are more frequently found in the bodies of plague victims which have begun to decompose. They are also observed in artificial cultures. These irregular forms (involution forms) are important in the quick recognition of plague. The bacillus stains very readily, best with methylene blue or with a momentary exposure to carbol-fuchsin. Better results are obtained by fixing the spread in alcohol one minute, rather than heating it. The Romanowsky stain gives good results. It is distinctly Gram-negative (contrary to the original statement of Kitasato). Capsules may be demonstrated on bacilli in the peritoneal exudate of guinea-pigs and mice, less easily in cultures. It is non-motile and flagella have not been demonstrated. Spores have not been observed and cultures are killed at 60° C. in 10 to 40 minutes. It is also easily destroyed by chemical germicides, for example, by 5 per cent carbo-lic acid in 1 minute. Mere drying at 35° to 37° C. kills the bacillus in two to three days, but at 20° C. it may withstand drying for 20 days. It may live for months in frozen material.

Cultures are readily obtained on ordinary media, best at a temperature between 25° and 30° C. Growth is moderately slow. Gelatin is not liquefied. On agar containing 3 per cent of sodium chloride, irregular involution forms are produced in 24 to 48 hours. Long chains are produced in broth. It does not form gas from sugars but does produce acid from dextrose, levulose, mannite and galactose, not from lactose or dulcitol.

The toxins of the plague bacillus are in part soluble and in part intimately combined with the bacterial cell. Filtrates of young broth cultures are without toxic properties but older broth cultures (14 days) yield a toxic filtrate. The bacterial cells killed

by heat produce fatal poisoning in guinea-pigs and rabbits. The poisons obtained so far are much less powerful than the soluble toxin of *B. diphtheriae* or the endotoxins of the typhoid and cholera germs.

Rodents, especially rats and guinea-pigs, are very susceptible to inoculation, even a needle prick carrying the minutest quantity of a virulent culture being sufficient to kill in a few days. At autopsy the adjacent lymph nodes are found greatly swollen and surrounded by hemorrhagic edema. The spleen is greatly swollen. Everywhere are enormous numbers of the bacilli. Infection by feeding gives positive results in about half the experiments. Inhalation of the bacilli produces typical pneumonic plague in rats. Monkeys are susceptible and present lesions similar to human plague.

Bubonic plague can be recognized in descriptions of epidemics in very ancient records. Rufus of Ephesus who lived at the time of Trajan (A. D. 98) mentions specifically a very fatal acute bubonic plague ("pestilentes bubones"). Great epidemics occurred in Europe in the 6th century (527-565 A. D.), in the fourteenth century (1347-1350 A. D.). Each of these was followed by smaller outbreaks persisting in the latter epidemic up to about 1850. It is estimated that 25 million persons died of the plague in the "Great Mortality" of the 15th century. Another pandemic of plague began in 1893. Its progress has been slow and undoubtedly hampered by the prophylactic measures made possible by the discovery of Yersin and Kitasato. It exists as a persistent infection among rodents or human beings, or both, in central Asia, central China, northern India, Arabia, southern Egypt, and, more recently, seems to have established itself in California. Outbreaks of plague in man in new localities have usually been preceded or associated with mortality among rodents, especially rats. When an epidemic begins in a seaport town, the sewer rats (*Mus decumanus*) are first attacked. Two to three weeks later the house rats (*Mus rattus*) begin to die, and about four weeks later the epidemic of human plague begins. The transmission

from animal to animal and from animal to man is accomplished very largely by the agency of fleas. Rat fleas are rarely found on man or at large in human habitations as long as their normal hosts are at hand, but when the rats sicken and die of plague, then the fleas leave and becoming hungry they bite human beings and thus inoculate them with plague bacilli.

In its permanent endemic centers, plague exists as an acute and chronic disease of rodents. It spreads from these regions through the agency of the wandering rats traveling along the routes of commerce and especially in ships. The infected rat, arrived at its destination, sets up an epizootic among its own species, which later spreads to other animals and to man through the agency of fleas, producing the bubonic form of the disease. The infection may then be transmitted from man to man by fomites and directly by contact, and by infectious material suspended in the air, giving rise to the pneumonic form of the disease. A persistent epizootic of chronic plague among rodents in a new region may give rise to a new permanent endemic center.

In man the disease occurs in two principal forms, the bubonic type, in which the portal of entry is on the skin or mucous membrane and the disease is manifested by swelling of the neighboring lymph nodes, and the pneumonic type in which the organisms are inhaled or aspirated into the lung. Both of these forms result in general bacteremia, as a rule. The bubonic form is largely due to inoculation of the skin by bites of insects (fleas), while the pneumonic form is transmitted more directly. Other clinical types of the disease occur. The death rate is 30 to 90 per cent in the bubonic and 98 to 100 per cent in the pneumonic type. In the bacteriological diagnosis, the morphology of the organism in the tissues and in cultures, its effect upon rats and guinea-pigs, and, finally, agglutination of the newly isolated culture with a known immune pest serum are important points.

Immunity, at least a relative immunity, follows recovery from the plague. Artificial immunity can be induced by injection of attenuated living cultures and by the injection of killed bac-

teria (Haffkine's method). Many modifications of the latter are recommended and they constitute the practical method of vaccination against plague. Haffkine employs broth cultures incubated at 25 to 30° C. for six weeks under a covering of sterile oil. The cultures are killed at 65° C., and preserved with carbolic acid. The dose is 0.1 to 0.5 c.c. for children and 3 to 4 c.c. for an adult man. It may be repeated after ten days. Good results have followed the use of this prophylactic in India. Kolle suspends two-day agar cultures in broth or salt solution and kills at 65° C. by one to two hours exposure. Five-tenths per cent carbolic acid is then added. The dose injected is the product of one agar culture. The vaccination should be taken by any physician who expects to handle plague bacilli, even if only in the laboratory.

Horses have been immunized by Yersin, injecting first killed bacilli, later highly virulent bacilli, and finally the filtrates of old broth cultures intravenously. The serum of these horses in a dose of 20 c.c. confers a transient passive immunity, and has seemed to be of value in the treatment of a few cases of plague. Its preparation is so difficult and its potency so low that it has not come into general use. The serum has also been injected along with killed bacilli to confer immunity (combined active and passive immunization).

The restriction and prevention of plague require measures adapted to the special conditions existing. In general they include precautions to exclude infected animals, wholesale destruction of rats and other rodents and the artificial immunization of the human population when confronted by the disease. The eradication of the endemic centers presents a problem of great complexity, requiring the recognition and destruction of the infected species of animals.

**Bacillus (Micrococcus) Melitensis.**<sup>1</sup>—Bruce in 1887 discovered this organism in the spleen of persons suffering from Malta

<sup>1</sup> This organism is classed as a micrococcus by most authors. It is here classed as a bacillus because of its general resemblance in many of its characters to *B. pestis*. None of the Gram-negative parasitic cocci resemble it in respect to physiological characters or in the remarkable ability to change its host.

fever and obtained pure cultures. Inoculation of monkeys with pure cultures gives rise to a disease resembling in detail<sup>1</sup> Malta fever in man.

The organism is spherical or oval, 0.3 by 0.4 $\mu$  in size, and is classed as a micrococcus by many bacteriologists. In gelatin cultures the cell is somewhat longer and resembles that of a true bacillus. The organisms are single, grouped in pairs or sometimes in short chains of four to five cells. Capsules and spores have not been observed. It is non-motile. Flagella have been detected by Gordon but other investigators have failed to confirm the observation. The organism stains readily and is Gram-negative.

Cultures are obtained on ordinary media and growth is possible between the extremes of 6° and 45° C. The colonies develop in one to three days at 37° C. and are very homogeneous. Gelatin is not liquefied and neither gas nor acid is produced in media containing the various sugars. The organism is killed by moist heat at 57° C. in 10 minutes, by dry heat at 95° C. in 10 minutes and in 1 per cent carbolic acid in 15 minutes. It survives drying for several months and retains its vitality in culture without transplantation for several years if drying is prevented.

Many mammals are susceptible, including guinea-pigs, rabbits, monkeys, rats and mice. Horses, cows, sheep and goats are not only susceptible to inoculation but also contract the disease naturally. In all animals the course of the infection is usually chronic and characterized by an irregularly remittent fever. Death is a common outcome in monkeys. Often the subcutaneous injection or the feeding of a minute quantity of the culture is sufficient to infect, but for the smaller laboratory animals intracerebral inoculation may be necessary.

Malta fever in man is a chronic disease characterized by an irregularly remittent fever. The spleen is enlarged and often the liver as well. Positive agglutination of a known culture of *B. melitensis* by the patient's serum in dilution of 1 to 1000 is an

<sup>1</sup> Eyre in Kolle and Wassermann, Handbuch, 1912, Bd. IV, S. 432.

important aid in diagnosis, and isolation of the organism from the circulating blood, or from the spleen, and its identification makes the diagnosis certain. Positive cultures are more often obtained from the spleen, but the puncture of this organ by the inexperienced is not without danger. Blood cultures should be made during a febrile period and preferably late in the afternoon. Death occurs in 1 to 2 per cent of the cases.

Careful investigations have shown that infection with *B. melitensis* is endemic among the goats of Malta, from which animals is obtained the milk supply of the region. The micro-organisms are excreted in the milk. Monkeys fed such milk acquire the disease, and human epidemics of Malta fever have followed the use of such milk under conditions closely resembling those of critical experimentation. Other methods of transmission have been tested with negative results.

Immunity follows recovery from the disease, but artificial immunization is not yet a practical success.

## CHAPTER XXII

### BACTERIACEÆ: THE COLON, TYPHOID AND DYS- ENTERY BACILLI

**Bacillus Coli**<sup>1</sup>.—This organism was probably observed by several investigators previous to 1886 but it was either neglected or its significance was misinterpreted. The first important study of it was made by Escherich in that year, who discovered it in the feces of healthy infants and obtained it alone on the aërobic gelatin plates cultures inoculated with this material.

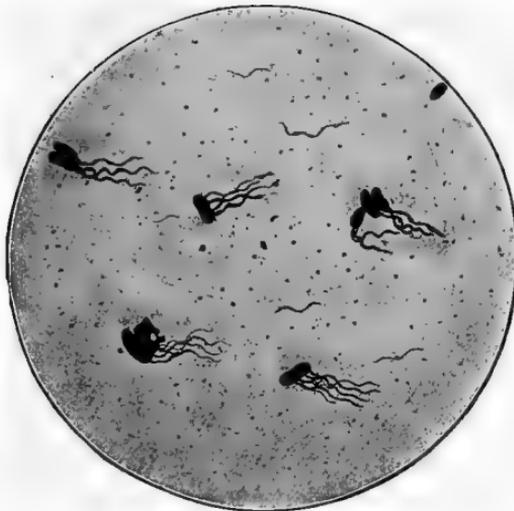


FIG. 133.—*Bacillus coli* showing flagella. (From McFarland after Migula.)

*B. coli* lives and grows in the intestinal tract of man and mammals, and organisms closely resembling it have been found in the intestinal canal of other vertebrates. It is discharged in large

<sup>1</sup> For the organisms of the colon-typhoid-dysentery group the Committee of the Society of American Bacteriologists has adopted the generic name *Bacterium* Ehrenberg 1838, emended Jensen 1909. Its use in this sense may lead to confusion with the genus *Bacterium* Migula, especially in the minds of beginning students. The old generic name, *Bacillus*, is therefore here retained.

numbers in the feces and some of these bacilli may continue their growth in the external world for a time. The organism is  $0.4$  to  $0.7\mu$  wide and  $1$  to  $6\mu$  long, with rounded ends, usually single but sometimes occurring in threads. It is motile but not very active, and many cells, even in young cultures, may be motionless.

There are four to eight peritrichous flagella. Spores have not been observed. The bacillus stains readily and is Gram-negative.

Cultures develop rapidly at  $37^{\circ}$  C. on all ordinary media. The colony is white, opaque, often somewhat heaped up in the



FIG. 134.—*Bacillus coli*. Superficial colony on a gelatin plate two days old.  $\times 21$ .  
(From McFarland after Heim.)

center and thinner near the edge. It may be round with smooth outline or the border may be lobulated. Under the low-power lens the colony appears brown, finely granular near the periphery and more coarsely granular near the center. It is soft and moist, easily removed from the medium and easily suspended as a diffuse cloud in water. Gelatin is not liquefied. *B. coli* ferments dextrose and lactose with the production of gas as well as acid. It coagulates milk in 24 to 48 hours at  $37^{\circ}$  C. and renders it acid, produces considerable indol in pepton solution and grows abundantly on potato, often producing a brown color.

Intraperitoneal injection of cultures into guinea-pigs and rats

causes fatal peritonitis. Subcutaneous injection may also cause death but frequently results in a local abscess.

The cultures of *B. coli* on ordinary media are practically free from soluble poisons, but there is some evidence that soluble poisons may be produced by this organism under special conditions.<sup>1</sup> The bacterial cell substance is poisonous.

As it grows in the intestine the colon bacillus is a harmless commensal but with a distinct tendency to invade the living tissue and become pathogenic whenever the normal resistance is lowered. The bacilli doubtless pass through the intestinal wall in very small numbers during absorption of the food and are destroyed in the normal body fluids and tissues in a few hours. In intestinal disturbances the invasive properties and the virulence are increased. In many other regions of the body the colon bacillus gives rise to inflammation, often purulent in character. It is a common cause of cystitis and pyelitis, and is an important agent in the causation of peritonitis following perforation of the intestine. Generalized infection with *B. coli* is rather uncommon. The bacilli frequently enter the blood stream from the intestine during the death agony, and are often present in the heart's blood at autopsy, especially if this is delayed.

The detection of *B. coli* in any material is ordinarily regarded as evidence of fecal contamination. Examinations of drinking water and of shell liquor from oysters are, perhaps, the most frequent applications of this principle. Fermentation tubes of dextrose broth are inoculated with measured quantities of the liquid to be tested, 0.01 c.c., 0.1 c.c. and 1 c.c. Those cultures in which gas is produced are plated on litmus lactose media and the typical colonies transplanted to gelatin, milk, fermentation tubes of dextrose broth and agar slants, and for final identification the agglutination test with a known colon-immune serum may be employed.

**Bacillus (Lactis) Aërogenes.**—Escherich described this organism in 1886 as distinct from *B. coli*. It is non-motile, is usually

<sup>1</sup> See Vaughan and Novy: Cellular Toxins, Phila., 1902, p. 220.



FIG. 135.—Friedländer's pneumobacillus; gelatin stab culture, showing the typical nail-head appearance and the formation of gas bubbles, not always present. (From McFarland after Curtis.)

capsulated and its colonies are thicker and less spreading. In other respects it does not differ materially from *B. coli* and many authorities regard it as a variety of this species. *B. aërogenes* was found by Escherich in the upper part of the small intestine. It is commonly present in ordinary cow's milk and has been found in the urine in cystitis<sup>1</sup> and pyelitis.

**Bacillus (Bacterium) Pneumoniae.**—

This organism was obtained by Friedländer in 1883 on gelatin plates inoculated with material from cases of pneumonia and was confused by him with the organisms which he observed microscopically in abundance in his material. The latter were undoubtedly pneumococci (See *Diplococcus pneumoniae* p. 266). *B. pneumoniae* is rather common in the upper air passages and occurs in the lungs in some cases of pneumonia. It is non-motile, capsulated and Gram-negative, and in nearly all respects quite like *B. aërogenes*. The nail-shaped culture in gelatin stab is regarded as specially typical.

**Bacillus (Bacterium) Rhinoscleromatis.**—

This organism was described by von Frisch in 1882. It is readily obtained, often in pure culture, by incising the lesion of rhinoscleroma and spreading the blood thus obtained on an agar surface.<sup>2</sup> It is also found in abundance by microscopic

<sup>1</sup> Luetscher, *Johns Hopkins Hosp. Bull.*, 1911, Vol. XXII, pp. 361-366.

<sup>2</sup> Wright and Strong: *New York Med. Journ.*, 1911, Vol. XCIII, pp. 516-519.

examination of sections of rhinoscleroma tissue. *B. rhinoscleromatis* is capsulated, non-motile and in morphology and cultural characters indistinguishable from *B. pneumoniae*. It is Gram-negative when stained by the usual technic. Its etiological relation to rhinoscleroma is somewhat uncertain.

Rhinoscleroma is a disease characterized by the occurrence of circumscribed grayish nodules in the mucous membrane of the nose, which tend slowly to extend without ulceration. Histologically the lesion is composed of granulation tissue and fibrous tissue with lymphocytic infiltration. Many of the cells appear swollen and vacuolated, so-called lace-cells, and in and near these the bacilli are present in large numbers. The disease occurs in Europe and has been seen in a number of Russian immigrants to the United States.

**Bacillus (Mucosus) Capsulatus and Bacillus Ozenæ** also occur on the mucous membranes of the upper air passages. They do not appear to be specifically different from *B. pneumoniae* of Friedlaender.

**Bacillus Enteritidis.**—Gaertner in 1888 isolated this organism from the spleen of a man who died in an epidemic of meat poisoning in which 57 persons were made ill. The meat was derived from a cow, sick at the time of slaughter, and this same organism was found in the meat which had not been sold. The bacillus is of the same size and shape as *B. coli*, but is more actively motile and has more flagella. It ferments dextrose with the production of gas, does not ferment lactose nor coagulate milk, nor does it produce an amount of indol appreciable by testing with sulphuric acid and nitrite. Its cultures are highly toxic, even after they have been boiled.<sup>1</sup> A typhoid-immune serum agglutinates *B. enteritidis* in fairly high dilutions. The cases of food poisoning in which it was found were characterized by vomiting and diarrhea and at autopsy by severe enteritis and swelling of the lymph follicles of the intestine. Food poisoning of this type seems to be rather common.<sup>2</sup>

<sup>1</sup> Vaughan and Novy: Cellular Toxins, 1902, p. 207.

<sup>2</sup> Anderson, Poisoning from *Bacillus enteritidis*. The Military Surgeon, 1912, Vol. XXXI, pp. 425-29. See also Marshall's Microbiology, 1920.

**Bacillus Suipestifer (B. Salmonii).**—This organism occurs in the intestinal contents of hogs and in the blood in the late stages of hog cholera, and was for a time believed to be the cause of this disease. More recent studies indicate that the etiological factor is a filterable virus (See page 392.) *B. suipestifer* resembles *B. enteritidis* very closely.

**Bacillus Icteroides** was described by Sanarelli in 1897 as the cause of yellow fever, a disease now known to be due to a different organism (page 374). It cannot be specifically distinguished from *B. suipestifer*.

**Bacillus Psittacosis** was found by Nocard in 1892 to be the cause of an epidemic pneumonia transmitted to man from diseased parrots. It resembles *B. coli* but may be distinguished by inoculating parrots, for which it is extremely virulent.

**Bacillus Typhi Murium.**—Löffler in 1890 found this organism to be the cause of a fatal epizootic among laboratory mice. It forms gas and acid from dextrose, does not produce indol nor coagulate milk. Mice are very susceptible and the organism has been employed as a practical means of destroying mice. It seems, however, not to be altogether harmless for larger animals and for man, and it is believed that some of the paratyphoid fever following food poisoning in man has been due to this particular organism.

**Bacillus (Fæcalis) Alkaligenes.**—This organism is occasionally found in human feces and is of importance because of the possibility of mistaking it for the typhoid bacillus, which it resembles in most respects. It does not produce acid from any of the sugars nor is it agglutinated by typhoid serum. It is not known to cause disease.

**Bacillus Paratyphosus.**—In certain irregular fevers in man resembling typhoid fever there have been found bacteria somewhat intermediate in character between *B. coli* and *B. typhosus*. These have sometimes been cultured from the blood stream; at other times from the feces. The presence of specific agglutinins in the patient's blood is further evidence of the pathogenic relationship

of these organisms. They ferment dextrose with the formation of gas but do not coagulate milk. Two serologically distinct types are recognized, paratyphoid A and paratyphoid B. The diseases in which they occur are sometimes traceable to meat poisoning. *B. enteritidis* and *B. typhi murinum* possibly occur in the circulating blood of man at times as paratyphoid bacilli. *B. psittacosis* is usually regarded as a paracolonic bacillus. **Bacillus Typhosus.**—Eberth in 1880 and Koch in 1880 observed this organism in the spleen and mesenteric lymph glands

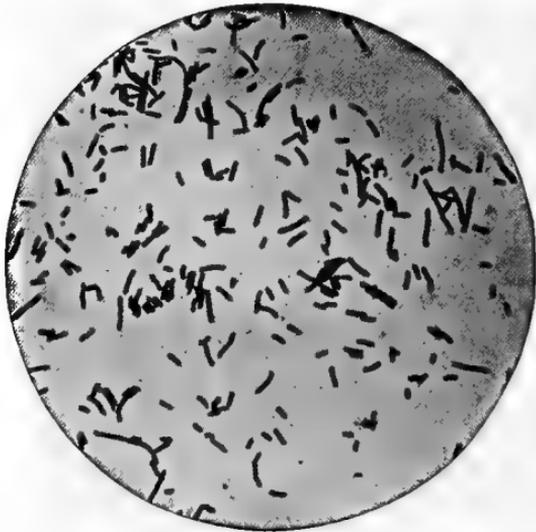


FIG. 136.—Bacillus of typhoid fever. X 1000.

of persons-dying of typhoid fever. Gaffky in 1884 obtained the first pure cultures. Metchnikoff and Besredka in 1911 succeeded in causing typical typhoid fever in anthropoid apes (chimpanzees) by feeding them cultures of *B. typhosus*, thus adding conclusive proof of the causal relationship of this organism to typhoid fever to the abundant strong evidence previously at hand.

*B. typhosus* is found in the intestinal contents, mesenteric lymph glands, spleen, blood and urine of patients suffering from typhoid fever. It is 0.5 to 0.8 $\mu$  in width and 1 to 4 $\mu$  in length,

<sup>1</sup> *Annals de l'Institut Pasteur*, 1911, Vol. XXV, 193-221.

commonly occurring single or in short threads, stains readily with anilin dyes and is Gram-negative. It is actively motile and



FIG. 137.—*Bacillus typhosus* showing flagella. (After McFarland.)

possesses 10 to 20 peritrichous flagella. Spores have not been observed.

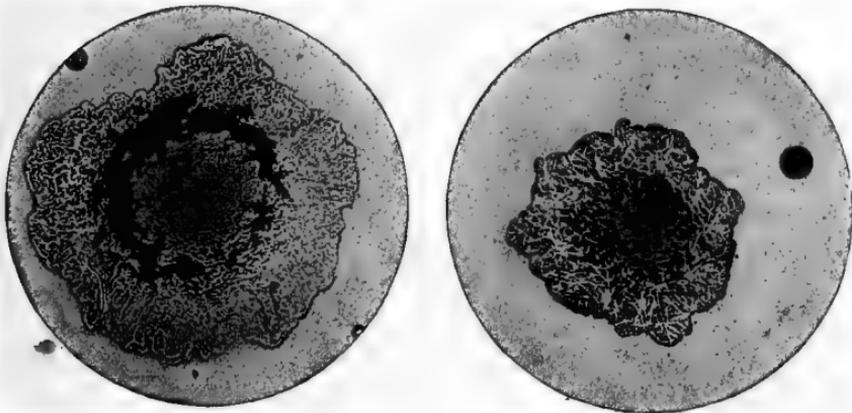


FIG. 138.—Colonies on gelatin plate two days old of (A) *Bacillus coli*, and (B) *Bacillus typhosus*, (From Jordan after Helm.)

The organism grows readily on ordinary media but not so luxuriantly as *B. coli*. The colony is smaller but relatively more

spread out and thinner than that of *B. coli*, and in semi-solid media the growth of *B. typhosus* may diffuse for quite a distance because of its active motility. Dextrose is fermented with the production of acid but without gas. Lactose is not fermented. Litmus milk is rendered slightly acid and later becomes alkaline without coagulation. On potato the growth is almost invisible. In Dunham's pepton-salt solution, indol is not produced in sufficiently large quantities to be detected, but indol can be demonstrated in old cultures in 5 per cent pepton. Growth is most rapid at 37°-39° C., but occurs also at room temperature.

*B. typhosus* is killed by moist heat at 60° C. in 10 to 15 minutes, and by 5 per cent carbolic acid or 1-1000 mercuric chloride in three to five minutes, when exposed in aqueous suspension. It resists drying for several days and may be alive in dry dust. The longevity of *B. typhosus* in surface waters has been studied by several investigators without full agreement. In general *B. typhosus* would seem to survive in such water only for three to ten days except it be taken up by aquatic animals, such as the shellfish, when it may persist for several weeks. In soil and in frozen material the bacillus may live a much longer time. Freezing and thawing destroys a large percentage of the bacilli in a given liquid but does not destroy them all.

The poisons are intimately associated with the cell substance, and it is not often that culture filtrates are found to be toxic. The dead germ substance is somewhat poisonous, and when it is disintegrated by physical comminution or by digestion with dilute alkali at a high temperature, or by the action of serum<sup>1</sup> upon it, there are set free quite powerful poisons or perhaps different quantities of the same poison.

The various small laboratory animals are very susceptible to intraperitoneal inoculation with *B. typhosus* and usually die in 24 to 48 hours with acute peritonitis and bacteremia. The disease produced bears no resemblance to typhoid fever in man. In chimpanzees a very typical attack of typhoid fever has been

<sup>1</sup> Zinsser: *Journ. Exp. Med.*, 1913, Vol. XVII, pp. 117-131.

caused by feeding the organisms, with resulting lesions in the intestine, bacilli in the blood and spleen, and a continued fever.

Typhoid fever exists generally throughout the temperate zone, is present throughout the year but most prevalent in the fall. The usual mode of infection is undoubtedly through food and drink. The bacilli swallowed survive in part the action of the gastric juice and so gain the lumen of the duodenum. The first multiplication seems to occur here<sup>1</sup> in a location fairly free from bacteria in health. The infection extends along the wall of the intestine, involving especially the lymphatic structures, solitary glands and Peyer's patches. The bacteria pass into the lymph stream to be carried to the mesenteric nodes, spleen and into the blood. At the onset of definite symptoms of typhoid fever the bacilli have usually reached the general blood circulation. Subsequently the infection reaches the gall bladder, perhaps by extension along the common bile duct and cystic duct or perhaps by the blood stream through the liver; the organisms also pass through the kidney and multiply in the contents of the urinary bladder. They are present in the rose spots on the skin. The bacilli are often present in the feces in small numbers, the abundance of other organisms making their isolation and recognition difficult. At times localized inflammations due to *B. typhosus* develop elsewhere in the body, as in the lungs. It is evident therefore that the bacilli may leave the body of the patient through many channels, but chiefly with the urine and feces. Even after recovery the patient may continue to discharge virulent bacilli for months or years. It is estimated that one per cent of recovered cases are persistent carriers of the infectious agent.

The bacteriological diagnosis of typhoid fever depends upon isolation and recognition of the germ or detection of specific substances in the blood produced by the patient as a reaction to the presence of *B. typhosus*. *B. typhosus* is sought by blood culture (see page 104) diluting the blood with large amounts of broth (200 c.c. of broth to 2 c.c. of blood) as well as inoculating tubes

<sup>1</sup> Hess: *Journ. Infect. Diseases*, 1912, Vol. XI, pp. 71-76.

of bile and the usual agar plates; by cultures from the rose spots, and by cultures inoculated with duodenal fluid. These methods are likely to be successful very early in the disease. Later it is well to make cultural examination of the feces and urine, especially just before discharging a recovered patient.

The detection of *B. typhosus* in feces requires special care. It is essential that the primary plate cultures should be made upon some medium which will specially favor the detection of colonies of this organism. Several different technical methods have been developed of which the most important are those of Krumwiede and his coworkers and of Russell and his followers at the Army Medical School. Krumwiede<sup>1</sup> and Pratt employ an agar containing brilliant green, which exercises a relative inhibition upon most of the other fecal bacteria, thus favoring the development and detection of *B. typhosus*. Russell's method as employed in the U. S. Army utilizes Endo's medium.<sup>2</sup> The fecal material is mixed with broth to form an opaque suspension. A drop of this suspension is transferred to the surface of an Endo plate and is spread over the surface by means of a sterile bent glass rod. After incubation at 37° C. for 24 hours the typhoid, paratyphoid and dysentery bacilli appear as small, clear, almost colorless, transparent

<sup>1</sup> Krumwiede, Pratt and McWilliams, *Journ. Infectious Diseases*, 1916, 18, 1.

<sup>2</sup> For Endo's medium a stiff lactose agar is prepared containing Liebig's extract 5 grams, salt 5 grams, pepton 10 grams, lactose 10 grams and agar 30 grams in 1000 c.c. of water. This is sterilized in flasks containing 100 c.c. each. When needed the contents of a flask are liquefied, enough sodium hydroxide is added to make the reaction 0.2 per cent acid to phenolphthalein and to it are then added 10 drops of saturated alcoholic solution of basic fuchsin, and approximately 20 drops of a freshly prepared solution of sodium sulphite, or just sufficient to decolorize the fuchsin. The amounts of fuchsin and of sulphite may be altered to suit different lots of medium and it is well to test out several different quantities upon known cultures of typhoid, paratyphoid and various dysentery bacilli before deciding upon the exact amounts of fuchsin and of sulphite of particular samples to be used. Both of these substances, but especially the sulphite, are subject to variation in composition as they are obtained in the market. The material is well mixed and poured into 8 or 10 Petri dishes, allowed to solidify and dried in the incubator to remove water from the surface before use. Large Petri dishes, 15 cm. in diameter, are preferable.

colonies while the colon and aerogenes bacilli will have produced larger colonies colored pink to deep red or even showing a metallic luster of the fuchsin. In every test it is wise to inoculate one plate with a fecal suspension to which a known culture of the organism in question has been added so that there may be known standard colonies for comparison. The suspected colonies are then transplanted to Russell's double-sugar medium, an agar<sup>1</sup> containing 1 per cent lactose, 0.1 per cent glucose and Andrade's indicator. This medium is slanted so that the lower end of the tube is entirely filled with agar for a depth of one half inch with the inclined surface above this. Each colony is transplanted by stabbing the inoculating needle deep into the cylindrical butt of the tube and also stroking the inclined surface, the result being a combined streak and stab-culture. After incubation for 24 hours any of these cultures which show a growth characteristic of the typhoid bacillus, that is, pink butt and almost colorless upper portion under the streak, without any gas bubbles, are tested for agglutination against a known anti-typhoid agglutinating serum. The examination is thus completed in two or three days.

The specific antibody ordinarily sought in the blood is the typhoid agglutinin. A few drops of blood in a Wright's capsule suffice for the microscopic test (see page 218). A young active culture (broth three hours) of a known *B. typhosus* is used, and the serum is tested in dilutions of 1:20, 1:40 and 1:80, observed for an hour. Normal serum rarely shows any clumping in any of these dilutions at the end of an hour. This agglutination test is of little or no value if the patient has received typhoid vaccine within a year.

Dreyer<sup>2</sup> and his coworkers have devised a technic for measuring

<sup>1</sup> The double-sugar medium is a 2 to 3 per cent agar, neutral to litmus, to which has been added 1 per cent lactose and 0.1 per cent glucose. On this medium *B. typhosus* does not change the color when it is growing on the surface, but produces a red (acid) color about the stab. See Russell, *Journ. Med. Resch.*, 1911, Vol. XX, pp. 217-229.

<sup>2</sup> Dreyer and Inman, *Lancet*, July 31, 1915, p. 225; Dreyer and Torrens, *ibid.*, 1915, ii, p. 1369; Dreyer, Walker and Gibson, *ibid.*, Feb. 13, 1915, p. 324; Dreyer and

the agglutination titre of a serum more accurately than has previously been possible. A standard agglutinable culture is employed. This is a formolized suspension of *B. typhosus* prepared so as to possess a standard opacity and a definite agglutinability. By this method it has been possible to demonstrate a distinct increase in agglutinins from the sixteenth to the twenty-fourth day of typhoid fever. Professor Dreyer and his followers have employed this test to diagnose enteric fevers (typhoid and paratyphoids) in patients who had been previously inoculated with the corresponding bacterial vaccines. Pappenheimer<sup>1</sup> has demonstrated conclusively, however, that such a rise in agglutinins in inoculated men is not a reliable criterion for diagnosis. In such cases the diagnosis of typhoid fever can be proven only by detection of the typhoid bacillus, or by the demonstration of the typical pathological changes at autopsy.

Transmission of the disease takes place in a variety of ways. To the best of our knowledge, the typhoid bacilli come only from human individuals infected with them. Some of these actually suffer from typhoid fever, while others are merely healthy carriers of the infection. From them as centers the bacilli are distributed by contact and by intermediate object. *B. typhosus* is able to live for a considerable time in the external world, probably for one to three weeks in ordinary surface waters and longer in soil. It is able to grow and multiply in some foods, especially milk. Water supplies contaminated with feces and urine from patients or from healthy carriers have unquestionably been an important factor in the causation of typhoid fever in the past, and the provision of a supply of drinking water free from all suspicion of recent mixture with sewage is the first step in the control of this

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Walker, *ibid.*, Sept. 2, 1916, p. 419; Walker, *ibid.*, Nov. 25, 1916, p. 896; Dreyer, Gibson and Walker, *ibid.*, Apr. 8, 1917, p. 766; Walker, *ibid.*, Vol. I, p. 17. Walker, *ibid.*, April 14, 1917, p. 568; Dreyer and Inman, *ibid.*, March 20, 1917, p. 365. Fennel, *Journ. Amer. Med. Assn.*, 1918, 70, p. 590.

<sup>1</sup> Pappenheimer, Trench Fever—Report of Commission, Medical Research Committee, American Red Cross, Oxford Press, 1918, pp. 80-142.

disease in a community. The infected oyster from a sewage-polluted oyster bed is another source of typhoid fever. The contamination of food by permanent carriers of the bacilli is difficult to control. All possible means need to be employed to prevent these persons from handling foods prepared for consumption, and especially milk. Flies (*Musca domestica*) are important aids in the transfer of bacilli from discharges containing them, especially from open privies, to foods exposed for sale or being prepared in neighboring unscreened kitchens.

The prevention of typhoid fever by restricting the distribution of the bacilli has been only partially successful in civil life and in armies on a war footing it has proven wholly ineffective. Vaccination to prevent typhoid fever was first extensively practised by Wright in the British army. Russell,<sup>1</sup> following the method developed by Wright and Leishman, has prepared a vaccine with which practically the whole U. S. army has been inoculated. The vaccine is a suspension of *B. typhosus* in salt solution, standardized by microscopic count of the bacterial cells, sterilized by heating at 53° to 56° for an hour and preserved by the addition of 0.25 per cent trikerosol. Three injections are given subcutaneously at intervals of 10 days, 500 million bacilli at the first dose and 1000 million at each of the following doses. The results in the U. S. army have been remarkably good, rivaling those obtained with the use of vaccinia in the prevention of small-pox.

Experience has shown, however, that the immunity conferred in this way is not always adequate to prevent the occurrence of typhoid fever. A considerable number, in the aggregate, of cases of bacteriologically proved typhoid fever occurred in the American Expeditionary Forces in France in 1918 and 1919 and in a few instances definite outbreaks of the disease occurred, such that they might be termed small epidemics. Nevertheless it may justly be concluded that typhoid vaccination has relegated

<sup>1</sup> Russell: *Boston Med. and Surg. Journ.*, 1911, Vol. CLXIV, pp. 1-8; Harvey Lecture, 1913.

typhoid fever to a very minor position as a cause of illness and death in armies, whereas in previous wars it has often shown itself the chief cause of death.

**Bacillus (Bacterium) Dysentericæ.**—Shiga in 1898 isolated this organism from the feces of patients suffering from dysentery, showed that it is agglutinated by the blood of dysenteric patients in high dilutions and not by normal human blood.

*B. dysentericæ* is about  $0.6\mu$  in width by 2 to  $4\mu$  in length, usually single and non-motile. It stains readily and is Gram-negative. Involution forms are common in older cultures. The organism grows readily on ordinary media and its cultures resemble those of *B. typhosus* very closely. Gelatin is not liquefied; no indol is produced in pepton solution; no gas is formed from any of the sugars; milk is rendered slightly acid and then alkaline without coagulation. It differs from the typhoid bacillus in failing to ferment mannite and maltose.

When cultures are injected intravenously into rabbits severe diarrhea is produced, which may be bloody. The animal usually dies in a few days, and if it recovers often exhibits paralysis of the hind legs. Similar results are obtained by the injection of dead bacilli, indicating that the effect is toxic rather than infectious. Kittens and puppies have been infected by introducing dysentery bacilli into the stomach, resulting in diarrhea with the intestinal lesions of dysentery. The toxins seem to be intimately bound up in the cells in young cultures, but readily set free into solution after the bacilli are killed. Culture filtrates, of which 0.02 c.c. suffices to kill a rabbit in 24 hours, have been obtained.

Acute epidemic dysentery is the disease in which this organism is found. The infectious agent is found on the membrane of the large intestine, which is diffusely inflamed, often covered with a fibrinous exudate, or by a pseudo-membrane. Later numerous ulcers may be formed. The bacillus is also present in the feces, especially during the first few days of the disease. It may be found by plating on Endo's medium by the same method as has

been described for typhoid bacilli. After the first week of the disease, search for the bacillus is less promising. The bacilli are only very rarely found in the blood or internal organs. The blood of the patient agglutinates the bacillus of Shiga in dilutions of 1 to 50 or 1 to 100. The mortality is about 25 per cent, but variable in different epidemics.

Horses have been immunized with cultures of *B. dysenteriae* and the serum of these animals has been found to be antitoxic as well as bactericidal. Its use in treatment has given promising results and seems to cause a reduction in the death rate of about 50 per cent.

**Paradysentery Bacilli.**—Flexner in 1899 isolated a bacillus from cases of dysentery in the Philippines which at the time was considered to be the same as the Shiga bacillus. Kruse, although he found the Shiga bacillus in epidemic dysentery, found a somewhat different organism in "asylum dysentery" or pseudo-dysentery, which proved to be identical with the Flexner bacillus. Between 1901 and 1903 a number of strains of bacilli resembling somewhat *B. dysenteriae* were isolated by different investigators from epidemics of diarrheal disorder, especially in the Eastern United States. The paradysentery bacilli are indistinguishable from *B. dysenteriae* in morphology or in cultures on ordinary media. They are all much less toxic to rabbits than the Shiga bacillus, and they all ferment mannite with the production of acid, while the Shiga bacillus does not.

The bacteria considered in this chapter are all inhabitants of the alimentary canal (mouth, pharynx, intestine) of man or other mammals. They are small bacilli, Gram-negative, without spores and without the ability to liquefy gelatin. They vary from each other in motility, possession of flagella, possession of capsules, and in their ability to form poisonous substances and to ferment various carbohydrates. Media containing various carbohydrates along with an indicator such as litmus to show the production of acid, and contained in fermentation tubes so as to measure the production of gas, are very useful in differentiat-

ing<sup>1</sup> the various types of bacteria in this group. Thus, in a broth containing maltose, *B. typhosus* produces acid, *B. coli* produces acid and gas, and *B. dysenteriae* produces neither. Specific agglutination with the serum of an animal immunized with a known culture constitutes the most important test in the identification of unknown forms falling within this group. This test may be used with the capsulated species after they have lost the tendency to form capsules through propagation on artificial media.<sup>2</sup> For a detailed discussion of the classification and fermentative reactions of the colon-typhoid group reference may be had to the paper of Winslow,<sup>3</sup> Kligler and Rothberg, with which is included an extensive bibliography.

<sup>1</sup> Hiss has devised a very useful medium for this purpose which obviates the necessity of using the fermentation tube to detect the gas. His serum-water medium is made by mixing beef serum, 1 part, with distilled water, 2 to 3 parts, and steaming 15 minutes to destroy enzymes. Pure litmus solution (about 1 part of a 5 per cent solution to 100 parts of the medium) is then added to produce a deep blue color. The medium is divided into several portions and 1 per cent of the desired carbohydrate is added to its respective portion. The sugar serum-water media are then sterilized at 100° C., on three days. Fermentation is shown not only by the reddening of the litmus but also by coagulation of the liquid medium, and gas production is shown by bubbles caught in the coagulum. (Hiss and Zinsser: Text-book of Bacteriology, 1910, p. 132.)

<sup>2</sup> Fitzgerald: Proc. Soc. Biol. and Med., 1913, Vol. X, pp. 52-53.

<sup>3</sup> Winslow, Kligler and Rothberg, *Journal of Bacteriology*, 1919, 4, 429-503.

## CHAPTER XXIII

### BACTERIACEÆ: BACILLUS MALLEI AND MISCELLANEOUS BACILLI

**Bacillus (Bacterium) Mallei.**—Löffler and Schütz in 1882 obtained pure cultures of this organism from glandered horses and produced glanders by the injection of these pure cultures.

The bacillus is 0.3 to 0.5 $\mu$  wide and 2 to 5 $\mu$  long, usually straight with rounded ends, but sometimes irregular in shape. Filamentous and branched forms have been observed in cultures.



FIG. 139.—*Bacillus mallei* from an agar culture.  $\times 1100$ . (After Park and Williams.)

It is not motile. Spores have not been observed. *B. mallei* is stained with moderate difficulty and often stains unevenly like the tubercle and diphtheria bacilli. After being stained, the bacterium is easily decolorized in weak acid or alcohol; it is also Gram-negative. Cultures develop on ordinary media, better on glycerinated media, at temperatures ranging from 22° to 42° C.; best at 37° C. On potato at 37° C. a viscid yellowish-brown

growth develops surrounded by a greenish stain on the potato. Gelatin is not liquefied. The organism is killed by moist heat at 55° C. in 10 minutes, and in 2 to 5 minutes by 5 per cent carbolic acid or 1 to 1000 mercuric chloride. It survives drying for only a few weeks and dies out quickly in water. Many mammals are susceptible to inoculation, including horses, guinea-pigs, cats and dogs. Cattle are immune. Man is susceptible and human glanders frequently ends in death.

Mallein is analogous to tuberculin. A culture in glycerin broth incubated for six weeks is steamed and filtered, and the filtrate evaporated to one-tenth the original volume is the mallein. This substance is toxic to animals suffering from glanders but not poisonous to healthy animals.

Glanders is a disease most common in horses, mules and asses. It begins as an inflammation of the nasal mucosa with localized nodular infiltrations which later ulcerate. The infection may become generalized at once causing acute glanders and death in one to six weeks, or it may progress very slowly and persist for years as chronic glanders. The chronic type is common in horses. After apparent recovery from the disease, nodules containing living bacilli may be found in the lungs. Histologically the glanders nodule consists of granulation tissue infiltrated with leukocytes and tending to become purulent at the center. The bacilli leave the body in the nasal secretion and in the discharge from ulcers. Infection of equines takes place most frequently by ingestion of food soiled by these discharges. In man the disease seems to result from inoculation of small wounds in the skin. It often runs an acute course terminating in death, but chronic glanders with recovery also occurs in man. A few sad laboratory accidents in which workers have become inoculated with glanders have emphasized the necessity for caution in handling this organism.

The bacteriological diagnosis depends upon (1) identification of *B. mallei*, (2) reaction of the animal to mallein, (3) agglutination reaction, and (4) complement fixation. For the recognition of the bacillus, some of the suspected material is suspended in

broth and injected into the peritoneal cavity of a male guinea-pig (method of Straus). If *B. mallei* is present a general inflammation of the peritoneum develops and after three or four days the testicles of the animal become swollen, inflamed and later suppurate. They may burst through the scrotum. Cultures should be made from this pus on plates of glycerin agar and the colonies transplanted to potato at 37° C. Very few other organisms give rise to a similar pathological picture in the guinea-pig. At the same time the mallein test is carried out by injecting 0.2 c.c. of the concentrated mallein diluted with 0.25 per cent solution of carbolic acid into the suspected horse. The presence of glanders is indicated by a rise in temperature of 2° to 5° F., signs of general intoxication, and especially by swelling and inflammation at the site of injection. For the agglutination test the serum is diluted to 1:500 to 1:3000. Positive results with lower dilutions may apparently be given by normal horses. The complement-fixation test follows the principles of Wassermann test for syphilis, a culture of *B. mallei* being employed as antigen.<sup>1</sup> Attempts at immunization have not been practically successful.

**Bacillus (Bacterium) Abortus.**—Bang and Stribolt, working in Denmark in 1897, isolated this organism from the uterus of a cow suffering from the disease known as contagious abortion, and reproduced the disease by inoculating healthy cows with these cultures. The same organism was isolated by MacNeal and Kerr<sup>2</sup> in 1910 from aborting cows in the United States. The organism is of interest because of its behavior toward oxygen when first isolated. It fails to grow in the air or in hydrogen, but grows in a partial pressure of oxygen somewhat below that of the atmosphere. The bacillus is pathogenic for a number of different mammals, and in guinea-pigs it causes granulomatous lesions resembling somewhat those of tuberculosis.<sup>3</sup> The organism

<sup>1</sup> Mohler and Fichorn: Twenty-seventh Annual Rep. Bur. Anim. Industry, U. S. Dept. Agr., 1910; reprinted as *Circular* 191 (1912).

<sup>2</sup> MacNeal and Kerr, *Journ. Infectious Diseases*, 1910, 7, p. 469.

<sup>3</sup> Smith and Fabyan: *Centr. f. Bakt.*, I, Abt. Orig., 1912, Bd. LXI, S. 549-555. Fabyan, *Journ. Med. Rsch.*, 1912, Vol. XXV, p. 441-488.

occurs rather frequently in market milk. It is not known to infect man.

**Bacillus (Bacterium) Acne.**—This minute non-motile organism, first described by Gilchrist, is constantly present in the papules and pustules of the common skin affection, acne vulgaris. Cultures are most readily obtained by expressing, with careful asepsis, some of the cheesy pus from a recent papule and mixing it with 2 c.c. of ascitic fluid in a test-tube. Dilutions from this are made to similar amounts of ascitic fluid in series (about five tubes in all). To the first tube one adds 8 c.c. of sterile glucose broth and covers this with a layer of paraffin oil (albolene). To each of the remaining tubes are then added 8 c.c. of melted glucose agar cooled to 50° C., the contents of each tube mixed without introducing air bubbles and then quickly solidified by immersion in cold water. The colonies of *B. acne* develop at 37° C. after five to ten days, beginning about 8 mm. beneath the surface, and they grow best in a narrow zone about 5 mm. in depth. The colonies attain a large size (3 mm.) and an abundant supply of bacillary substance for preparation of vaccine may be obtained by thrusting a sterile glass capillary into such a colony. In its behavior to oxygen when first isolated the organism exhibits the same peculiarity as the bacillus mentioned in the preceding paragraph.

Sometimes the agar cultures fail. In that event one may repeat the series of ascitic-glucose-agar dilution cultures by inoculating with sediment from the ascitic-broth tube, which has been incubated ten days. This broth culture usually develops an abundant growth of staphylococci for the first few days but after ten days the cocci will have disintegrated to a considerable extent and *B. acne* will usually have become the most numerous organism in the sediment.

**Bacillus Fusiformis (Vincenti).**—In an ulcerative disease of the tonsils, known as Vincent's angina there occur very large numbers of fusiform rods, 0.3 to 0.8 $\mu$  in thickness and 3 to 10 $\mu$  long, associated with spiral filaments with rather coarse windings.

These associated organisms also occur in other ulcerative conditions of the mouth and pharynx and rarely elsewhere in the body. The spiral filaments are evidently ordinary mouth spirochetes. The fusiform bacillus is an anaerobe and it has been grown in artificial culture.<sup>1</sup>

**Bacillus (Lactobacillus) Bifidus.**—Tissier in 1898 showed that the Gram-positive bacillus predominant in the stools of healthy nurslings is not a form of *B. coli* as had been supposed since the investigations of Escherich (1886) but is an entirely different organism. He obtained cultures by making a series of dilutions (five to ten tubes) in tall tubes of glucose agar by the method of Veillon (see page 116). The colonies develop best about 1 to 2 cm beneath the surface after three to eight days at 37° C. In these colonies many of the bacilli show dichotomous branching. Bifid forms are also sometimes seen in stools and in mixed cultures in broth. The organism produces a strong acid reaction and the cultures soon die out. The bifid forms are doubtless involutions due to presence of unfavorable amounts of acid.

**Bacillus (Lactobacillus) Bulgaricus.**—This organism is a rather large rod 1 by 6 $\mu$  approximately. It occurs in milk and milk products and is especially abundant in milk fermented at 40° C. for three or four days. Colonies may be obtained on plates of milk agar (1:2) incubated at 37° C. in hydrogen. A high degree of acidity (lactic acid) is produced in the cultures of this organism, and it is employed to some extent in the preparation of acid-milk-beverages.

**Bacillus (Proteus) Vulgaris.**—Hauser in 1885 discovered this organism in putrefying infusions of animal matter. It is an actively motile rod 0.6 $\mu$  in thickness and exceedingly variable in length, with abundant flagella. Spores have not been observed. It is universally distributed in the soil and is abundant in putrefying flesh. Gelatin is rapidly liquefied. Food poisoning in man has been ascribed to this organism. It is also capable of infecting laboratory animals when injected in large doses.

<sup>1</sup> Tunncliff: *Journ. Infectious Diseases*, 1906, 3, p. 148; *ibid.*, 1912, 10, p. 1.

**Bacillus Pyocyaneus (Pseudomonas Pyocyanea).**—Gessard in 1882 isolated this organism from green pus. It is a slender rod, actively motile. A soluble blue-green pigment is produced in the cultures. Gelatin is liquefied. Guinea-pigs are susceptible to intraperitoneal inoculation. In man the organism is most common in the pus from wounds, where its presence is considered as only mildly deleterious. The bacillus has also been found in otitis media and a few cases of fatal generalized infection with *B. pyocyaneus* have been described.

**Bacillus Fluorescens var. Putidus.**—This non-pathogenic actively motile rod is common in putrefying material. It produces spores when grown on quince jelly. The greenish-yellow pigment is soluble in water. Gelatin is not liquefied. A number of different fluorescing bacilli have been found in the soil and surface waters. Some of them liquefy gelatin.

**Bacillus Violaceus.**—This is a non-pathogenic water bacterium which produces a pigment of deep violet color. It is actively motile and liquefies gelatin rapidly. The pigment is not soluble in water. Several different bacteria are known which produce a violet pigment.

**Bacillus Cyanogenus (Pseudomonas Syncyanea).**—This non-pathogenic actively motile organism produces a bluish-black pigment which is soluble in water. Gelatin is not liquefied. *B. cyanogenus* sometimes causes trouble in dairies as its growth in milk imparts a blue color to it.

**Bacillus Prodigiosus.**—This small oval organism grows rapidly at room temperature on ordinary media and is occasionally observed on foodstuffs such as moist bread and potatoes. Ordinarily it is encapsulated and non-motile, but it sometimes possesses flagella. Gelatin is rapidly liquefied. A red pigment is produced at room temperature but not at 37° C. This pigment is insoluble in water. Large doses of *B. prodigiosus* injected into animals sometimes give rise to signs of intoxication.

## CHAPTER XXIV

### SPIRILLACEÆ AND THE DISEASES CAUSED BY THEM

**Spirillum Rubrum.**—Esmarch discovered this organism in the body of a dead mouse. It is of chief interest as a harmless example of spiral bacterium for class study. It grows rather slowly at room temperature without liquefying gelatin. A dull red pigment, insoluble in water, is produced even in the absence of oxygen. Growth occurs at 37° and also in the refrigerator at 5° to 10° C. When grown at temperatures above 20° C. the organism is a relatively short, slightly bent rod and its spiral nature is not very evident. At 10° C. beautiful long spirals are produced in broth cultures. It is actively motile.

**Spirillum Cholerae (Vibrio Cholerae).**—Koch in 1883 discovered this organism in the intestinal discharges of patients suffering from Asiatic cholera, and continuing his studies in India in the same year established this organism as the probable cause of cholera. It occurs in the intestinal contents and feces of cholera patients, often in great abundance, rarely in the feces of healthy persons, and has been found at times in surface waters, and in drinking water during epidemics of cholera.

*Sp. cholerae* is a curved cylinder about 0.4 $\mu$  in thickness and 1.5 $\mu$  in length. In older cultures in broth long spiral forms occur. There is considerable variation in shape in cultures older than 48 hours. The organism is actively motile and possesses a single flagellum at one end. Those short spirals showing more than one flagellum are not to be regarded as true cholera germs. Spores have not been observed. The spirillum stains readily and is Gram-negative.

It grows well and rapidly on ordinary media. The reaction needs to be distinctly alkaline to litmus as the organism is very

sensitive to acids. Colonies appear on gelatin at 22° C. in about 24 hours as circular disks with somewhat irregular border and granular interior. A few hours later the gelatin begins to liquefy. In pepton-salt solution both indol and nitrate are formed, so that the addition of sulphuric acid gives rise to the red color due to nitroso-indol. This has been called the cholera-red reaction, but it is of course not a specific test for this organism. In milk there occurs abundant growth without apparent change in the medium. In broth, growth is extremely rapid and a pellicle forms in 24

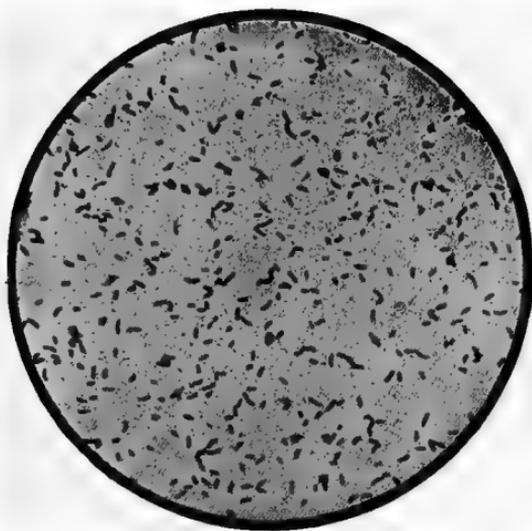


FIG. 140.—*Cholera vibrios*, short forms. (From Kolle and Schürmann after Zettnow.)

hours. The rapid growth in pepton solution (pepton 1 per cent, salt 0.5 per cent) and the tendency for the organisms to collect at the surface are utilized in practical enrichment for purposes of diagnosis. The spirillum is an obligate aërobie. It is very easily killed. If dried on a cover-glass at 37° C., the organisms are all dead in two hours. It seems impossible, therefore, for the infection to be distributed in dry dust. Moist heat at 56° C. kills the cholera spirilla in 30 minutes. They are also easily killed by chemical germicides. Milk of lime is recommended for the disinfection of excreta. The organism lives for several weeks

in surface waters but certain waters, as for example the Ganges River, destroy the cholera spirilla very quickly. This property has been ascribed to a weak acidity of the water.



FIG. 141.—*Cholera vibrios*, longer forms at higher magnification, showing long flagella. (From Kolle and Schürmann after Zettnow.)

Animals are not naturally susceptible to cholera. Koch gave to a guinea-pig 5 c.c. of a 5 per cent solution of sodium carbonate



FIG. 142.—Involution forms of the spirillum of cholera. (Van Ermengem.)

through a tube, and then 5 to 10 c.c. of water containing cholera spirilla. The animal then received 1 c.c. of tincture of opium

per 200 grams of body weight, injected into the peritoneal cavity. In this way a condition resembling cholera in man was induced, and the animals died in 24 to 36 hours. Autopsy revealed severe enteritis, and abundant cholera spirilla in the intestine. Similar results may be obtained, however, when other organisms are substituted for the cholera germs in this procedure. Intravenous injection of cultures into rabbits, and feeding of virulent cultures to very young rabbits gives rise to rather typical cholera in many of the animals. Intraperitoneal injection of cultures into guinea-pigs gives rise to fatal peritonitis. Pigeons are relatively immune.

The poisons of the cholera germ are intimately connected with the substance of the living cell. Culture filtrates are slightly or not at all poisonous. The dead bacterial cells are poisonous, but the poison in them is a very labile substance and readily altered by heat. It seems to become soluble when the cell disintegrates, and this may explain the poisonous properties sometimes observed in the filtrates of older cultures.

Immunity to this organism was obtained by Pfeiffer by injecting non-fatal doses into guinea-pigs. When a small amount of culture is injected into the peritoneal cavity of such an immune animal, the bacteria become quickly clumped together and are then rapidly disintegrated and dissolved in the peritoneal fluid. This is known as Pfeiffer's phenomenon and was the first example of cytolysis to be observed. The solution of the bacteria sets free their poison and if a very large dose has been injected the animal may be killed by this poison regardless of his immunity to the living germs.

Asiatic cholera seems to have existed in India for many centuries and there are reliable records of its occurrence there in the sixteenth, seventeenth and eighteenth centuries. The first recognized great world invasion of cholera began in 1817 and ended in 1823. Succeeding pandemics occurred in 1826-1837, 1846-1862, and 1864-1875. The fifth invasion began in 1883 and ended shortly after the great outbreak at Hamburg in 1892. The sixth epidemic began in 1902 and has involved

Egypt, Russia, Turkey and Italy. The fifth and sixth invasions have been very much restricted, largely without doubt because of the modern methods founded upon knowledge of its causation. Cholera was epidemic in the United States in 1833-35, 1848-54, 1871-73, and there were a few cases in 1893 and again in 1910. This disease occurs as a protracted epidemic in which the infection passes from person to person, and as an explosive epidemic in which many people are stricken at once as a result of contamination of the public water-supply.

The causal relationship of *Spirillum cholerae* to human Asiatic cholera is no longer questioned. Several laboratory workers among them R. Pfeiffer and E. Oergel, have suffered typical attacks of the disease as a result of accidental laboratory inoculation. Dr. Oergel received some peritoneal fluid from an inoculated guinea-pig into his mouth and he died of cholera. Pettenkoffer and Emmerich, in order to disprove the supposed causal relation of this organism to cholera, took some alkaline water and then water containing a minute quantity of a fresh culture. The former investigator had a severe diarrhea and the latter a severe and dangerous attack of typical cholera from which he eventually recovered. The organism was recovered from the stools in all these instances.

The cholera spirilla enter the body with the food and drink and if they escape the germicidal action of the gastric juice they may establish themselves in the intestine. In an acute case of cholera they multiply here enormously and induce a severe enteritis in which large quantities of fluid are secreted into the lumen of the intestine and discharged from the rectum along with bits of desquamated epithelium and enormous numbers of cholera spirilla. The germs do not pass through the intestinal wall, but they multiply on and in the intestinal epithelium as well as in the intestinal contents. The general symptoms, shock, coma and the ultimate death, seem to be due in part to the absorption of poisons from the intestine and in part to the severe local irritation in the abdomen.

The bacteriological diagnosis depends altogether upon the recognition of the cholera germ in the feces. During an epidemic of the disease a probable diagnosis in the individual case may be made by mere microscopic examination of stained preparations of the mucous flakes in the stools. The presence of abundant curved rods arranged parallel to each other is sufficient for a probable diagnosis. The problem presents itself in a different phase when it is necessary to recognize the first case of cholera in a given locality. Here it is necessary to follow up the microscopic diagnosis by cultures on gelatin plates, agar plates and in pepton solution, and the identification of the cultured organisms by agglutinating them with a known cholera-immune serum in high dilution (1 : 1000). The serum should be powerful enough in a dilution of 1 : 10,000 to agglutinate very definitely the culture used in producing it. The examination of immigrants for the detection of cholera carriers also requires culture work. The stool should be passed naturally, but a dose of salts is permissible if there is too great delay. About 1 gram of feces is mixed with 50 c.c. of sterile pepton solution<sup>1</sup> in a flask, and this is incubated at 37° C. for six to eight hours. A stained preparation is then made from the surface film of the flask. If no curved rods are found in it, the specimen is probably negative. A loopful of the surface film should nevertheless be transferred to a tube of pepton solution which is incubated for six hours and again examined microscopically. If curved rods are found microscopically on the surface film of either the first or second culture, the problem of differentiating between the cholera vibrio and other similar organisms is presented. Plate cultures on gelatin at 22° C. and on agar at 37° C. should be made and at the same time the transplantation to fresh pepton solution should be continued at six-hour intervals. After eighteen hours, one examines the plates for typical colonies and subjects these to agglutination tests with specific serum of high titre. The bacteria from the surface film of the pepton solution are also tested in the same way. A rapid clearing of the

<sup>1</sup> Pepton 10, NaCl 10, NaNO<sub>3</sub> 0.1, NaCO<sub>3</sub> 0.2, distilled water 1000.

microscopic field in the agglutination preparations warrants positive diagnosis.<sup>1</sup>

Similar principles are followed in attempting to find cholera germs in drinking water. A solution of pepton 100 grams, salt 100 grams, potassium nitrate 1 gram and sodium carbonate 2 grams in distilled water 1000 c.c. is prepared, filtered, distributed in 10 flasks each of 1000 c.c. capacity, and sterilized. To each flask containing 100 c.c. of this sterile solution, one adds about 900 c.c. of the suspected water and incubates the mixture at 37° C. for six to eight hours. Subcultures and microscopic preparations are made from the surface films and any curved bacteria observed are tested as described above.

The prophylaxis of cholera no longer rests upon the enforcement of quarantine regulations, for it is now known that convalescents may carry the vibrio alive in their intestines for many weeks. The exclusion of the disease depends upon the bacteriological examination of every person coming from infected regions before he is allowed to land at his destination. A water-supply system well protected from fecal pollution is an element of safety for any community. The Hamburg epidemic of 1892 illustrated this point. The unfiltered water taken from the Elbe near the harbor carried the infection and distributed it throughout the city of Hamburg. In the presence of an epidemic the best protection against contact infection is provided by immunization.

Ferran in 1884 first induced immunity to cholera in animals and in man by the subcutaneous injection of living cultures. Haffkine improved the method so as to make it reliable. He employed a first vaccine of attenuated virus and a second vaccine of high virulence with an interval of five days between the injections. Kolle introduced the use of killed cultures, employing a single injection of 2 mg. of growth from an agar culture suspended in 1 c.c. of salt solution and killed by heating an hour at 58° C. As a result of this treatment the agglutinins, bacteriolysins and

<sup>1</sup> Krumwiede, Pratt and Grund, *Journ. Infect. Diseases*, 1912, Vol. X, pp. 134-141.

opsonins for the cholera vibrio are increased. Practically such vaccination has resulted in a reduction in case incidence to about one-half and in mortality rate to about one-fourth that observed among the unvaccinated.

**Spirillum (Vibrio) Metchnikovi.**—This curved organism was found by Gamaleia in 1887 in the feces and in the blood of chickens suffering from enteritis. Morphologically and in cultures this organism resembles *Sp. cholerae* very closely. It has a single flagellum. The growth and liquefaction of gelatin seems to be somewhat more rapid in the case of *Sp. metchnikovi*, and it usually produces a larger amount of indol. Accurate differentiation is possible only by animal experimentation and by testing with anti-sera. A minute quantity of culture of *Sp. metchnikovi* introduced into the skin of a dove or chicken is sufficient to cause general bacteremia and death, whereas even large doses (4 mg.) of true cholera organisms introduced into such a skin wound are without effect. *Sp. metchnikovi* is also much more virulent for guinea-pigs. Agglutination and bacteriolytic tests with specific sera also differentiate the two organisms.

**Spirillum (Vibrio) Finkler-Prior.**—Finkler and Prior in 1885 isolated this organism from the feces in cholera nostras. Morphologically it resembles the cholera vibrio very closely. Indol is not produced. It is apparently non-pathogenic.

**Spirillum Tyrogenum (Vibrio Deneke).**—This organism was isolated from old cheese. It resembles the cholera vibrio but does not form indol and appears not to be pathogenic.

A large number of other cholera-like organisms have been isolated in the various examinations for the cholera germ. Some of these can be differentiated morphologically, as they possess more than one flagellum. Others fail to produce indol or show other cultural difference from the true cholera organism. In some instances differentiation depends almost altogether upon the agglutination test. This latter has come to be regarded as most important in the accurate recognition of the cholera organism and its differentiation from other vibrios.

## CHAPTER XXV

### SPIROCHÆTÆ

**Spirochæta Plicatilis.**—Ehrenberg in 1833 observed this long slender spiral organism in swamp water. It occurs commonly in stagnant water among the algæ which grow there and has also been found in sea water. The cell is about  $0.75\mu$  in thickness and 20 to  $500\mu$  in length. It moves by rotation and also by bending of the thread. Multiplication takes place by transverse division, sometimes occurring simultaneously at many points in a filament so that many short forms result. This organism is regarded as the type species of the genus *Spirochæta*.

A number of saprophytic spirochetes are known. Dobell<sup>1</sup> has made a careful study of several species, not only free-living but also parasitic spirochetes, directing special attention to their systematic relationships. He concludes that the spirochetes belong to the bacteria and that they agree with the bacteria in their structure in all respects except the organs of locomotion. Concerning the flagella he seems to be doubtful.

**Spirochæta Recurrentis.**—Obermeier in 1873 described the slender spiral organism first seen by him in 1868 in the blood in cases of relapsing fever. Ross and Milne observed a similar organism in man in Uganda in 1904 and Dutton and Todd in the same year demonstrated the presence of a spirochete in the blood in the African tick fever of the Congo. In 1905 a similar organism was found in a case of relapsing fever in New York City. The disease has also been recognized in Russia and in India. The spirochetes have been successfully inoculated into monkeys and into rats, and various strains from different parts of the world have thus been made available for comparative study in

<sup>1</sup> *Archiv. f. Protistenkunde*, 1912, Bd. XXVI, pp. 117-240.

the same laboratory. There are certain differences between these spirochetes of human relapsing fever, and several distinct varieties (or species?) are recognized. We shall consider them as varieties of *Sp. recurrentis*.

**Spirochæta Recurrentis var. Duttoni.**—This is the spirochete of Congo tick fever discovered by Dutton and Todd in 1904. It is about  $0.45\mu$  in thickness and 24 to  $30\mu$  in length. The organism has been cultivated by Noguchi<sup>1</sup> in ascitic fluid containing sterile tissue and covered by paraffin oil. The African tick fever caused

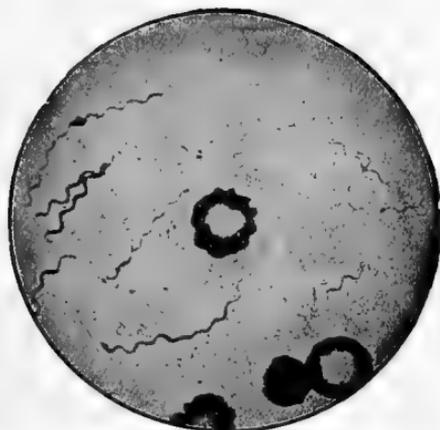


FIG. 143.—Spirochætæ of relapsing fever in blood of a man. (After Kolle and Wassermann.)

by this organism is one of the most fatal of the relapsing fevers. The tick remains infective for a very long time and also transmits the infection to its offspring through the egg. Other insects,<sup>2</sup> fleas and lice, are also capable of transmitting the infection.

**Spirochæta Recurrentis var. Rossii (Kochi).**—This organism occurs in the blood of relapsing fever of East Africa. It resembles *Sp. duttoni* very closely. Noguchi obtained cultures readily in ascitic fluid containing sterile tissue.

**Spirochæta Recurrentis var. Novyi.**<sup>3</sup>—This organism is more slender than the two preceding varieties, measuring about

<sup>1</sup> *Journ. Exp. Med.*, 1912, Vol. XVI, pp. 199-210.

<sup>2</sup> Nuttall, *Johns Hopkins Hosp. Bull.*, 1913, Vol. XXIV, pp. 33-39.

<sup>3</sup> Novy and Knapp: *Journ. Inf. Diseases*, 1906, Vol. III, pp. 291-393.

0.31 in thickness. The relapsing fever in which it occurs has been observed in South America. Noguchi has obtained cultures by the same methods as he employed for *Sp. rossii*, but the cultivation is more difficult.

Several other varieties of spirochetes, which cause relapsing fever in man, have been recognized. The spirochete concerned in any case seems to be able to infect several species of insects and

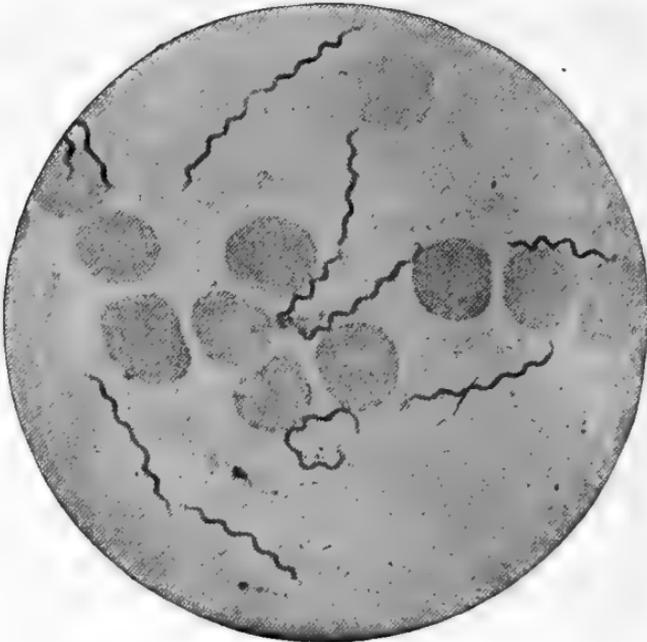


FIG. 144.—*Spirochaeta recurrentis* (*novyi*). Organisms of different lengths in the blood of a white rat.  $\times 1500$ . (After Novy and Knapp.)

to be transmitted to a new mammalian host by them. Furthermore one species of insect seems to be capable of transmitting any one of these spirochetes.<sup>1</sup>

The diagnosis of relapsing fever depends upon recognizing the characteristic spirochetes in the blood during the febrile attack. Their recognition offers little difficulty, as a rule, but they may be overlooked by a beginner. In doubtful cases it is well to search the fresh drop of blood not only by direct central illumination

<sup>1</sup> Nuttall: *Johns Hopkins Bull.*, 1913, Vol. XXIV, pp. 33-39.

with a yellow light but also by means of dark-field illumination, and to examine thin films made by mixing India ink 3 parts with the blood 1 part and spreading very thin. Finally thin blood films should be stained and examined. The inoculation of white rats with 1 to 5 c.c. of blood conveys the infection to them and the parasites appear in the blood of the animal 2 to 4 days after inoculation. The spirochetes may vanish from the blood with marvelous rapidity.

**Spirochæta Anserina.**—Sacharoff in 1890 discovered this spiral organism in the blood of geese suffering from a serious disease in the Caucasus. Ducks and chickens are also susceptible. The spirochete is about  $0.5\mu$  thick by 10 to  $20\mu$  long. It is considered by Nuttall to be identical with the *Sp. gallinarum* of Marchoux and Salimbeni.

**Spirochæta Gallinarum.**—Marchoux and Salimbeni in 1903 discovered this organism in the blood of diseased chickens at Rio Janeiro. The organism is  $0.5\mu$  thick and 15 to  $20\mu$  long. The disease is transmitted by means of the fowl tick *Argas minutus* (*persicus?*), most effectively when the tick is kept at a temperature of  $30^{\circ}$  to  $35^{\circ}$  C. In cold climates the disease is unknown. Leishman and Hindle have studied very carefully the changes which the spirochetes pass through in the body of the insect. They found numerous exceedingly minute "coccoid bodies" in the cells of the Malpighian tubules. These minute bodies are considered<sup>1</sup> to be the products of a fragmentation of spirochetes and to be capable of again growing into typical spirochetes. If the view is correct these bodies necessarily play an important part in the infection of the vertebrate host and in the inheritance of the infection in the insect species.

**Spirochæta Muris.**—This is a very short spirochete which occurs naturally in a non-fatal relapsing fever of rats and mice. It possesses one or sometimes two flagella on each end and multiplies by simple transverse fission. The infection in rats and probably also in mice is evidently world wide.

<sup>1</sup> Nuttall: Harvey lecture, 1913.

In 1915, Futaki and Takaki<sup>1</sup> found a spirochete apparently identical with *Spirochæta muris* in rat-bite fever (Sodoku) of man. To the organism they gave the name *Spirochæta morsus-muris*.

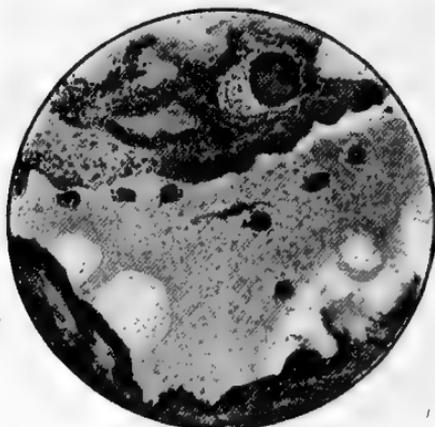


FIG. 145.—*Spirochæta (morsus) muris* in lung of mouse inoculated with blood from human rat-bite fever. Silver impregnation.  $\times 1500$ . (After Futaki, Takaki, Taniguchi and Osumi.)

The disease assumed some importance in the armies during the period of trench warfare. It is transmissible to various animals,

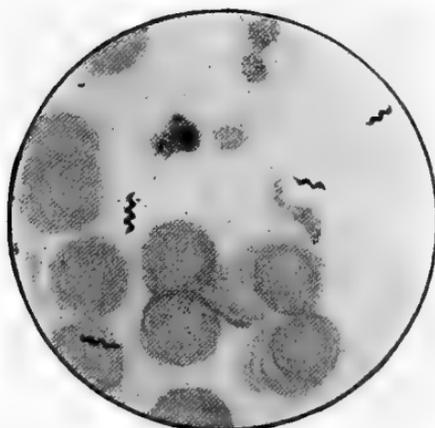


FIG. 146.—*Spirochæta (morsus) muris* in blood of guinea-pig with experimental rat-bite fever. Giemsa's stain.  $\times 1250$ . (After Futaki and associates.)

the guinea-pig being perhaps most satisfactory for study of the experimental disease.

<sup>1</sup> Futaki, Takaki, Taniguchi and Osumi: *Journ. Exp. Med.*, 1916, 23, p. 249; *ibid.*, 1917, 25, p. 33.

**Spirochæta (Leptospira) Icterohæmorrhagiæ.**—This organism was discovered by Inada and Ido<sup>1</sup> in the human disease known as infectious jaundice or Weil's disease. The organism is a long slender somewhat irregular spiral. It occurs in the blood and in greater abundance in the substance of the kidney. It grows as

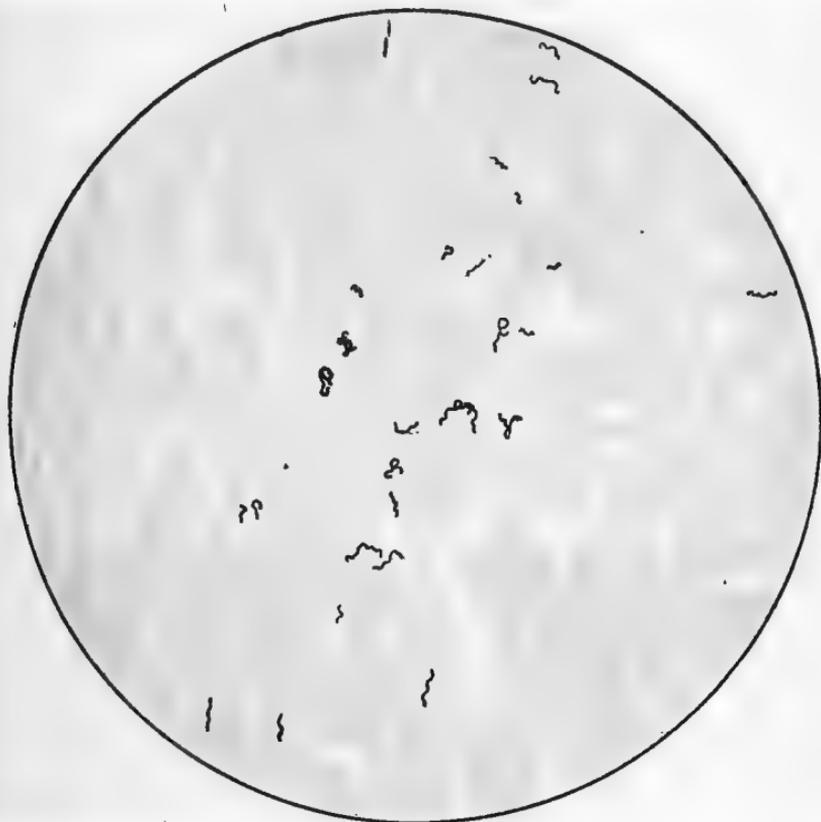


FIG. 147.—*Leptospira icterohemorrhagiæ* in section of human liver. Drawing of a silver-impregnated specimen. (After Inada, Ido, Hoki, Kaneko and Ito.)

an aërobe in diluted rabbit serum at temperatures ranging from  $10^{\circ}$  to  $37^{\circ}$  C., which suggests that insects may act as reservoirs for the virus.<sup>2</sup> Wild rats frequently harbor the parasite within their kidneys and excrete it with the urine. The mode of transfer

<sup>1</sup> Inada, Ido, Hoki, Kaneko and Ito: Journ. Exp. Med. 1916, 23, p. 377.

<sup>2</sup> Noguchi: Journ. Exp. Med., 1918, 27, pp. 575, 593, 609.

blood (black vomit). It is frequently fatal. Permanent immunity follows recovery. Reed, Carroll, Lazear and Agramonte,<sup>1</sup> in 1901, showed that the virus is present in the blood at least during the first two or three days of the attack, that it will pass through a porcelain (Chamberland B) filter and that it is naturally transmitted from man to man by the mosquito *Aedes (Stegomyia) calopus*, which becomes capable of inoculating the disease about twelve days after sucking blood which contains the virus. The mosquito probably remains infective as long as it lives and may be regarded as an essential agent in the spread of yellow fever. Prophylactic measures based upon this deduction have been remarkably successful in the suppression of the disease.

The newer work of Noguchi suggests that there may be vertebrate animals which serve as reservoirs of the yellow fever virus in the tropics.

**Spirochæta (Leptospira) Hebdomadalis.**—Ido, Ito and Wani<sup>2</sup> have found this organism, which resembles *Spirochæta icterohæmorrhagiæ* in form and motion, in blood of patients suffering from the Japanese seven-day fever, Nanukayami. It has been successfully inoculated into young guinea-pigs.

**Spirochæta Gallica.**—Couvry and Dujarric de la Rivière<sup>3</sup> have found a small spirochete in the blood in trench fever and have suggested the name *Spirochæta gallica* for it. They also found the spirochete in the liver and kidneys of guinea-pigs inoculated with the human blood. Their results suggest the probable causal relationship of this organism to trench fever, but critical confirmation has not yet appeared.

Trench fever [*Febris quintana (Wolhynica)*] was probably the most important epidemic disease of the world war up to 1918. MacNee in 1915 proved that it was transmissible by blood in-

<sup>1</sup> The publications of Reed, Carroll and their associates have been issued as a volume entitled Yellow Fever, U. S. Senate Document No. 822, 61st Congress 3rd Session, 1911.

<sup>2</sup> Ido, Ito and Wani: *Journ. Exp. Med.*, 1919, 29, p. 199.

<sup>3</sup> *Comptes rendus Soc. Biol.*, 1918, 81, p. 22.

oculation. Early in 1918<sup>1</sup> the Trench Fever Commission,<sup>1</sup> Medical Research Committee, American Red Cross, confirmed the inoculation experiments of MacNee and demonstrated that the virus resides in greatest concentration in the blood plasma and that it is transmitted from man to man by natural infestation with body lice (*Pediculus humanus*). The direct inoculation experiments described in detail by Baetjer<sup>2</sup> and the louse transmission experiments described in detail by MacNeal and Peacock<sup>3</sup> leave no room for doubt upon these points. This portion of the work of the commission has been confirmed in all essentials by the simultaneous and independent research of the Research Committee<sup>4</sup> of the British War Office, working in England under the presidency of Sir David Bruce.

**Spirochæta Pallida (Treponema Pallidum).**—Schaudinn and Hoffmann in 1905 observed this slender spiral organism in primary syphilitic lesions, in fluid obtained from swollen lymph glands in syphilis and in the liver and spleen of a still-born syphilitic fetus. The occurrence of the organism in syphilitic lesions was quickly and abundantly confirmed by other workers. Cultures were obtained in collodion sacs by Levaditi and McIntosh in 1907. Schereschewsky, and Muhlens and Hoffman obtained cultures in gelatinized horse serum. Noguchi<sup>5</sup> has carried out the most successful cultural work and has succeeded for the first time in causing syphilitic lesions in animals by the inoculation of pure cultures.

*Sp. pallida* occurs naturally only in human syphilis. It is a slender spiral 0.2 to 0.35 $\mu$  in thickness and 3.5 to 15.5 $\mu$  in length. Its curves are narrow and very regular. It is actively motile,

<sup>1</sup> Strong, Swift, Opie, MacNeal, Baetjer, Pappenheimer and Peacock: Medical Bulletin, Am. Red Cross, March, 1918, 1, p. 376; Trench Fever, Oxford University Press, 1918, pp. 446 +VIII.

<sup>2</sup> Trench Fever, Oxford Press, 1918, Chapter VII, p. 61-74.

<sup>3</sup> Trench Fever, Oxford Press, 1918, Chapters X and XI, p. 143-274.

<sup>4</sup> Transmission of Trench Fever by the Louse: *British Med. Journ.*, Mar. 23, 1918.

<sup>5</sup> *Journ. Exp. Med.*, 1911, Vol. XIV, p. 99; 1912, Vol. XV, p. 90.

as are all the spirochetes, and has a very slender flagellum at each end. The usual motion is that of rapid rotation on the longitudinal axis with progression, but at times there is gross bending of the filament, especially when the organism is living under unfavorable conditions. The mode of division

is a somewhat vexed question as it is in regard to the whole group of spirochetes. Transverse and longitudinal division have been described. Probably the weight of authority<sup>1</sup> now favors transverse division as the sole mode of multiplication, although able adherents to the opposite view are not lacking. The refractive index of the filament is not very much greater than that of serum, so that the unstained organism is difficult to see by direct illumination. Dark-field illumination is more satisfactory. *Sp. pallida* in film preparations stains with difficulty by ordinary methods. Schaudinn employed Giemsa's modification of the Romanowsky stain. Good results are obtained by staining with solutions of the Romanowsky

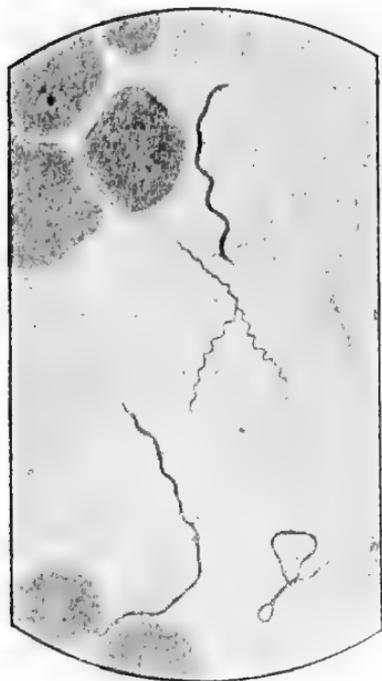


FIG. 151.—Film preparation from a genital syphilitic papule; in the center are two specimens of *Spirocheta pallida*, the other three are specimens of *Spirocheta refringens*. (After Schaudinn and Hoffmann.)

staining principles in methyl alcohol provided an excess of methylene-violet be present (see p. 44). Tunnicliff<sup>2</sup> recommends staining with a mixture of saturated alcoholic solution of gentian violet, 1 part, in 5 per cent carbolic acid, 9 parts. Thin films are essential but staining process requires only a few seconds.

<sup>1</sup> *Journ. Exp. Med.*, 1911, Vol. XIV, p. 99; 1912, Vol. XV, p. 90.

<sup>2</sup> *Journ. A. M. A.*, 1912, Vol. LVIII, p. 1682.

Probably the most satisfactory stain is that of Fontana.<sup>1</sup> In pieces of tissue the spirochete is best stained by the method of Levaditi. For this purpose thin (1 mm.) pieces of syphilitic tissue are fixed in formalin (10 per cent) for 24 hours or longer and hardened in 95 per cent alcohol for a day. The alcohol is then removed by soaking in distilled water and the tissue is transferred to a fresh 1 to 3 per cent solution of silver nitrate in distilled water. This is placed at 37° C. in the dark for three to five days. The tissue is next washed in distilled water and placed in reducing fluid, consisting of pyrogallic acid 3 grams, formalin (40 per cent formaldehyde) 5 c.c. and distilled water 100 c.c., for one to two days. It is then washed in distilled water, dehydrated, embedded in paraffin and sectioned. The spirochetes are stained a dense black by this method. The sections may be stained to show histological structure also, by applying methylene blue or toluidin blue to them after they have been fixed on the slide.

Cultivation of *Sp. pallida* has been most successfully practised by Noguchi.<sup>2</sup> He has grown the organism in a mixture of serum and water, to which naturally sterile tissue was added, and in ascitic-fluid agar with similar bits of tissue, always under strict anaërobic conditions. The technic of culture is somewhat difficult and the original papers should be consulted in detail. Inoculation of the cultures into rabbits and monkeys has caused typical syphilitic lesions.

Noguchi's luetin is prepared by grinding the solid medium rich in spirochetes in a mortar and emulsifying it in a small amount of fluid. This is then heated to 60° C. for an hour and preserved by the addition of 0.5 per cent carbolic acid. The final preparation contains many dead unbroken spirochetes.

<sup>1</sup> Three solutions are required (a) Glacial acetic acid 1, formalin 20, distilled water 100; (b) Phenol 1, tannic acid 5, water 100; (c) Silver nitrate (25 per cent solution) 5 c.c., ammonia water 1 drop. The thin smear is dried in air. Treat with (a) one minute; wash in water; cover with (b) and steam one-half minute; wash in distilled water; cover with (c) and steam one-half minute; wash; blot; dry; mount in balsam. Medical War Manual No. 6, U. S. Army, 1919, p. 30.

<sup>2</sup> *Journ. Exp. Med.*, 1911, Vol. XIV, p. 99; 1912, Vol. XV, p. 90.

Syphilis is an inoculation disease which has been widely prevalent throughout the civilized world since the early part of the 16th century. Transmission takes place by direct contact and in the great majority of instances by venereal contact, although

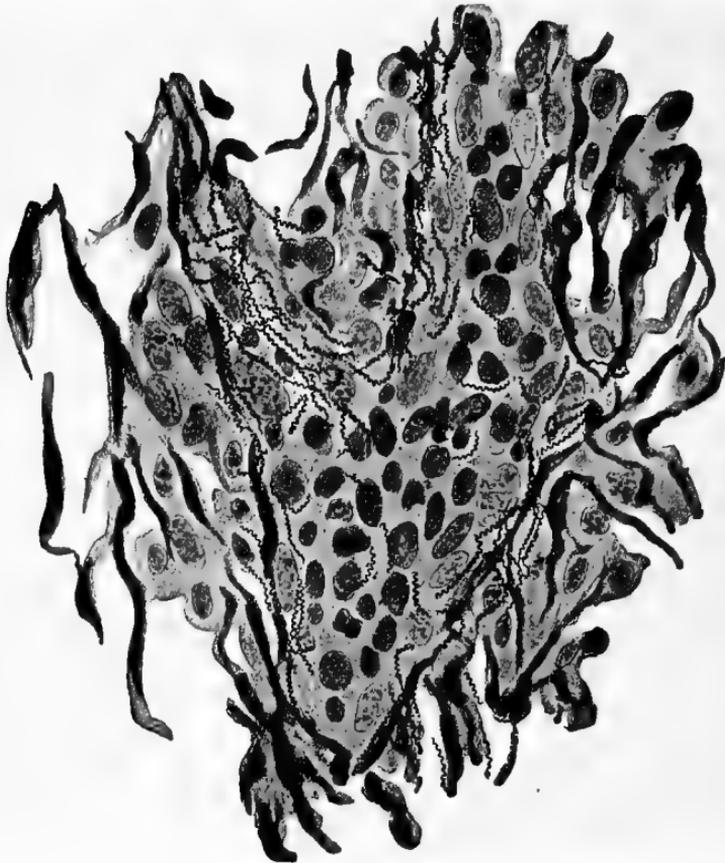


FIG. 152.—*Spirochæta pallida* stained by Levaditi method. The section shows an infarcted lymph vessel at the junction of two branches. The lumen is filled with leukocytes. The spirochetes follow the lymph vessel for the most part, but are also penetrating into the surrounding tissue. (From Dofstein after Ehrmann.)

many authentic cases of transmission by means of intermediate objects are known. The spirochete is able to live for some hours outside the body if drying is prevented. The primary lesion develops at the point of inoculation about two weeks after that event, first as a papule, which becomes vesicular and ulcerates,

remaining indolent for several weeks. The neighboring lymph glands become swollen. The secondary manifestations occur about a month later as a general macular or sometimes papular eruption on the skin, together with sore throat and ulcerated patches in mouth. The skin eruption does not itch. Subsequent to this stage there may be local necrotic lesions (gummata) in various parts of the body, or low-grade inflammatory changes in the meninges and central nervous system. Bacteriological methods of diagnosis are of assistance in some cases in all the various stages of syphilis. Early in the disease the spirochetes are relatively numerous, in certain locations at any rate, while later the parasites may be so few as to render their detection practically hopeless for diagnostic purposes. In these later stages, however, the presence of specific and other antibodies in the body fluids of the patient may often be recognized and this recognition employed as an aid in diagnosis.

Microscopic examination of a primary ulcer is best done by means of the dark-field illumination. For this purpose the ulcer (which should not have been treated with mercurials) is carefully cleansed and a few drops of freshly exuded serum collected in a glass capillary, and the usual slide-cover-glass preparation is made with this fluid. Permanent preparations are made most easily by mixing such serum with India ink on a slide and spreading the mixture in a very thin layer. Collargol, one part in nineteen parts of water, gives even more satisfactory preparations<sup>1</sup> than India ink. It is used in the same way. Thin films of the serum on slides or cover-glasses may be stained as directed above. Microscopic examination of fluid obtained by gland puncture or from secondary lesions on the skin or mucous membranes is carried out in the same way. Serious confusion in the recognition of the spirochete is likely to arise in the case of lesions in the mouth or pharynx, inasmuch as some of the normal mouth spirochetes are very similar in form to *Sp. pallida*. The presence of typical spirochetes in the juice aspirated from a lymph gland is practically

<sup>1</sup> Harrison: *Journ. Roy. Army Med. Corps*, 1912, Vol. XIX, p. 749.

diagnostic, and the recognition of typical organisms in genital chancres or lesions on the skin has important diagnostic value.

Inoculation of animals is of little practical use in diagnosis, but it has been possible by this method to demonstrate the frequent presence of *Sp. pallida* in the circulating blood in cases of untreated secondary syphilis.

The detection of antibodies in the blood of the patient is undertaken in two ways, first by the complement-fixation (Wassermann) test and second by the luetin test. For the complement-fixation<sup>1</sup> test, as performed at the Laboratories of the New York Post-Graduate Medical School and Hospital the following are employed:

1. The red blood cells are obtained by defibrinating fresh sheep's blood, filtering it through paper if necessary to remove fragments of clot, separating the cells in the centrifuge and washing them four times with 0.9 per cent salt solution. Finally 1 c.c. of the corpuscles as packed by the centrifuge is suspended in 19 c.c. of 0.9 per cent salt solution; 0.2 c.c. of this suspension is arbitrarily taken as the unit of red blood cells.

2. The complement is obtained by drawing 5 to 10 c.c. of blood from a large guinea-pig by cardiac puncture. This blood is transferred to a Petri dish, allowed to clot, incubated at 37° C. for 30 minutes and then refrigerated. The separated serum is then drawn off with a pipette and 2 c.c. of it are mixed with 18 c.c. of cold 0.9 per cent salt solution. This 10 per cent solution of guinea-pig's serum is kept in a cold place, preferably immersed in ice water. It is prepared on the day it is to be used. The unit of complement is contained in 0.1 c.c. of this solution. Two units (0.2 c.c.) are employed in the actual test.

3. The hemolytic amboceptor is prepared by injecting 2 c.c. of thoroughly washed (five times) sheep's corpuscles intravenously into a large rabbit at intervals of three days, until four injections have been given. Ten days after the last injection the animal is allowed to fast for 12 hours and the blood is then aseptically

<sup>1</sup> Smith, J. W., and MacNeal: *Journ. Immunology*, 1916, 2, p. 75; *Journ. Infect. Diseases*, 1917, 21, 233.

drawn from the carotid artery, allowed to clot and the serum separated by standing at  $37^{\circ}$  C. for two to five hours. The clear serum is transferred to small glass ampoules in amounts of 0.5 to 1.0 c.c. and hermetically sealed. These are then heated at  $56^{\circ}$  C. for 30 minutes and stored in the refrigerator. The hemolytic power of this serum is ascertained by titration. The unit is that amount which, when mixed with 0.2 c.c. (1 unit) of corpuscles and 0.1 c.c. (1 unit) of complement and sufficient salt solution (0.9 per cent) to make a total volume of 1 c.c., will cause complete laking of the red blood cells in exactly 1 hour after being placed in the incubator (water bath) at  $37^{\circ}$  C. The unit of amboceptor is ordinarily contained in 0.1 c.c. of a dilution of 1 part of serum in 500 to 1200 parts of salt solution. After the strength has been ascertained by trial of a series of different quantities of a strong (1:200) solution, the amboceptor dilution to be used on the same day is made up so that 0.1 c.c. contains 1 unit.

The amboceptor is quite permanent under ordinary refrigerator conditions, but when diluted it may deteriorate after a few days. The relation of complement, red blood cells and amboceptor is tested always immediately before undertaking a complement-fixation test. If the mixture of one unit of each of these in a total volume of 1 c.c. produces complete hemolysis at the end of an hour, the hemolytic system is considered satisfactory. If there is only a slight discrepancy this may be corrected by altering the final dilution of the amboceptor from its usual strength, within limits of 25 per cent. If the discrepancy is greater than this it is well to obtain a new sample of complement or of sheep's cells or of both. The hemolytic system should behave much the same from day to day when the technic is accurate.

4. The patient's serum is obtained from 5 to 10 c.c. of blood drawn from the elbow vein. The serum should be removed from the clot within 24 hours, freed from corpuscles and preserved with chloroform unless it can be tested promptly. Particular care is required when the serum is to be sent by mail. The serum is heated at  $54^{\circ}$  to  $56^{\circ}$  C. for 30 minutes just before use.

5. The antigen is an alcoholic extract of the heart muscle of beef. The clean, finely chopped heart muscle, 10 grams, is extracted in 100 c.c. of absolute ethyl alcohol at 37° C., with frequent shaking for two weeks. It is filtered through paper. The filtrate is then refrigerated a day and again filtered. This clear filtrate is the plain alcoholic antigen and it is sealed up in small ampoules or stored in a tightly stoppered bottle. The strength of antigen to be used must be ascertained by careful titration. A dilution of 1 c.c. of the antigen in 9 c.c. of salt solution is first prepared. Then various quantities, 0.1 c.c., 0.2 c.c., 0.3 c.c., 0.4 c.c. and 0.5 c.c. of this suspension are placed in separate tubes. To each tube is added 2 units of complement and sufficient salt solution to bring the total volume to 0.6 c.c. The tubes are placed in the ice-box overnight. Then one unit of corpuscles (0.2 c.c.) and two units of hemolytic amboceptor (0.2 c.c.) are added and the tubes are incubated an hour at 37° C. Of those tubes in which hemolysis is not complete, the one containing the least antigen marks the concentration at which the antigen is distinctly anti-complementary. The second test of the antigen is now undertaken. Various amounts of a 1 to 100 dilution, 0.01 c.c., 0.03 c.c., 0.05 c.c., 0.1 c.c. and 0.2 c.c., are measured into tubes. To each tube is then added 2 units of complement, 0.02 c.c. of serum from an active untreated case of syphilis and sufficient salt solution to make a total volume of 0.6 c.c. The tubes are left overnight in the ice-box. Then 1 unit of corpuscles (0.2 c.c.) and 2 units of hemolytic amboceptor (0.2 c.c.) are added and the tubes are incubated one hour at 37° C. Of the tubes showing no hemolysis (complete fixation), that one which contains the least antigen marks the lowest effective concentration of the antigen. This amount of antigen should be very much less than the anti-complementary amount ascertained in the first test. Ordinarily it is about  $\frac{1}{100}$  of this amount. The unit of antigen to be employed should be chosen so that it is several times greater than the least effective quantity but still not more than one-fifth to one-fourth the least anti-complementary amount. Having chosen

the tentative antigen unit, a third test is applied. One, two and four units of antigen are placed in tubes and a unit of corpuscles is added to each, together with sufficient salt solution to make the total volume 1 c.c., and these are incubated for an hour. The corpuscles should not be laked. If they are laked the antigen is itself markedly hemolytic. A satisfactory antigen should perform its specific function of fixing complement in the presence of a syphilitic serum in an amount which is at most  $\frac{1}{50}$  of the amount which is in itself either anti-complementary or hemolytic. It keeps well in the refrigerator as the alcoholic solution. The dilution for use should be freshly prepared by slowly adding the salt solution, a few drops at a time, to the alcoholic antigen in a test tube, with thorough shaking.<sup>1</sup>

The antigen is the element in the test which is designed to enter into chemical reaction with the specific substance in the patient's blood, which is present there as a result of active syphilis. During the course of this reaction, complement is absorbed or destroyed. The nature of the lipoidophilic substance<sup>2</sup> is unknown. It behaves in the test very much as a specific immune body would be expected to behave. Experience has shown that an antibody of this nature is rarely present in other conditions than active syphilis and that it is present in this disease. Upon the results of this experience we have to rely in ascribing diagnostic value to the test.

In performing a test for diagnosis, sera from several patients should be tested at the same time, and one, two or three sera, previously tested and found to fix complement in varying degrees, and at least one serum known to give a negative result, should be tested along with the new samples. Three tubes are used for each serum to be tested. The first incubation is carried out in the refrigerator over night and the second incubation, after the addition of the sensitized cells is at 37° C. in the water bath.

<sup>1</sup>The anticomplementary test upon one, two, three and four units of antigen must be repeated every time a diagnostic test is carried out and simultaneously with it using the identical reagents.

<sup>2</sup>Simon: *Infection and Immunity*, Phila., 1912, p. 272.

Tube No. 1	Tube No. 2	Tube No. 3
Complement 2 units (0.2 c.c.)	Complement 2 units (0.2 c.c.)	Complement 1 unit (0.2 c.c.)
Patient's serum 0.1 c.c.	Patient's serum 0.02 c.c.	Patient's serum 0.05 c.c.
Salt solution 0.3 c.c.	Antigen 1 unit (0.1 c.c.) Salt solution 0.28 c.c.	Antigen 1 unit (0.1 c.c.) Salt solution 0.25 c.c.

Mix thoroughly and leave overnight in ice box. Then add:

Sheep's corpuscles 1 unit (0.2 c.c.)	} 0.4 c.c. <sup>1</sup>	Sheep's corpuscles 1 unit (0.2 c.c.)	} 0.4 c.c. <sup>1</sup>	Sheep's corpuscles 1 unit (0.2 c.c.)	} 0.4 c.c. <sup>1</sup>
Hemolytic amboceptor 2 units (0.2 c.c.)		Hemolytic amboceptor 2 units (0.2 c.c.)		Hemolytic amboceptor 2 units (0.2 c.c.)	

Mix thoroughly and incubate at 37° C. in water bath for 1 hour, recording the progress of hemolysis at intervals of 15 minutes. Then refrigerate 16 hours and record the final reading.

Tube No. 1 should show complete hemolysis early in the second incubation. If this has behaved properly and the tests on the known sera have resulted as they did when previously tested, then the behavior of Tubes 2 and 3 is a measure of the amount of lipoidophilic substance in the serum of the patient. One distinguishes six different grades of reaction, from complete fixation (no trace of hemolysis) to no fixation (complete hemolysis). These are designated by the signs (++++) (+++) (++) (+) (±) and (-).

The luetin test is performed by injecting 0.05 c.c. of luetin intracutaneously in two places on the left arm and at the same time 0.05 c.c. of a control suspension, consisting of the medium

<sup>1</sup> The suspension of sheep's corpuscles containing 1 unit in 0.2 c.c. and the solution of hemolytic amboceptor containing 2 units in 0.2 c.c. are quickly mixed together in equal parts, and 0.4 c.c. of this homogeneous mixture is added at this point. This procedure results in a saving of time as well as greater accuracy.

without any growth of spirochetes, at two points on the right arm. Local inflammation on the left arm, appearing in two to ten days and sometimes resulting in the formation of a pustule, is regarded as a positive test. The test is often negative in the earlier stages of syphilis.

The various diagnostic tests for syphilis are now extensively employed. Microscopic search for the spirochete is of value in the untreated primary and secondary stages. The complement-fixation test becomes positive a few weeks after the appearance of the primary lesion and is generally regarded as indicating an active syphilitic process. The luetin test may be positive in latent or inactive syphilis when the Wassermann is negative. Further experience with the luetin test is necessary in order to determine its real significance.

**Spirochæta (Treponema) Refringens.**—This is a relatively gross spirochete which occurs in primary syphilitic lesions along with *Sp. pallida*. It seems to have no pathogenic properties. Noguchi<sup>1</sup> has obtained pure cultures of it and found them without pathogenic properties for rabbits and monkeys.

**Spirochæta (Treponema) Microdentium.**<sup>2</sup>—This is one of the common spirals of the mouth. It may be confused with *Sp. pallida*, which it resembles in size and shape. Pure cultures have been obtained by Noguchi. Other spirochetes of the mouth have also been cultivated by this investigator and there are probably several species of them.

<sup>1</sup> *Journ. Exp. Med.*, 1902., Vol. XV, p. 466.

<sup>2</sup> Noguchi: *Journ. Exp. Med.*, Vol. XV, pp. 81-89.

## CHAPTER XXVI

### THE FILTERABLE MICROBES

**The Virus of Foot-and-mouth Disease.**—This filterable organism occurs in the vesicles present in the mouth and on the feet of the diseased animals, and also in the milk of cows suffering from foot-and-mouth disease. The virus was shown to be filterable by Löffler and Frosch in 1898. It is rendered inert by heating to 50° C. for 10 minutes. Animals are immune after recovery from the disease. Cattle and swine are naturally susceptible and a few cases of the disease have occurred in man. Nothing definite is known concerning morphology or cultures. The infection seems to be transmitted with the food as well as by inoculation.

**The Virus of Bovine Pleuro-pneumonia.**—This organism is present in the affected lungs and in discharges from the respiratory tract of cattle suffering from pleuro-pneumonia. Nocard filtered the virus through a Chamberland "F" filter in 1899. It is rendered inert by heating at 58° C., but retains its virulence in glycerine for weeks and resists freezing. Cultures have been obtained by the collodion-sac method by Nocard and Roux. The organisms in such cultures are extremely minute and variable in form. Some of them are spirals and others approximately spherical. Immunity follows recovery from the disease, and has been induced artificially by inoculation with cultures and also by inoculation with virulent exudate from the lung of a dead animal into the subcutaneous tissue of the tail of the animal to be immunized.<sup>1</sup>

**The Virus of Cattle Plague (Rinderpest).**—This organism occurs in the blood, organs and excretions of cattle suffering from the disease. It was shown to be filterable by Nicolle and Adil-

<sup>1</sup> Kolle and Wasserman, Handbuch, 1912. Bd. I. S. 028.

Bey in 1902, and is able to pass through the Chamberland "F" filter. The virus resists drying for four days and remains active for two or three months when spread on hay in a dark place. It is destroyed by distilled water in five days, by glycerin in eight days and rendered avirulent in a few hours by admixture of bile. The disease is an acute febrile disorder characterized by severe inflammation of the mucous membranes and rapid emaciation. It is usually fatal. Immunity follows recovery and is induced artificially by injecting the bile of infected animals under the skin of the healthy cattle. In this way an active immunity is acquired without an evident attack of the disease.

**The Virus of Rabies.**—This organism exists in the central nervous system, the peripheral nerves, the salivary glands, the saliva and less frequently in other parts of the body of persons or animals suffering from lyssa or rabies. The virus was filtered by Remlinger in 1903. It may also be dialyzed through collodion sacs.<sup>1</sup> The virus is rendered inert by drying for two weeks, and by heating at 55° C. for 30 minutes, by admixture of bile in a few minutes, and by the gastric juice in 5 hours. It remains virulent in glycerine for several months. Negri in 1903 described certain bodies which seem to occur in the central nervous system invariably and exclusively in this disease. They are especially numerous in the ammon's horn of the brain in cases of street rabies. Preparations should be made from the gray matter of the brain. A bit of this tissue is carefully spread on a slide by exerting moderate pressure upon it with a second slide or a cover-glass and at the same time moving it along the surface of the first slide. The film is fixed in pure methylic alcohol and stained with Giemsa's solution, or it may be stained directly without fixation with Leishman's stain. The Negri bodies are round and somewhat irregular in outline, from 1 $\mu$  to 27 $\mu$  in diameter, and usually inside the nerve cells. In the interior of the larger bodies, smaller spherical structures of variable size and number may be seen. The exact nature of the Negri bodies is uncertain. Some stu-

<sup>1</sup> Poor and Steinhardt, *Journ. Infect. Dis.*, 1913, Vol. XII, pp. 202-205.

dents of rabies regard them as protozoa, while others consider them to be products of cell degeneration. The evidence to decide the matter is not yet at hand. They seem to occur only in rabies and to be constantly present in this disease.

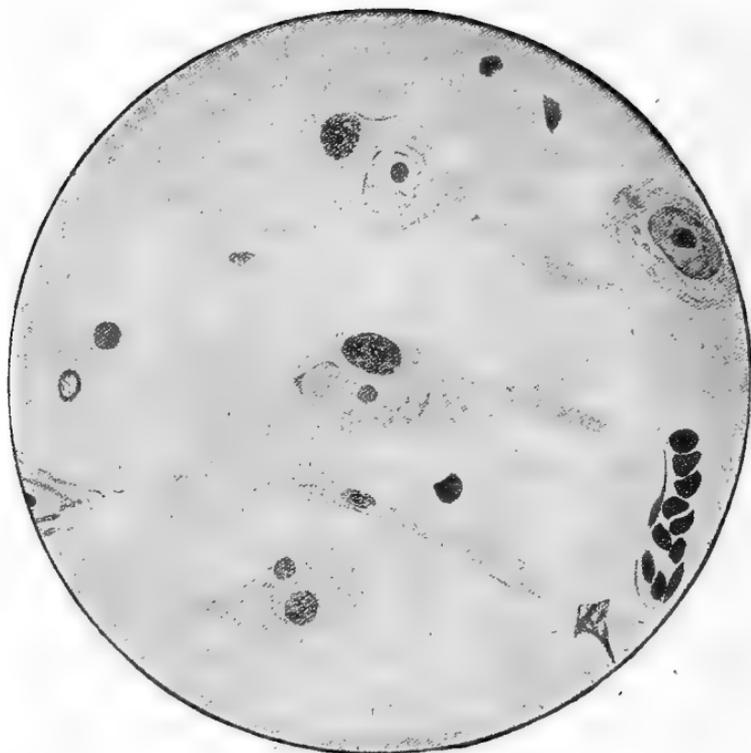


FIG. 153.—Section through the *cornu ammonis* of brain of a rabid dog; stained by the method of Lentz. Five Negri bodies of different sizes are shown, enclosed within the ganglion cells. The smallest contains only three minute granules. (After Lentz, *Centralbl. f. Bakt.*, 1907, Abt. I, Vol. XLIV, p. 378.)

Lyssa or rabies<sup>1</sup> is primarily a disease of dogs but it occurs in other mammals as well, usually as a result of dog bites. In animals inoculated directly into the brain with the most virulent material (fixed virus), the symptoms of rabies appear in 4 to 6 days and death occurs on the seventh day. Inoculation with the saliva or nervous tissue of a mad dog (street virus) rarely causes

<sup>1</sup> For a general discussion of rabies see Cumming: *Journ. A. M. A.*, 1912, Vol. LVIII, pp. 1496-1499.

symptoms before three weeks and the onset may be delayed for a year. In fact many persons and animals bitten by rabid dogs may fail to develop the disease at all. This variability depends upon the virulence and the amount of virus and especially upon the part of the body into which it is introduced. Bites upon the face or hands, because of the rich *nerve supply* and the lack of protection by clothing, are especially dangerous. After the disease has developed so as to cause symptoms, death is inevitable in the present state of our knowledge.

Rabies may be diagnosed in an animal by observing the course of the disease, by autopsy and by inoculation of test animals and observation of the course of the disease in them. If the suspected animal be caged, the question of rabies may be settled in a few days, for, if he is mad, the raging stage will be quickly followed by the characteristic paralysis and death. If the animal has been killed, a careful autopsy may reveal the absence of food from the digestive tract and the presence there of abnormal ingested material (grass, wood or stone), highly suggestive of rabies. Microscopic examination of the central nervous system may reveal the Negri bodies, characteristic of the disease. For confirmation of the diagnosis a portion of the brain or spinal cord, removed without contamination, should be injected into the brain of guinea-pigs and rabbits and the effects observed. This last test carried out by an experienced observer is the most trustworthy of all.

The Pasteur treatment of rabies is designed to induce immunity after the person has been bitten and before the disease has had time to develop. Pasteur<sup>1</sup> first demonstrated the possibility of this by experimental work on dogs, and the subsequent use of the method in man has been remarkably successful and the disease is practically always prevented if the treatment is begun directly after infliction of the infecting wound. The first essential is thorough cauterization of the wound, best with concentrated nitric acid under anesthesia. The patient is then injected sub-

<sup>1</sup> Vallery-Radot: *The Life of Pasteur*, 1911, Vol. II, p. 188.

cutaneously with emulsions of the spinal cords which have been removed from rabbits dying of rabies after inoculation with the fixed virus, and which have been dried by hanging in bottles over caustic soda for some time. The first injection is prepared from cords hung for 14 and 13 days, the second from cords hung 12 and 11 days, and so on until the three-day cord is reached on the seventh or eighth day of the treatment. The series from five-day down to three-day cords is then repeated several times, the whole treatment lasting about 21 days. The course of treatment is varied somewhat according to the urgency of the case and the severity of the wounds inflicted. It is most effectively carried out at special Pasteur institutes devoted to this work, but the material for injection may be shipped for some distance when necessary.

**The Virus of Hog Cholera.**—Dorset, Bolton and McBryde, continuing the investigations of de Schweinitz, demonstrated in 1905 the presence of a filterable agent in the blood of hogs suffering from hog cholera, capable of causing the disease upon injection into healthy animals. It passes through the Chamberland "B" and "F" filters. It leaves the body in the urine and probably also in other excretions, and seems to enter the new victim with the food and drink. The virus resists drying for three days, remains alive in water for many weeks and in glycerine for eight days. It is destroyed at 60° to 70° C. in an hour.

King, Baeslack and Hoffman<sup>1</sup> have found a short, rather thick, actively motile spirochete, *Spirochæta suis*, in the blood in forty cases of hog cholera, together with abundant granules which may, perhaps, represent a stage of this organism. The spirochete has not been found in healthy hogs. It seems probable that this organism may prove to be the causative agent of the disease, but further evidence is necessary to demonstrate this relationship.

Hog cholera is an extremely contagious disease of hogs, frequently fatal, characterized by fever and by ulcerations in the intestine. Immunity follows recovery and is induced artificially

<sup>1</sup> *Journ. Infect. Dis.*, 1913, Vol. XII, pp. 30-47; pp. 206-215.

by the injection of serum from a hyperimmune hog (passive immunity) and by the injection of such serum together with virulent blood from a hog sick with the disease (combined passive and active immunity).

**The Virus of Dengue Fever.**—Ashburn and Craig showed in 1907 that the virus of this disease exists in the blood of the patients and that it is filterable. The disease is probably transmitted by the mosquito *Culex fatigans*. Apparently the analogy to yellow fever is rather close.

**The Virus of Phlebotomus Fever.**—Doerr in 1908 demonstrated a filterable virus in the blood of persons suffering from the benign three-day fever of Malta and Crete. The disease is rather widely distributed in tropical countries. It is transmitted by the sand-fly *Phlebotomus papatasi*.<sup>1</sup>

**The Virus of Poliomyelitis.**—Several investigators, among them Flexner and Lewis, demonstrated in 1899 the presence of a filterable virus in the central nervous system of patients suffering from infantile paralysis. The virus also occurs in the nasal mucus and in the blood. It survives in glycerine for a month, also resists freezing for weeks, and is rendered inert at 45° to 50° C. in 30 minutes. It is quickly destroyed by hydrogen peroxide and by menthol.

Flexner and Noguchi<sup>2</sup> have obtained cultures of the organism in ascitic fluid containing sterile tissue and covered with paraffin oil, and in this medium rendered solid by admixture of agar. The colonies are made up of minute globose bodies 0.15 to 0.30μ in diameter. Similar bodies have been identified in the nervous tissue from cases of the disease. It seems probable that this structure is a living organism and the microbic cause of poliomyelitis, especially as inoculation of monkeys with the cultures has given rise to the disease. Flexner and his coworkers<sup>3</sup> regard this

<sup>1</sup> Brit. Journ. Roy. Army Med. Corps, XIV, pp. 1920, Vol. 236-258.

<sup>2</sup> Journ. A. M. A., 1913, V. LX, p. 362.

<sup>3</sup> Amoss, H. L.: Survival of poliomyelitic virus in brain of rabbit, *Journ. Exp. Med.*, 1918, 27, p. 443; Smillie, W. G., Cultivation experiments on globoid bodies of poliomyelitis, *ibid.*, 1918, 27, p. 319.

organism as a distinct species and as the microbic cause of the disease. Another group<sup>1</sup> of investigators claim that the globoid bodies are diminutive forms of a coccus, which can be grown aërobieally on blood agar. Their evidence is not considered entirely convincing.

Poliomyelitis or infantile paralysis occurs in epidemics and also sporadically, attacking children and young adults. It is characterized by digestive disturbance and fever, which may be very mild, followed by paralysis of one or more extremities as a rule. Death may occur, but recovery with permanent paralysis is a frequent result. The mode of transmission is unknown.

**The Virus of Measles.**—Goldberger and Anderson<sup>2</sup> in 1911 reported the successful inoculation of monkeys with the blood, nasal and buccal secretions of patients sick with measles and also with Berkefeld filtrates. Later investigation<sup>3</sup> has failed to confirm the claims of these authors although several successful inoculations of monkeys have been reported.<sup>4</sup>

**The Virus of Typhus Fever.**—Nicolle, Conor and Conseil in 1910 transmitted typhus fever to monkeys by means of serum which had passed through a Berkefeld filter. Ricketts and Wilder failed to obtain infective filtrates in their study of Mexican typhus. Typhus is an acute febrile disease, widely distributed but not very prevalent in any locality. Apparently it is not contagious<sup>5</sup> but is transmitted from man to man by body lice (*Pediculus vestimenti*). Immunity follows recovery.

Plotz<sup>6</sup> and his associates have reported the finding of an anaërobic organism in the blood in typhus, *Bacillus typhi-*

<sup>1</sup> Nuzum, J. W.: *Journ. Infectious Diseases*, 1918, 23, p. 301, p. 309; Rosenow, E. C. and others, *ibid.*, 1918, 22, p. 281, p. 313, p. 379.

<sup>2</sup> Goldberger and Anderson; *Journ. A. M. A.*, 1911, 57, p. 971.

<sup>3</sup> Sellards and Wentworth: *Bull. Johns Hopkins Hops.*, 1919, 30, p. 57.

<sup>4</sup> Hektoen: *Journ. A. M. A.*, 1919, 72, p. 177.

<sup>5</sup> Wilder: *Journ. Infect. Dis.*, 1911, Vol. IX, p. 9. Ricketts and Wilder: *Journ. A. M. A.*, 1910, Vol. LV, pp. 309-311.

<sup>6</sup> Plotz, Olitsky and Baehr: *Journ. A. M. A.*, 1916, 67, p. 1507.

*exanthematici*, which they consider the cause of typhus. Further independent confirmation of their results should be awaited.

**The Virus of Small-pox.**—The virus of this disease was shown to be filterable by Casagrandi in 1908. The vaccine virus, which is generally considered to be the same organism, had been previously filtered. The organism passes through the coarser Chamberland filters. The virus resists drying for several weeks and remains active in glycerine for eight months, but is quickly rendered inert by bile and by sodium oleate. It is also destroyed by heating at 58° C. for 15 minutes. Cell inclusions, which were described by Guarnieri in 1892, are considered by some to represent forms of the pathogenic agent.

Small-pox is an acute disease of man characterized by a general eruption on the skin, at first papular, then vesicular and pustular. It is highly contagious by direct association and by fomites and is readily transmitted by placing bits of crust from dried pustules on the nasal mucous membrane or on a scratch in the skin. Cow-pox is a milder disease which occurs naturally in cows, and has also been produced by inoculating calves with small-pox virus. An attack of either small-pox or cow-pox is followed by immunity to both diseases. Cow-pox in man is a comparatively mild disease. Inoculation results in the formation of a single pustule, rarely surrounded by secondary vesicles, with slight illness for a few days. Edward Jenner in 1798 discovered that cow-pox resulting from artificial inoculation (vaccination) confers an immunity to small-pox. Vaccination is now very generally practised in enlightened communities and in such places small-pox is practically unknown. The inoculation is best done by making a very slight superficial linear incision, about 5 mm. long, in the epidermis and rubbing into it the vaccine virus. The whole procedure should result in only a faint tinge of blood. When the vesicle appears it should be carefully protected from violence. A normal vaccination causes little inconvenience and is usually completely healed in about 4 weeks after inoculation. Failure

of the inoculation is not a proof of immunity. The vaccination should be repeated until it does take.

**The Virus of Chicken Sarcoma.**—Rous in 1910 discovered a tumor in a chicken which is histologically a typical spindle-cell sarcoma and which he has been able to reproduce in other chickens, not only by transplantation but also by inoculation of an agent which can be separated from the tumor cells<sup>1</sup> by filtration through Berkefeld filters, as well as by inoculation with tumor tissue which has been dried and powdered and preserved in the dry condition for months. The filterable microbe, or filterable agent as Rous conservatively calls it, is rendered inert by heating at 55° C. in 15 minutes, also by the admixture of chicken bile or saponin. Two other sarcomata of the fowl have been shown to be due to a filterable agent by the same investigator.

Our conceptions of the nature of filterable agents is at present beginning to become more definite. They are no longer regarded as necessarily beyond the possibility of morphological study and there is good reason to hope that the development of improved methods of study and their careful application may be able to establish not only the important physiological properties of these agents but their form and perhaps to some extent their structure as well. The beginning already made is full of promise for the future.<sup>2</sup> Filterability does not necessarily mean invisibility.

In recent years it has become fashionable to accept a filterable virus as the cause of an infectious disease in which a visible microbe cannot easily be found, even though the evidence of filterability is far from convincing. In some instances the reported work with filterable viruses has been shown to be unreliable.<sup>3</sup>

<sup>1</sup> Rous and Murphy: *Journ. Exp. Med.*, 1913, Vol. XVII, pp. 219-231. Previous papers are cited there.

<sup>2</sup> A number of other diseases have been shown to be caused by filterable agents. A brief mention of these together with references to the literature will be found in the article by Wolbach: *Journ. Med. Resch.*, 1912, Vol. XXVII, pp. 1-25.

<sup>3</sup> Bradford, Bashford and Wilson: *British Med. Journ.*, May 17, 1919, 2, pp. 599-604; Arkwright: A criticism of certain recent claims to have discovered and cultivated the filter-passing virus of trench fever and of influenza, *Brit. Med. Journ.*, Aug. 23, 1910, 2, p. 233.

## CHAPTER XXVII

### MASTIGOPHORA<sup>1</sup>

**Herpetomonas Muscæ (Domesticæ).**<sup>2</sup>—This flagellate protozoön is commonly found in the intestine of the house fly (*Musca domestica*). The cell body is spindle shaped (Fig. 154) and 15 to 25 $\mu$  in length. The flagellum is of about equal length and contains two stainable filaments which terminate near the deeply staining blepharoplast situated in the anterior part (flagellated end) of the cell. From this blepharoplast a delicate thread extends in the cytoplasm toward the posterior end. The nucleus (trophonucleus) is at the center of the cell. Multiplication takes place by longitudinal division.

**Leptomonas (Herpetomonas) Culicis.**<sup>3</sup> In the digestive tract of mosquitoes, flagellated organisms occur which bear a confusing resemblance to trypanosomes. They multiply abundantly in the blood which the insect ingests and are most easily found in the mosquito near the end of digestion of a blood meal (48 to 96 hours after feeding). The body is 16 to 45 $\mu$  in length and 0.5 to 2 $\mu$  in width. Artificial cultures have been obtained in the condensation water of blood-agar and these have been purified by streaking

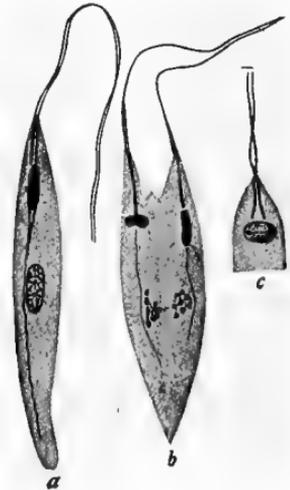


FIG. 154.—*Herpetomonas muscæ*. *a*, Normal individual; *b*, dividing form; *c*, mode of division of the blepharoplast. (From Doflein after Prowazek.)

<sup>1</sup> Only a few protozoal forms can be considered and those very briefly. The interested student should consult Doflein: *Protozoenkunde*, III Auflage, Jena, 1911.

<sup>2</sup> Prowazek, Arb. Kais. Gesundheitsamt., 1904, Bd. XX, S. 440.

<sup>3</sup> Novy, MacNeal and Torrey: *Journ. Inf. Dis.*, 1907, Vol. IV, p. 223.

on blood-agar plates. The organism is not known to be capable of infecting vertebrates.

Somewhat similar flagellates are found in the alimentary tract of various insects, where they may be easily mistaken for developmental stages of hematozoa. *Trypanosoma* (*Herpetomonas*) *grayi* which is found in the tsetse fly *Glossina palpalis* may be mentioned as another example.

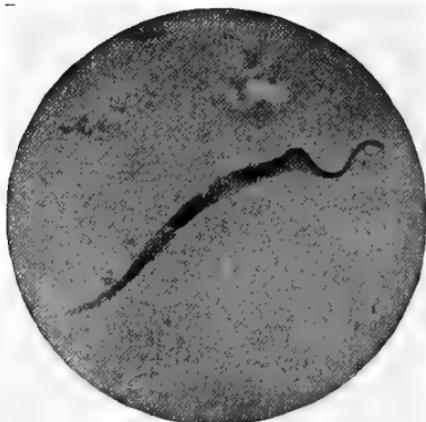


FIG. 155.—*Leptomonas culicis* from the digestive tract of a mosquito.  $\times 1500$   
(After Novy, MacNeal and Torrey.)

**Trypanosoma Rotatorium.**—This organism is the type species of the genus *Trypanosoma*, as this name was first applied to it by Gruby in 1843. It is commonly found in small numbers in the blood of frogs. The form of the cell varies from that of a slender spindle to a very broad and thick structure (Fig. 156). The width varies from 5 to  $40\mu$  and the length from 40 to  $80\mu$ . These various forms are probably stages in the growth of the parasite but it is not impossible that they represent different species parasitic in the same animal. When the larger forms are well stained the typical structures of a trypanosome are distinctly evident. The large nucleus (triphonucleus) lies near the middle of the body and closer to the undulating border. Posterior to it is the smaller and more deeply stained blepharoplast. Close to the latter a small clear colorless area is commonly seen. The flagellum

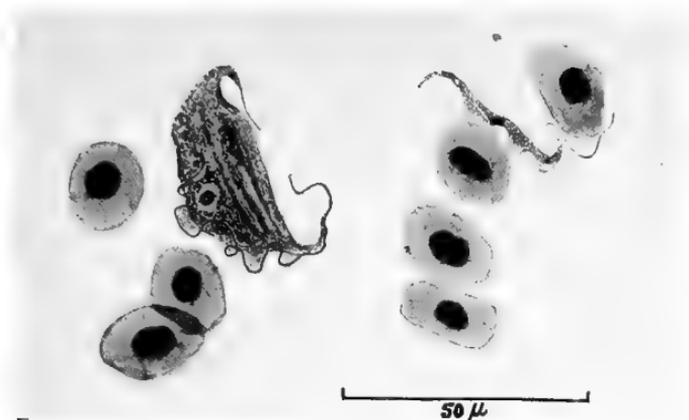


FIG. 156.—*Trypanosoma rotatorium* in blood of a frog; drawn from a preparation stained by Romanowsky method after dry fixation. The smaller form is feebly stained.

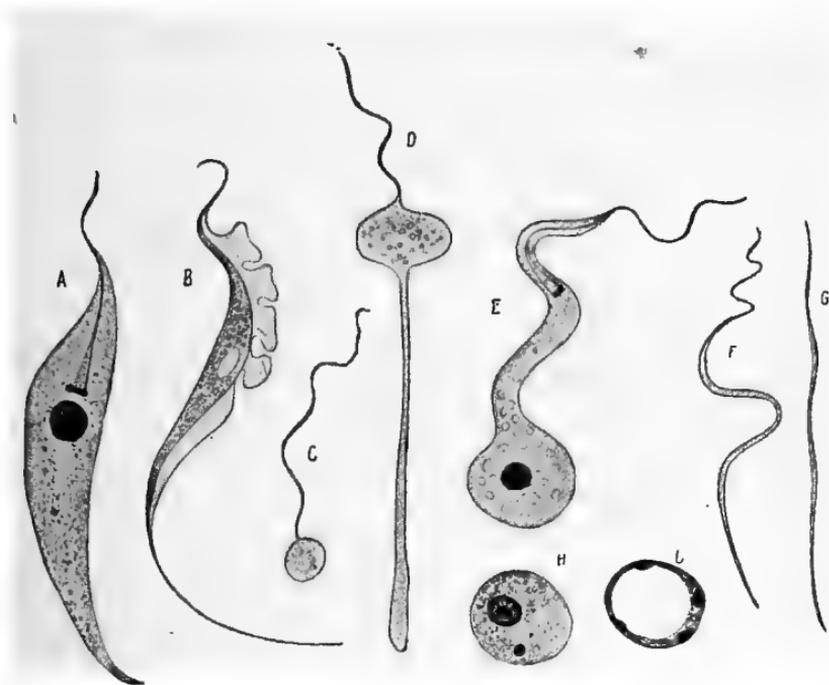


FIG. 157.—*Trypanosoma rotatorium*. The various forms which occur in artificial culture. A, Crithidia form; B, trypanosome form; C, spherical form; D and E, club forms; F and G, spirochete forms; H, resting stage; J, resting stage with vacuole and double nucleus. (After Doflein.)

originates near the blepharoplast and extends along the convex border of the cell, which is drawn out into a well-developed thin undulating membrane, to the anterior end of the cell and beyond it as a free flagellum. The posterior tip of the cell is usually drawn out to form a slender process. The other border of the cell is nearly straight and the cytoplasm near it usually shows

definite evidence of longitudinal striation, indicating the presence of elementary muscular structures, so-called myonemes. The slender form resembles very closely the shape of mammalian trypanosomes.



FIG. 158.—*Trypanosoma lewisi*.  $\times$  2500. (From Doflein after Minchin.)

Cultures of *Tr. rotatorium* were first obtained by Lewis and H. U. Williams in the condensation fluid of slanted blood-agar. Various forms of the organism occur in the cultures. Many of these are doubtless degenerating cells. The mode of transmission from frog to frog is unknown but it is probably accomplished by means of leches.

**Trypanosoma Lewisi.**—This organism, the common rat trypanosome, appears to have been seen as early as 1845, but its modern study dates from its rediscovery by Lewis in 1879. It occurs in the blood of wild rats throughout the world, from 1 to 40 per cent being infected. In the rat the parasite passes through a short period, 8 to 14 days, of rapid multiplication, which is followed by a period, usually several weeks or months, in which the organism persists without evident increase in numbers; further multiplication beginning upon transfer to a new host. In the adult or resting stage, the trypanosomes are quite uniform, 1.5 to 2 $\mu$  wide by 27 to 28 $\mu$  in length, including the flagellum (Fig. 158). When blood containing these adult forms is injected

into a healthy young rat the multiplication forms of the parasite appear after about three days. These forms show a great variety of size and shape and they stain more deeply than the adult stage (Fig. 159). Numerous dividing parasites are also present, some of them showing multiple division with the formation of rosettes. The division is longitudinal and essentially unequal, as one cell retains the old flagellum while a new one is formed for the other



FIG. 159.—*Trypanosoma lewisi*. Various forms in the blood of a rat six days after inoculation.  $\times 1125$ . (After MacNeal.)

daughter cell. The rosettes arise by successive longitudinal divisions, and an unbroken rosette contains one cell with the old flagellum larger than the others (Fig. 160).

The infection is readily transmitted to young rats by the injection of blood containing the parasites. Under natural conditions transmission is due to insects, especially fleas and lice.<sup>1</sup> The

<sup>1</sup> Swellengrebel and Strickland: Parasitology, 1910, Vol. III, pp. 360-389.

trypanosomes multiply in the digestive tract of these insects, producing various forms, many of them resembling herpetomonas and leptomonas. Fleas remain infective for a long time.

Cultures of *Tr. lewisi* were obtained by MacNeal and Novy<sup>1</sup> in 1902-03, in the condensation fluid of inclined blood-agar, and the infection was reproduced by inoculation of these cultures.

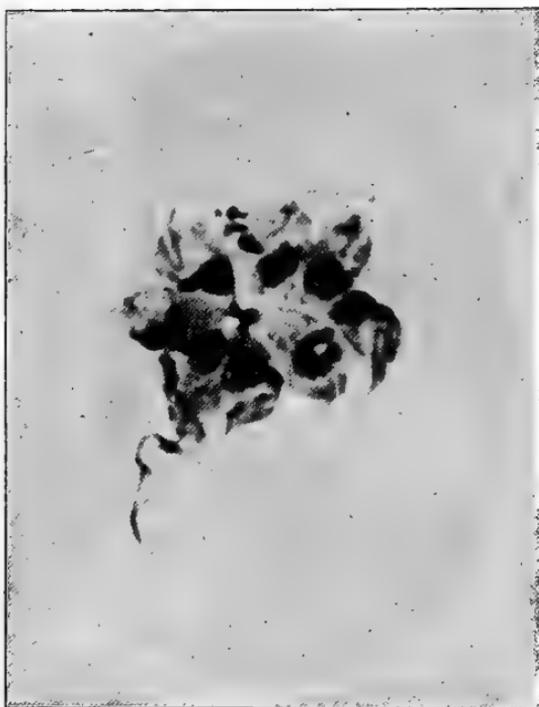


FIG. 160.—*Trypanosoma lewisi*. Eight-cell rosette in division. Note the long original or parent whip on one of the cells. Several cells show a second flagellum growing out preparatory to a further division.  $\times 2250$ . (After MacNeal.)

The size and shape of the organism in culture is quite variable. The actively dividing forms are usually grouped in rosettes with flagella directed centrally, and the cells themselves are pear-shaped or oval. Herpetomonad forms are common.

The infection with *Tr. lewisi* rarely results in death of the rat.

<sup>1</sup> Contributions to Medical Research, dedicated to Victor Clarence Vaughan, 1903, pp. 549-577.

Other species of animals are not readily infected. Immunity follows recovery. Artificial immunity has been produced by Novy, Perkins and Chambers<sup>1</sup> by the injection of a pure culture which had been propagated for six years on artificial media and had lost its virulence.

There are many other relatively harmless trypanosomes parasitic in the blood of various mammals.

**Trypanosoma Brucei.**—Bruce in 1895 discovered this organism

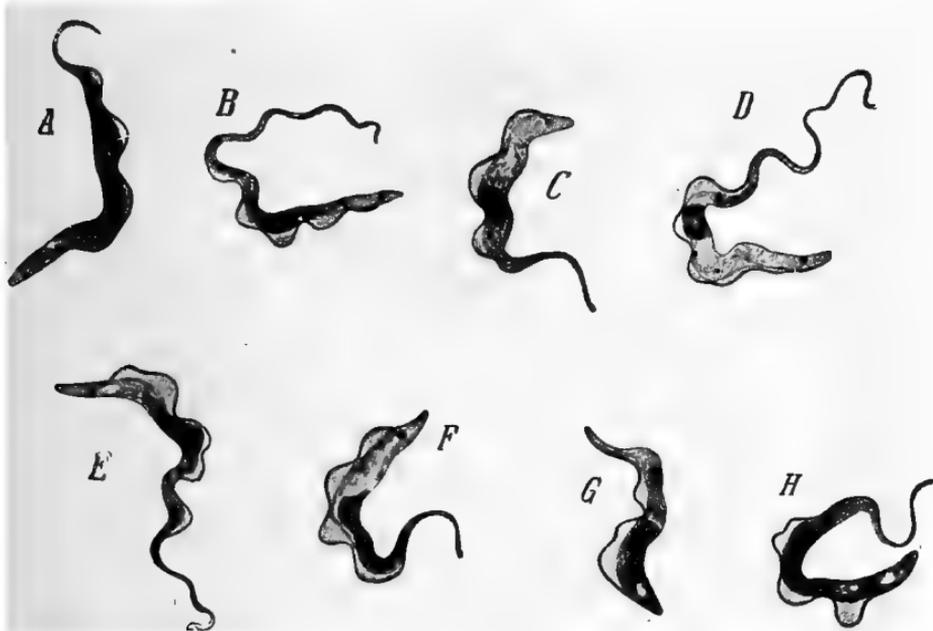


FIG. 161.—The most important trypanosomes parasitic in vertebrates. A, *Tr. lewisi*; B, *Tr. evansi* (India); C, *Tr. evansi* (Mauritius); D, *Tr. brucei*; E, *Tr. equiperdum*; F, *Tr. equinum*; G, *Tr. dimorphon*; H, *Tr. gambiense*. All magnified  $\times 1500$ . (From Doflein after Novy.)

in the blood of horses suffering from Nagana, the Tsetse-fly disease of Zululand. Pure cultures have been obtained in the condensation fluid of inclined blood-agar by Novy and MacNeal and the injection of pure cultures into animals produces the disease and death.

*Tr. brucei* is 1.5 to 5 $\mu$  wide and 25 to 35 $\mu$  long, including the

<sup>1</sup> *Journ. Inf. Dis.*, 1912, Vol. XI, pp. 411-426.

flagellum. The nucleus lies near the center of the cell. It is oval or somewhat irregular in outline and usually occupies the whole width of the cell. Near the blunt posterior end of the cell is a

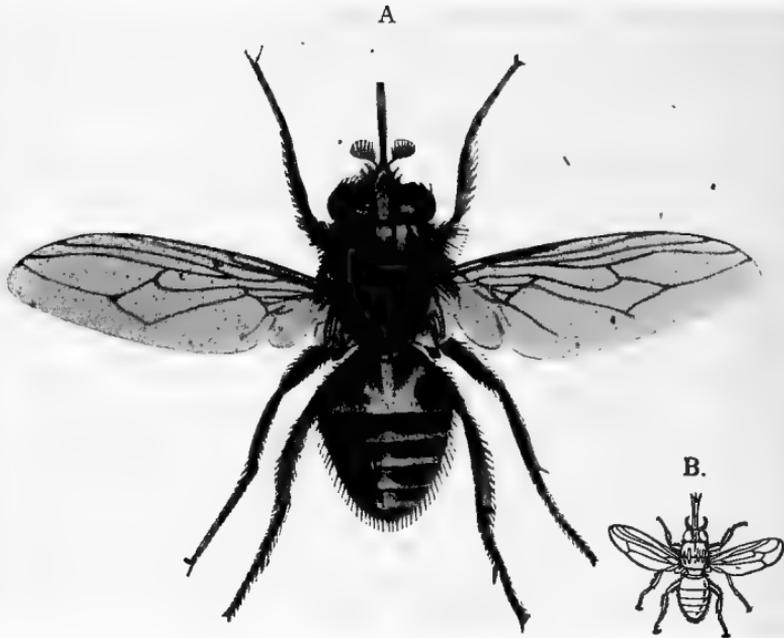


FIG. 162.—*Glossina morsitans*. A, Magnified. (After Doflein.) B, Sketch showing natural size. (From Doflein after Blanchard.)

spherical granule, the blepharoplast. Near this the flagellum originates and it extends forward along the convex border of the cell, which is drawn out into a thin undulating membrane, and



FIG. 163.—*Glossina morsitans*; lateral view of the resting fly. A, Before feeding. B, After sucking blood. (From Doflein after Austen.)

extends beyond the anterior end of the cell as a free flagellum. The cytoplasm anterior to the nucleus often contains many coarse granules. The general shape of the trypanosome as seen in the

blood of the infected animal is fairly uniform. There is, however, considerable variety in size, internal structure and staining properties. Multiplication takes place by unequal longitudinal division, much the same as in *Tr. lewisi*, but the dividing cell has the same general form as the others and multiple division figures are less common. The larger cells are usually in process of division. Trypanosomes with feebly staining cytoplasm and others with very abundant coarse granules also occur. The former are probably degenerating and disintegrating cells.

*Tr. brucei* is taken up by the blood-sucking tsetse fly, *Glossina morsitans* and in about 5 per cent of these it multiplies in the alimentary canal and penetrates into the body cavity, causing a generalized infection of the fly. After about three or four weeks the salivary glands are invaded and the fly is then able to infect other animals by biting them, and it remains infective for a long time, probably as long as it lives. Other insects may possibly serve to transmit the parasite. The infection is also readily transmitted from animal to animal by the injection of infected blood.

Cultures are obtained with some difficulty, but most readily by inoculating inclined blood-agar,<sup>1</sup> 2:1, and incubating at 28° C. The primary cultures should not be transplanted until they are about three weeks old, and they usually fail to infect animals if injected into them. The virulence is regained in the subcultures. Culture filtrates are not toxic. The poison of trypanosomes seems to be set free as a result of their disintegration in the body fluids.<sup>2</sup>

Nagana occurs naturally in a great variety of the quadrupeds and is usually fatal. Man is not susceptible. Mice and rats die in 6 to 14 days after inoculation. Guinea-pigs may show one or more relapses, the disease lasting for two to ten weeks.

<sup>1</sup> The agar employed should contain the extractives of 125 grams of meat, 1 gram pepton, 5 grams salt and 25 grams of agar in 1000 c.c. It is liquefied, cooled to 50° C. and mixed with twice its volume of warm defibrinated rabbit's blood and then allowed to solidify in an inclined position.

<sup>2</sup> MacNeal: *Journ. Inf. Dis.*, 1904, Vol. I, p. 537.

Diagnosis may be made by microscopic examination of the blood when the parasites are numerous. At other times it is well to inject 5 to 10 c.c of blood into a white rat. The distinction of *Tr. brucei* from other species of trypanosomes causing similar diseases is not easy and may require prolonged study.

Immunity of susceptible animals has not yet been achieved, but inoculation with attenuated cultures produces a relative immunity in small laboratory animals.<sup>1</sup>

**Trypanosoma Evansi.**—This organism was discovered by Griffith Evans in 1880 in the blood of horses and various other

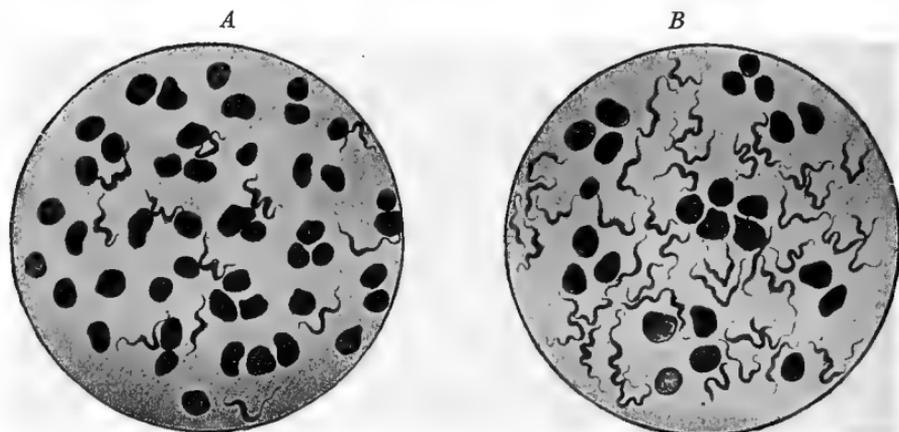


FIG. 164.—*Trypanosoma equiperdum*. Blood of an inoculated rat. A, after four days; B, after eight days. (After Doflein.)

animals suffering from the disease known in India as Surra. The trypanosome resembles *Tr. brucei* in most respects but is recognized as a distinct species. Surra is apparently transmitted by various flies, *Tabanidæ*, *Stomoxys*, and also by fleas.

**Trypanosoma equiperdum** was found by Rouget in 1896 in the blood of horses suffering from dourine. The infection is transmitted by coitus and probably also in other ways. Dourine occurs in southern Europe and northern Africa. A few cases have been observed in Canada and in the United States. Small laboratory animals are susceptible to inoculation.

<sup>1</sup> Novy, Perkins and Chambers: *Journ. Inf. Dis.*, 1912, Vol. XI, pp. 411-426.

**Trypanosoma Equinum.**—Elmassian in 1901 observed this organism in the blood of horses suffering from Mal de Caderas in South America. It possesses a very minute blepharoplast, a morphological character which distinguishes it from most other trypanosomes. Small laboratory animals are susceptible.

Several other species of trypanosomes have been described, which cause fatal diseases in quadrupeds. Most of these have been found in Africa.

**Trypanosoma Gambiense.**—Dutton and Todd in 1901 observed this organism in the blood of an Englishman in Gambia. The parasite had been previously seen by Forde. The disease, which resulted in death after two years, was called trypanosoma fever. Castellani in 1903 observed trypanosomes in the cerebrospinal fluid of patients suffering from sleeping sickness in Uganda. This organism is now known to be the same as the *Tr. gambiense* of Dutton, and sleeping sickness is recognized as the terminal stage of trypanosoma fever.

*Tr. gambiense* is very similar in form to *Tr. brucei* but the posterior end is on the average somewhat more pointed. The length varies between 15 and 30 $\mu$  and the width from 1 to 3 $\mu$ . The significance of the different forms found in the blood is not definitely known. Multiplication takes place in the same way as in *Tr. brucei*. In the tsetse fly, *Glossina palpalis*, the trypanosomes slowly disintegrate and disappear during the first four days after the infected blood is ingested, and in most of the flies this results in extermination of the trypanosomes. In 5 to 10 per cent of the flies the parasites are not completely destroyed, but the early diminution in their number is followed by an abundant multiplication of the trypanosomes in the stomach and intestine of the insect. After 18 to 53 days these flies become capable of infecting new animals by their bite and remain infectious for a very long time. The parasites are found in the salivary glands<sup>1</sup> when the fly becomes capable of causing the disease. A great

<sup>1</sup> Bruce, Hamerton, Bateman and Mackie: Proc. Royal Soc., 1911, Ser. B, Vol. LXXXIII, pp. 338-344; pp. 345-348; pp. 513-527.

diversity of form is observed in the trypanosomes within the fly but the significance of the different types is not yet fully understood.

Many of the mammals are susceptible to inoculation with *Tr. gambiense*. White rats usually relapse 2 or 3 times before finally succumbing to the infection, whereas they usually die within 2 weeks when inoculated with *Tr. brucei*. The virulence of the organism is somewhat variable.

Attempts to cultivate *Tr. gambiense* in artificial media have not been fully successful. It has been possible to obtain multipli-

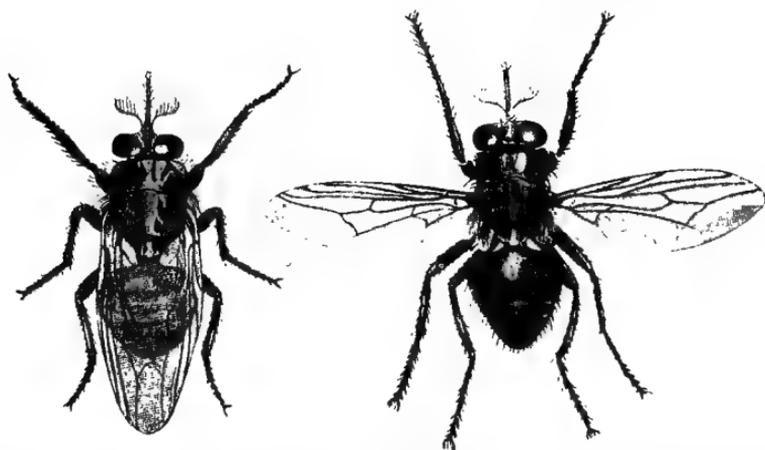


FIG. 165.—*Glossina palpalis* in natural resting position, and with wings outstretched. (After Dozlein.)

cation of the organisms and to keep them alive for several weeks on blood-agar but such cultures are not virulent and cannot be kept up indefinitely.<sup>1</sup>

Human trypanosomiasis is a most important and widespread disease in equatorial Africa. Symptoms appear long after the infection has taken place. The disease manifests itself in two forms, the trypanosoma fever and the sleeping sickness. Trypanosoma fever is an irregularly remittent fever lasting for several days at each attack, accompanied by a macular eruption, and

<sup>1</sup> Thomson and Sinton: *Annals of Trop. Med. and Parasitol.*, 1912, Vol. VI, pp. 331-356.

always associated with a general enlargement of the lymph nodes. The trypanosomes are numerous in the blood during the febrile period and become very scarce during the intermissions. The fever leads to emaciation and death, sometimes without inducing the terminal coma and sometimes with the production of typical sleeping sickness. The sleeping sickness is characterized by prolonged coma and progressive emaciation. At intervals the patient may be aroused and given nourishment, but eventually this is no longer possible. At this stage the trypanosomes are present in the cerebrospinal fluid. Bacterial infection of the

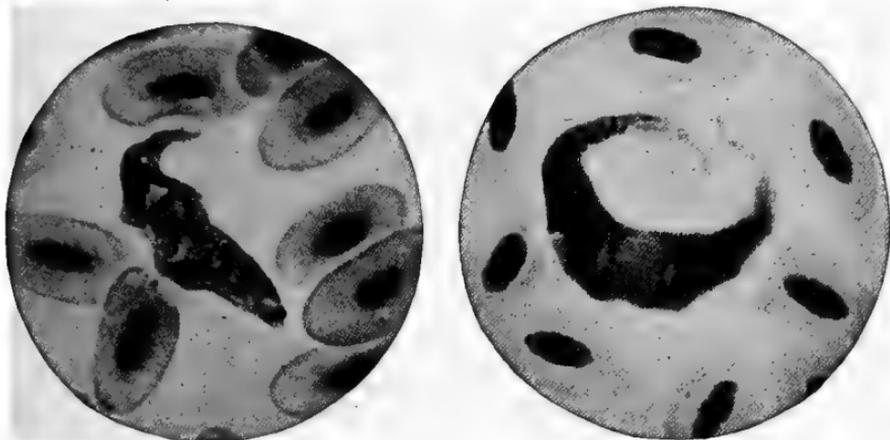


FIG. 166.—*Trypanosoma avium* in the blood of common wild birds.  $\times 1500$ .  
(After Novy and MacNeal.)

meninges often takes place as a terminal event. It is conservatively estimated that 100,000 natives have died of trypanosomiasis in Africa from 1900 to 1910. There have been several cases in Europeans. Recovery seems to be rather uncommon but does occur.

**Trypanosoma Rhodesiense.**—Stephens and Fantham<sup>1</sup> have studied a case of human trypanosomiasis contracted in north-eastern Rhodesia, where *Glossina palpalis* does not occur. The parasite differs somewhat from *Tr. gambiense* and is regarded by

<sup>1</sup> Proc. Royal Soc., 1910, Ser. B, Vol. LXXXIII, pp. 28-33.

these authors as a distinct species. It seems to be transmitted by *Glossina morsitans*.<sup>1</sup>

**Trypanosoma Avium.**—Trypanosomes were probably seen in the blood of birds by earlier investigators, but the first accurate description of such observations is that of Danilewsky in 1885.



FIG. 167.—*Trypanosoma avium* in culture on blood agar,  $\times 1500$ . (After Novy and MacNeal.)

Infection with trypanosomes is very common in the ordinary wild birds. Novy and Mac Neal<sup>2</sup> examined 431 American birds representing 40 common species and found trypanosomes in 38 individuals, representing 16 species. The indicated prevalence

<sup>1</sup> Kinghorn and Yorke: *Annals of Trop. Med. and Parasitol.*, 1912, Vol. VI, pp. 269-285. Kinghorn, Yorke and Lloyd: *ibid.*, 1912, Vol. VI, pp. 495-503.

<sup>2</sup> *Journ. Infect. Dis.*, 1905, Vol. II, pp. 256-308.

of the infection, 8.8 per cent, is doubtless far below the actual percentage, as many of the birds were not tested by the cultural method. There are doubtless several species of bird trypanosomes but the most common form is *Tr. avium*. The length varies from 25 to 70 $\mu$  and the width from 4 to 7 $\mu$ .

Cultures are easily obtained by transferring the infected blood to tubes of blood-agar and incubating at 25° to 30° C. The protozoa grow abundantly and, by weekly transfers, may be kept under cultivation without special difficulty for an indefinite period. Injection of cultures into birds is only rarely followed by appearance of trypanosomes in the blood.

The parasites persist in the blood of the birds for many months and probably for years. They seem to be comparatively harmless. The mode of transmission from bird to bird is unknown.

*Trypanosoma avium* is a form of considerable importance in the study of systematic protozoölogy because of the confusion of trypanosomes and hemocytozoa by Schaudinn<sup>1</sup> in 1904, who regarded *Tr. avium* as merely an extracellular form of *Hæmoproteus noctuæ* (*danilewskyi*?) (see page 433). This misconception, together with the analogous assumption of similar relationship between spirochetes of birds and the leukocytozoön of Ziemann, *Hæmoproteus ziemanni*, made by Schaudinn at the same time, has exercised a profound influence upon the course of investigation in the groups of spirochetes, trypanosomes and hemocytozoa.

**Schizotrypanum Cruzi.**—Chagas discovered this organism in 1907. It occurs in the blood in the Brazilian human trypanosomiasis called coreotrypanosis. Multiplication takes place within endothelial cells, lymphocytes and other cells in the parenchymatous organs, and especially in the interior of muscle cells in the heart and skeletal muscles.<sup>2</sup> The dividing parasites are without flagella and resemble the intracellular forms of *Leishmania*. From these cysts the parasites escape into the blood, where they

<sup>1</sup> Arb. a. d. Kais. Gesundheitsamte, 1904, Vol. XX, pp. 387-439.

<sup>2</sup> Vianna: *Memorias do Instituto Oswaldo Cruz*, 1911, Vol. III, pp. 276-293. Abstract in *Sleeping Sickness Bull.*, 1912, Vol. IV, pp. 288-293.

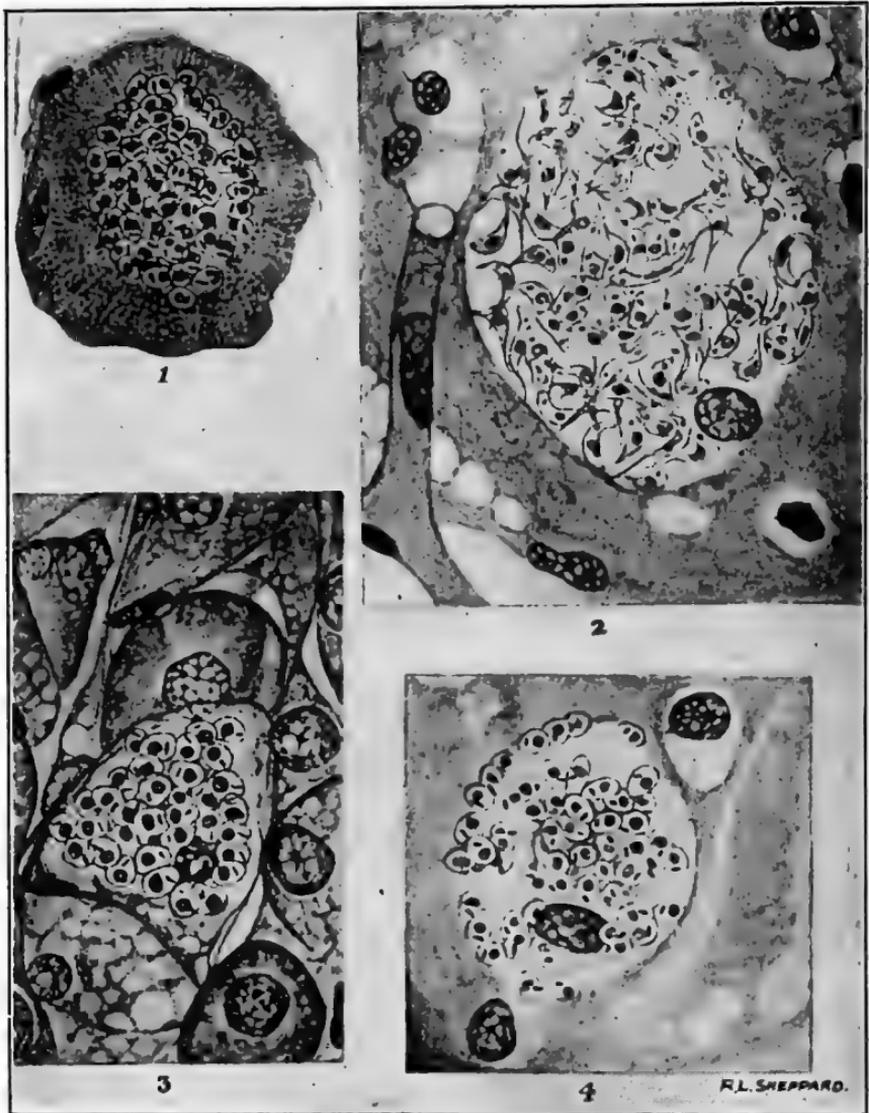


FIG. 168.—*Schizotrypanum crusi* developing in the tissues of the guinea-pig. 1. Cross-section of a striated muscle fiber containing *Schizotrypanum crusi*: Note dividing forms. 2. Section of brain showing a *Schizotrypanum* cyst within a neuroglia cell, containing chiefly flagellated forms. 3. Section through the suprarenal capsule, fascicular zone. 4. Section of brain showing a neuroglia cell filled with round forms of *Schizotrypanum*. (From Low, in *Sleeping Sickness Bulletin*, after Vianna.)

are found as trypanosomes in the blood plasma. Slender and thick forms occur here, the difference probably depending upon the age of the parasites.

Monkeys, rats, mice, young guinea-pigs and many other mammals are susceptible to inoculation. The infection is transmitted by a bug, *Conorhinus megistus*, in which the protozoön develops abundantly. The bedbug, *Culex lectularius*, also is capable of transmitting the disease.

Cultures are readily obtained on blood-agar and Chagas was able to infect animals with such cultures.

**Leishmania Donovanii.**—Laveran and Mesnil in 1903 described this protozoön which occurs inside cells in various parts of the body, but is especially abundant in the spleen and liver, in the disease

known in India as Kala-Azar or tropical splenomegaly. The organism is oval, 2 to 4 $\mu$  in diameter, finely granular and sometimes vacuolated. In the interior there is a large rounded nucleus and a smaller oval or rod-shaped blepharoplast, near which a third very slender short thread may usually be recognized as the rudiment of the undeveloped flagellum. These structures are doubled in the division stages. Multiple division also occurs. In the circulating blood the organism is found within lymphocytes and polynuclear leukocytes. Many of them may be found in a single cell.

Cultures are readily obtained by inoculating fluid (citrated) blood with blood or with spleen juice containing the parasites, or by inoculating the usual blood-agar. In artificial culture the cell elongates, the rudimentary whip extends into a true flagellum and the organism assumes the appearance of a typical leptomonas (herpetomonas). Little difficulty is experienced in keeping the cultures alive and flourishing.

The parasite has been supposed to be transmitted from man to man by bugs of the genus *Cimex*, but this hypothesis has been

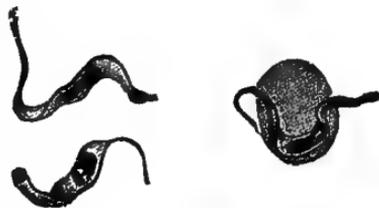


FIG. 169.—*Schizotrypanum cruzi* in human blood. (From Doflein after Chagas.)

rendered very uncertain by the work of Wenyon<sup>1</sup> and the Ser-



FIG. 170.—*Conorhinus megistus*, the insect carrier of *Schizotrypanum cruzi*. (From Doflein after Chagas.)

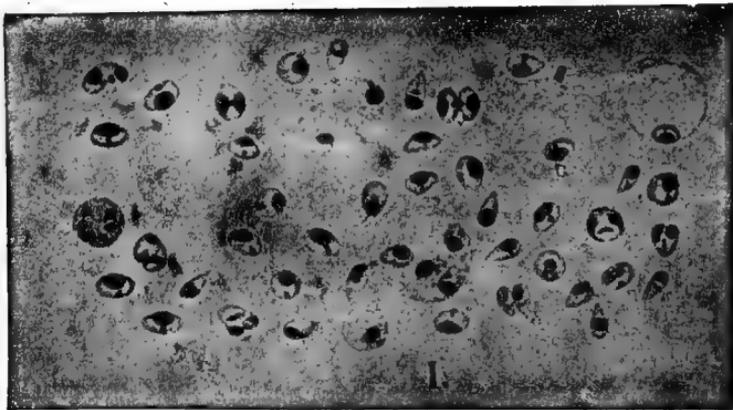


FIG. 171.—*Leishmania donovani* in the juice obtained by puncture of the spleen in kala-azar. (From Doflein after Donovan.)

gents.<sup>2</sup> The latter investigators were able to effect experimental transmission by means of the dog flea, *Ctenocephalus canis*,

<sup>1</sup> *Journ. Lond. Sch. Trop. Med.*, 1912, Vol. II, pp. 13-26.

<sup>2</sup> Sergent (Edm. & Et.), L'Heritier and Lemaire, *Bull. Soc. Path. Exot.*, 1912, Vol. V, pp. 595-597.

Kala-Azar is endemic in tropical Asia and northeast Africa, where it occurs among the poorer class of people, living in squalor. It is characterized by irregular fever, weakness and cachexia and especially by enormous enlargement of the spleen, often of the liver also. It is frequently fatal. Dogs and monkeys are susceptible to inoculation.

**Leishmania Tropica.**—This organism was first accurately described by J. H. Wright,<sup>1</sup> who found it in great abundance in the lesion known as Aleppo boil, Delhi boil or tropical ulcer. The parasites occur within the endothelial cells within the lesion and are very

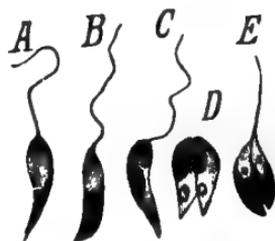


FIG. 172.—*Leishmania donovani*, various forms observed in artificial culture. (From Doflein after Chatterjee.)

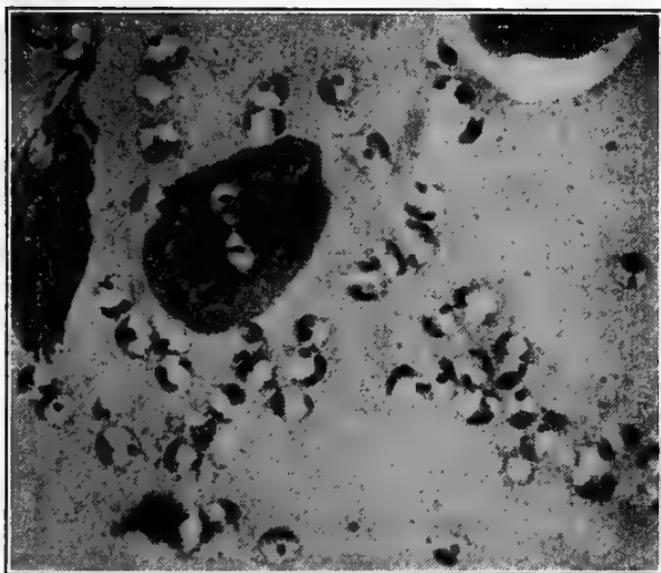


FIG. 173.—*Leishmania tropica*. Smear from a Delhi boil.  $\times 1500$ . (After J. H. Wright.)

numerous. *Leishmania tropica* resembles *L. donovani* very closely except in its pathogenic properties. Cultures on blood-

<sup>1</sup> *Journ. Med. Rsch.*, 1903, Vol. X, pp. 472-482.

agar have been obtained by Nicolle and are easily propagated at 22° C. Dogs and monkeys are susceptible to inoculation and the human disease is probably contracted from dogs through the agency of insects. The disease is relatively benign and recovery is followed by prolonged immunity. Inoculation has been practised in man in order to produce immunity.



FIG. 174.—*Leishmania tropica*, forms observed in cultures. (From Doflein after Nicolle.)



FIG. 175.—*Trypanoplasma cyprini*. Bl, Blepharoplast; N, nucleus.  $\times 2000$ . (After Doflein.)

***Leishmania Infantum*.**—Nicolle in 1908 observed this organism in the spleen, liver and bone marrow of children dying from splenomegaly in northern Africa. The disease resembles Kala-Azar in all respects except that the patients are all very young. Dogs are naturally infected with this parasite and are probably the source of the human disease. Cultures on blood-agar are readily obtained and kept up indefinitely without special difficulty.

**Trypanoplasma Borreli.**—Laveran and Mesnil in 1901 described this protozoön, which occurs in the blood of various species of fish. It resembles a trypanosome somewhat, but the blepharoplast is relatively large and from it two flagella originate, one extending forward immediately as a free whip while the other runs along the convex border, ensheathed in an undulating membrane, and extends at the posterior end as a free flagellum. Longitudinal division takes place in the circulating blood. Transmission seems to be accomplished by means of leeches. *T. cyprini* and *T. guernei* seem to be identical with *T. borreli*, but they may prove to be distinct species.



FIG. 176.—*Bodo lacertæ*. a, Sketched from life; b, drawn from a stained preparation. (From Doflein after Hartmann and Prowazek.)

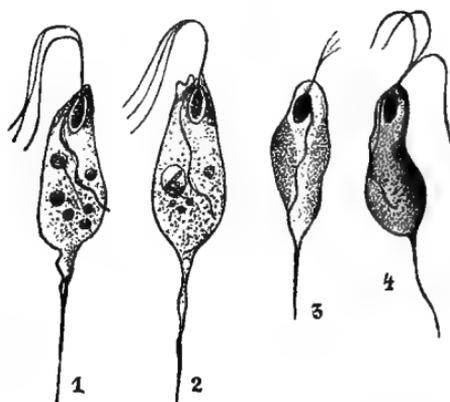


FIG. 177.—*Trichomonas hominis* from the mouth. (From Doflein after Prowazek.)

**Bodo Lacertæ.**—In the cloaca of various lizards a flagellate is almost constantly found. It is 2 to 4 $\mu$  wide and 6 to 12.5 $\mu$  long, lance-shaped and twisted at the posterior (pointed) end. The nucleus is near the anterior end. At its side is a granule resembling a blepharoplast and from this a thread extends to the anterior cell end of the cell, where it gives rise to two flagella.

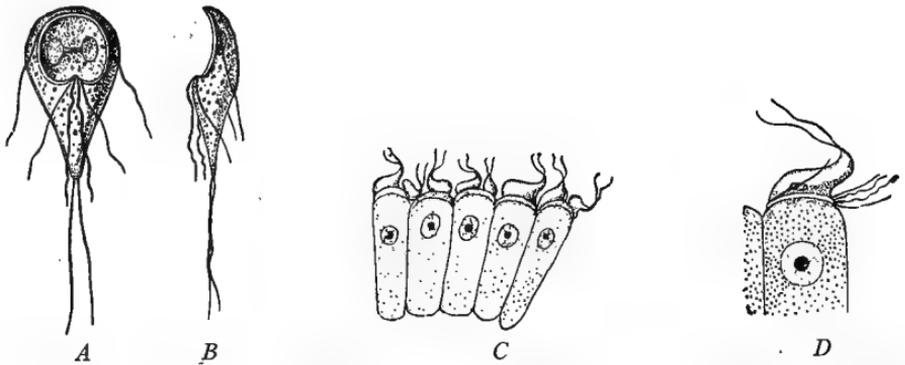


FIG. 178.—*Lamblia intestinalis*. A, Ventral aspect; B, lateral view; C, in position on epithelium; D, the same enlarged. (From Doflein after Grassi and Schewiakoff.)

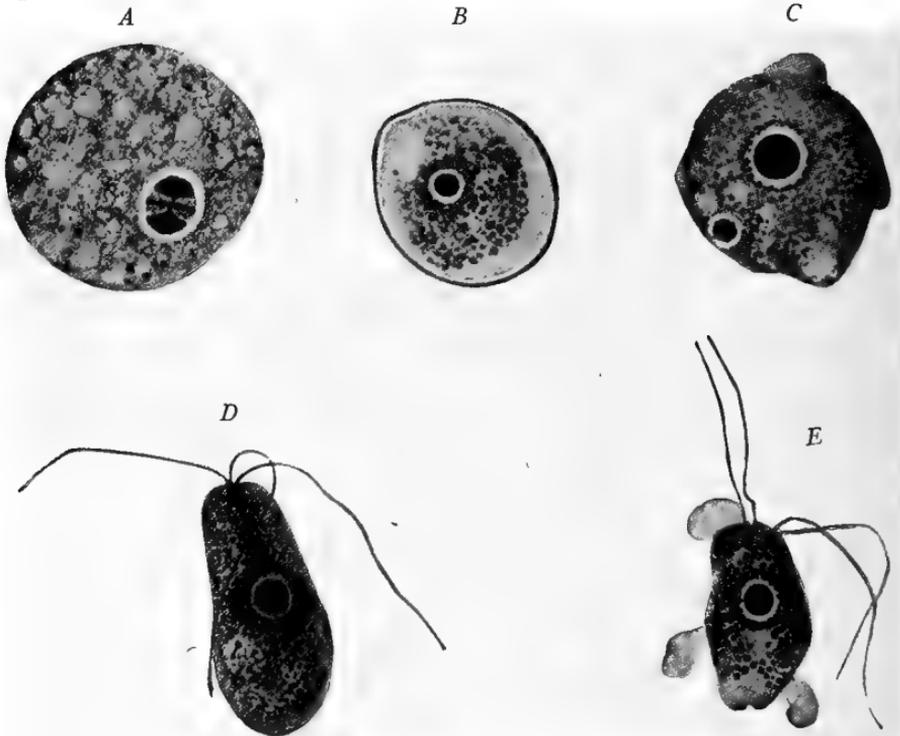


FIG. 179.—*Trimastix gamæba philippinensis*. A, Early stage of division of the nucleus. The polar caps are still united by a bridge. The equatorial plate has formed. B, Ordinary cyst. C, Vegetative form showing the nucleus and a second chromatin granule (split off from it?). D, Flagellated form showing remains of the rhizoplast between the nucleus and the basal granules. E, Flagellated form with pseudopodia. (After Whitmore.)

**Trichomonas Hominis.**—Davaine observed this parasite in 1854. It is common in the human digestive tract, especially in the stomach in anacidity and in the intestine in chronic digestive disturbances. The organism is 3 to  $4\mu$  wide and 4 to  $15\mu$  long, pear-shaped and provided with three free flagella, and a fourth thread which passes around one side of the cell in the margin of the undulating membrane. The parasite seems to be a harmless commensal, as a rule, but it may possibly bear some causal relation to diarrhea in some cases. Animals have not been successfully inoculated with it. *Tr. vaginalis* is very similar. It grows in the acid vaginal mucus. Other trichomonad forms occur in the intestines of animals, particularly in mice, in frogs and in lizards.

**Lambliia Intestinalis.**—The cell has the form of a turnip with a wide and deep excavation in front near the anterior rounded end, forming a suction cup. The body is bilaterally symmetrical. The length is 10 to  $21\mu$  and the width 5 to  $12\mu$ . There are eight flagella, each from 9 to  $14\mu$  long. The mode of multiplication is not fully known. Resistant cysts are formed, probably after sexual union of two individuals, and these escape with the feces and lead to the infection of new hosts. *Lambliia* lives in the duodenum and jejunum of man and many other mammals. It appears to be relatively harmless in most cases but the possibility that it may be a cause of digestive disturbance must be considered. It is often present in chronic dysenteries.

**Mastigamœba Aspera.**—This is a saprophytic form, described by Schulze, which possesses a single flagellum, but is also capable of extending finger-like projections of its cytoplasm, pseudopodia, just as an ameba does. Whitmore<sup>1</sup> has described a somewhat similar saprophyte, *Trimastigamœba philippinensis*, which is at times ameboid without flagella and at other times possesses three or possibly four whips. It divides and encysts like an ameba. The organism is readily cultivated on the alkaline agar of Musgrave and Klegg.

<sup>1</sup> *Archiv f. Protistenkunde*, 1911, Bd. XXIII, S. 81-95.

## CHAPTER XXVIII

### RHIZOPODA

**Amœba Proteus.**—This large saprophytic amœba may be considered as an example of the numerous species of free-living amebæ, the classification and identification of which is still in hopeless confusion. The organism is widely distributed in stagnant water and is easily cultivated in the laboratory in not too foul infusions containing bacteria and algæ. The cell is 50 to

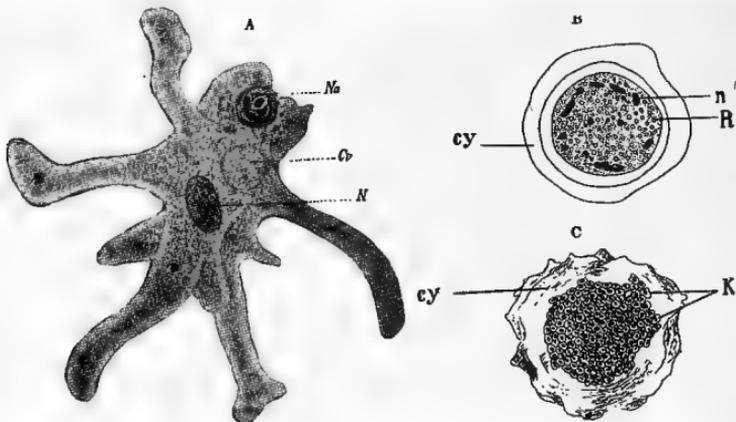


FIG. 180.—A, *Amœba proteus* engulfing a clump of small algæ (*Na*). *Cv*, contractile vacuole; *N*, nucleus. B, Newly encysted amœba showing nuclear fragments; *cy*, cyst wall; *n*, nucleus; *R*, reserve food substance. C, Cyst containing many young amebæ beginning to escape; *cy*, cyst wall; *k*, young amebæ. (After Doflein.)

500 $\mu$  across, often possesses numerous thick, blunt pseudopodia. The ectoplasm and endoplasm appear distinctly different, the latter being filled with granules, crystals, vacuoles and food particles, such as algæ and bacterial cells, and possessing a contractile vacuole. The nucleus is lentil-shaped and the chromatin within it has a very typical arrangement in a central plate surrounded by a network on which the peripheral chromatin is symmetrically

placed. Binary division with mitosis of the nucleus seems to be the common mode of multiplication. Multiple division also occurs in the vegetative state. The resistant stage (cyst) is characterized by a thick, firm wall of several layers, within which the nucleus divides into 200 or more daughter nuclei. Each of these becomes surrounded by a little cytoplasm and, when the cyst bursts, wanders out as a young ameba. The life history is incompletely known.

Cultures of saprophytic amebæ are readily obtained upon agar plates. The medium contains agar 0.5 gram, tap water



FIG. 181.—*Endamoeba coli*. a, Free ameba; b, ripe cyst with eight nuclei. (From Doflein after Hartmann.)

90 c.c., ordinary nutrient broth 10 c.c. Cultures are incubated at 25° C. Williams<sup>1</sup> has succeeded in obtaining pure cultures free from bacteria, at 36° C. by employing agar smeared with naturally sterile brain substance.

**Endamoeba Coli.**—Loesch<sup>2</sup> in 1875 observed amebæ in the human large intestine in gastro-intestinal disturbance. The organism is very common in the human intestine, being found in 10 to 60 per cent of persons without digestive disturbances, when the examination is thorough.

The cell in the vegetative stage is variable in shape and size,

<sup>1</sup> *Journ. Med. Resch.*, 1911, Vol. XXV, pp. 263-283.

<sup>2</sup> *Virchow's Archiv*, 1875, Bd. LXV, S. 196-211.

the diameter measuring 10 to 70 $\mu$ . The protoplasm is slightly granular and shows distinctly an alveolar structure. The distinction between ectoplasm and endoplasm is apparent only in the pseudopodia. There is no contractile vacuole. Food substance is present in the cytoplasm, bits of vegetable material, bacteria and, rarely, red blood cells. The nucleus is round, vesicular and enclosed in a nuclear membrane. In its center is a relatively large mass of chromatin and there are numerous smaller masses of chromatin at the periphery beneath the nuclear membrane. Multiplication in the vegetative stage takes place by binary division as a rule, but multiple division preceded by repeated division of the nucleus also occurs.

*E. coli* discharges all food material from its cytoplasm before encystment so that the cell is clear and the nucleus plainly visible. A large vacuole in the cytoplasm usually makes its appearance and is present during the first and second division of the nucleus in the cyst. It is large in those cysts in which much chromatin escapes from the nuclei into the cytoplasm as chromidia, and it usually disappears when the four nuclei have been formed. A further division of the nuclei gives rise to eight and this is the usual number present in the fully developed cyst of *E. coli*, although rarely ten or even sixteen nuclei may be observed.<sup>1</sup> The self-fertilization, autogamy, described by Schaudinn as occurring early in encystment has not been observed by Hartmann, and its actual occurrence seems questionable. The developed cyst with eight nuclei is about 15 $\mu$  in diameter and is considered to be definitely characteristic of this species.

*E. coli* is generally regarded as a harmless commensal in the human intestine. It is however impossible to exclude the possibility that it may contribute to the aggravation of pathological conditions present in the digestive tract. (Compare with *Bacillus coli*.) Its common occurrence in healthy men speaks against its possessing any very specific and powerful pathogenic property.

<sup>1</sup>Hartmann and Whitmore: *Archiv. f. Protistenkunde*, 1912, Bd. XXIV, S. 182-194.

**Endamœba Dysentericæ.**—It is evident that the dysentery ameba was observed by Loesch in 1875, but a clear distinction between the harmless and the pathogenic forms of enteric amebæ

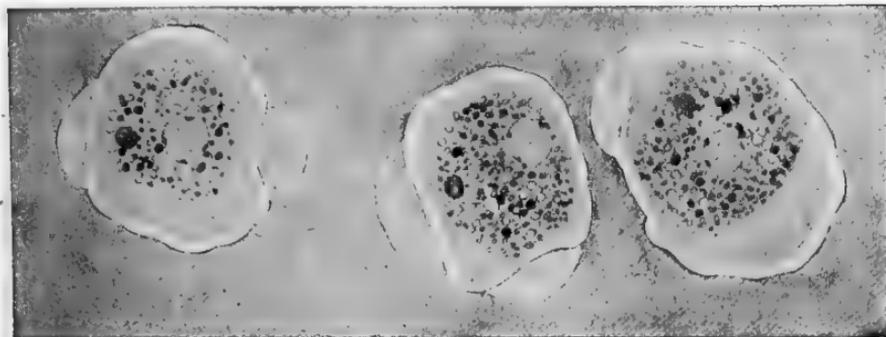


FIG. 182.—*Endamœba dysentericæ*. The same living individual drawn at brief intervals while moving. (From Doflein after Hartmann.)

was not made until 1903. It is yet not certain whether one or several species are included in the dysenteric amebæ. The name,

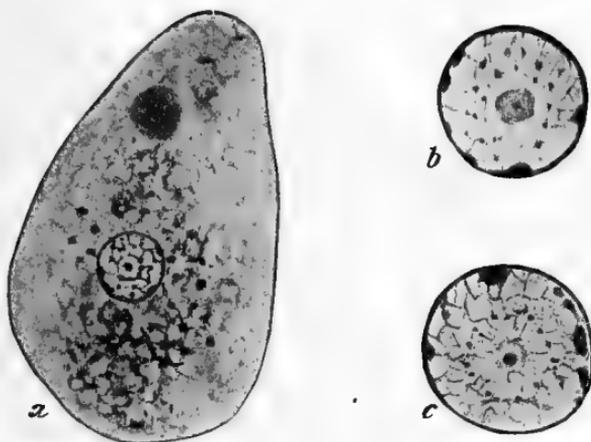


FIG. 183.—*Endamœba dysentericæ*. a, Vegetative cell containing a red blood cell (near upper end).  $\times 1300$ . b and c, Drawings of nuclei showing stages of the so-called cyclical changes.  $\times 2600$ . (From Doflein after Hartmann.)

*Endamœba dysentericæ* is synonymous with *E. tetragena* and *E. histolytica*. The organism occurs in the intestine and in the stools of persons suffering from amebic dysentery and very seldom in

other individuals. The cell is 8 to 60 $\mu$  in diameter. The ectoplasm is distinctly differentiated from the endoplasm even when the cell is motionless, and the lobose pseudopodia are made up entirely of the stiff highly refractive ectoplasm. The endoplasm contains food material consisting of bacteria, cell fragments and red blood cells. The nucleus is very distinctly visible in the living ameba. It is spherical and surrounded by a thick doubly-contoured nuclear membrane. The chromatin is usually distributed just beneath the nuclear membrane in largest amount and in the center there is a karyosome with definite centriole. The vegetative multiplication takes place by division into two daughter cells. Multiple division seems not to occur.



FIG. 184.—*Endamoeba dysenteriae*. Mature cyst containing four nuclei and a mass of chromidial substance. (After Hartmann.)

Cyst formation is rarely observed. The cysts are most likely to be found when the stool becomes formed in convalescence from an attack of dysentery and they may then be very numerous. The mature cyst contains four nuclei, and frequently contains also one or more large masses of chromidial substance which stain black with iron hematoxylin.

The forms of the organism commonly observed in the feces of dysentery are either the active vegetative cells<sup>1</sup> or degenerating forms, and the latter may lead to confusion unless their true nature is recognized.

The belief that amebæ bear a causal relation to dysentery is based upon the fact that certain types of amebæ, *E. dysenteriae* (*E. histolytica*) are found in the stools, as a rule, only in cases of dysentery; further, that these cases of dysentery, in which these amebæ occur, are characterized by definite clinical signs and typical anatomical changes in the intestine; and that these amebæ are found penetrating deeply into the mucosa of the intestine, and it is possible to produce ulcerative enteritis in experimental

<sup>1</sup> Hartmann: *Arch. f. Protistenkunde*, 1912, Bd. XXIV, S. 163-181.

animals by injecting feces containing amebæ into the rectum or by feeding fecal material containing cysts; and further, the fact that abscesses occur in the liver in amebic dysentery, in which the amebæ are present and in which it has been impossible to demonstrate the presence of bacteria. The causal relation seems highly probable, but it must be recognized that the evidence is very inconclusive and admits of other possible explanations. Even the relationships of the various forms seen in the microscopic preparations require a certain amount of speculation for their determination, and the possibility of error, even by the experienced protozoölogist, must be recognized and has been well illustrated by the divergent views of Schaudin<sup>h</sup> and of Hartmann in studying the same slides. Greater certainty would doubtless be derived from the study of artificial cultures if such could be made available.

Numerous cultures of amebæ have been obtained from the stools of cases of dysentery, and some from the pus of amebic abscesses of the liver, the growth taking place on agar in the presence of a single species of bacteria. With these cultures it has been possible to cause enteritis in monkeys. Such cultures have also been grown at 37° C. by A. W. Williams<sup>1</sup> in pure culture on agar streaked with brain substance and with blood, and in these cultures she finds that the amebæ approach in their structure the typical endamebæ, not only in nuclear structure and cyst formation, but also in the utilization of red blood cells as food. Whitmore<sup>2</sup> has carefully studied a number of cultures of amebæ obtained from cases of dysentery, one of them from a liver abscess, and has concluded that in every instance the amebæ were free-living saprophytic forms belonging to the genus *Amæba* and not in any case parasitic species.

<sup>1</sup> Soc. Amer. Bact., New York Meeting, Jan. 2, 1913. Science 1913, Vol. XXXVIII, p. 451; Williams, A. W., and Calkins, G. N., *Journ. Med. Rsch.*, 1913, Vol. XXIX, pp. 43-56.

<sup>2</sup> *Archiv f. Protistenkunde*, 1911, Bd. XXIII, S. 71-80; *ibid.*, pp. 81-95.

**Other Rhizopoda.**—The remaining orders of the Rhizopoda, namely Helizoa, Foraminifera, Radiolaria and Mycetozoa contain no parasitic forms of great importance to human pathology. *Plasmodium brassicae* which causes tumors on the roots of the cauliflower plant is of some interest.<sup>1</sup>

<sup>1</sup> See Doflein, *Protozoenkunde*, 1911, S. 672-678.

## CHAPTER XXIX

### SPOROZOA

**Cyclospora Caryolytica.**—Schaudinn in 1902 discovered this organism, which lives as a parasite in the nuclei of epithelial cells of the intestinal mucosa in the common mole. It is ingested in the form of spores, from which the slender young sporozoites escape in the intestine and penetrate the nuclei of epithelial cells. Here the parasite becomes rounded and enlarges; becoming quickly differentiated into either the male or female type. The former type of parasite has numerous refractive granules in its

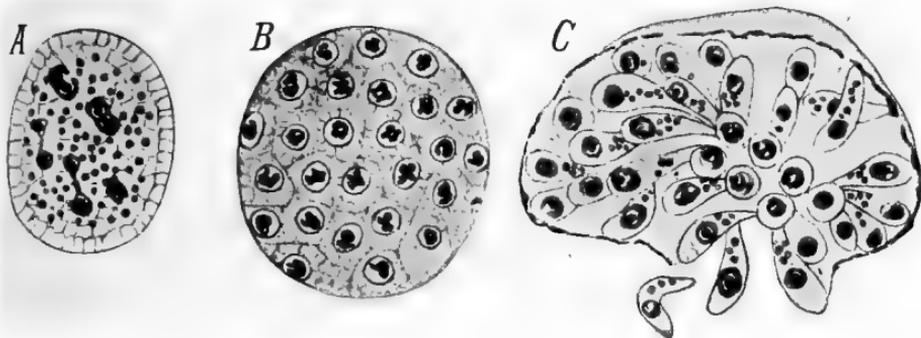


FIG. 185.—*Cyclospora caryolytica*. A, Male cells within the nucleus of the host cell. B and C, Reproduction by multiple division with final rupture of the host nucleus in (C). (From Doflein after Schaudinn.)

cytoplasm, while the female type has a clear cytoplasm. The parasites grow rapidly and segment after 4 to 8 hours, the females earlier than the males, and the cells resulting from this segmentation, so-called merozoites or agametes, penetrate new nuclei and go through the same development. Four to five days after infection of the mole, the parasites suddenly cease their asexual multiplication. The male parasites, microgametocytes, after rapid multiplication of nuclei, give rise to numerous microgametes

provided with two flagella. The female cells, macrogametocytes, enlarge slowly and produce numerous yolk-like granules in their

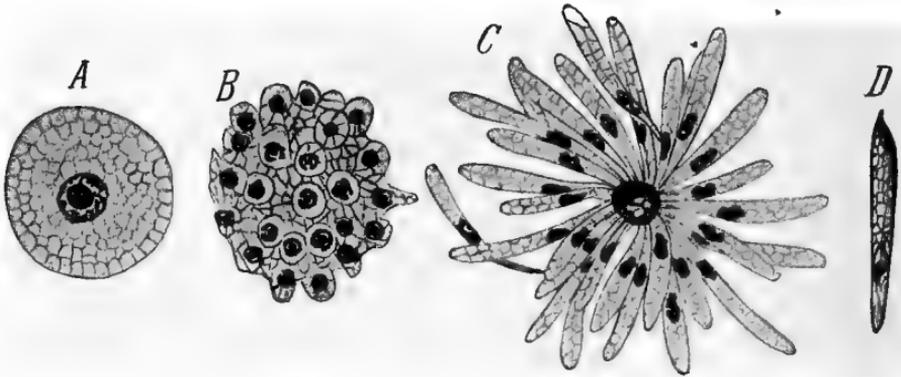


FIG. 186.—*Cyclospora caryolytica*. A, Female cell (agamete) within the host nucleus. B and C, Multiple division. D, A free young female agamete. (From Doflein after Schaudinn.)

cytoplasm. The nucleus undergoes two reduction (maturation) divisions, and one daughter nucleus remains while the others

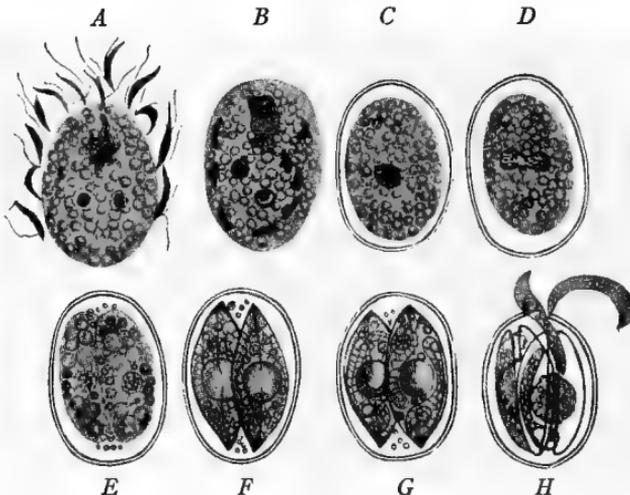


FIG. 187.—*Cyclospora caryolytica*. A, Fertilization. B, Fertilized cell. C, Fertilized cell (oocyst) with cyst wall. D, E, F and G, Division of the cyst contents to form two spores, each containing two sporozoites. H, Escape of the sporozoites. (From Doflein after Schaudinn.)

disintegrate. Several microgametes penetrate the matured macrogamete and one of them unites with the nucleus. A cyst wall

forms about the fertilized cell and within this the cell divides into two and later into four embryo parasites, which are enclosed in pairs in two spores within the cyst. This escapes with the feces of the mole and serves to infect a new host.

The invasion of the epithelium produces a severe diarrhea in the mole often resulting in death. If the animal survives for five days, until after the spores are formed, it then usually recovers.

**Eimeria Stiedæ** (*Coccidium Cuniculi*).—This very common parasite of the rabbit was first described by Lindemann in 1865. It lives and grows within the epithelial cells of the small intestine, of the bile passages and of the liver of rabbits suffering from coccidiosis, and its oöcysts are found in the intestinal contents and in the feces of such animals. The oöcyst is an elongated oval, variable in width from 11 to 28 $\mu$  and in length from 24 to 49 $\mu$ . It contains, when fully developed, four spores, each of which contains two embryo parasites or sporozoites. These gain entrance to the intestine of a new host along with the food and the pancreatic digestion makes an opening at one end where the wall is exceedingly thin, the micropyle, and through this opening the wedge-shaped sporozoites escape. They penetrate epithelial cells, in which the parasite becomes rounded and grows to a diameter of 20 to 50 $\mu$ , destroying the host cell. The nucleus divides many times and after it the cytoplasm, so as to form numerous spindle-shaped young cells, merozoites or agametes, which penetrate new epithelial cells and pass through the same cycle. This cycle of asexual multiplication, schizogony, is repeated many times and may lead to extensive destruction of intestinal mucosa, of the epithelium of the bile ducts and of liver substance. Some of the growing parasites become differentiated into sexual elements. The female cell, macrogametocyte, accumulates numerous large granules in its cytoplasm, and when full-grown the chromatin



FIG. 188.—*Eimeria stiedæ*. Oöcyst containing four spores, in each of which two sporozoites are developing. The micropyle is below. (From Doflein after Metzner.)

of the nucleus is reduced by expulsion of the karyosome. The matured cell, macrogamete, is then ready for union with the microgamete. The growing cell destined to give rise to the male sexual elements attains a large size and possesses a pale cytoplasm. It is called the microgametocyte. Its nucleus divides many times, the small nuclei accumulate near the surface of the cell and each escapes with a small portion of protoplasm as a slender motile microgamete. The penetration of one of these into the macrogamete produces the fertilized oöcyst, which forms a thick wall about itself and escapes to the external world. Here, the

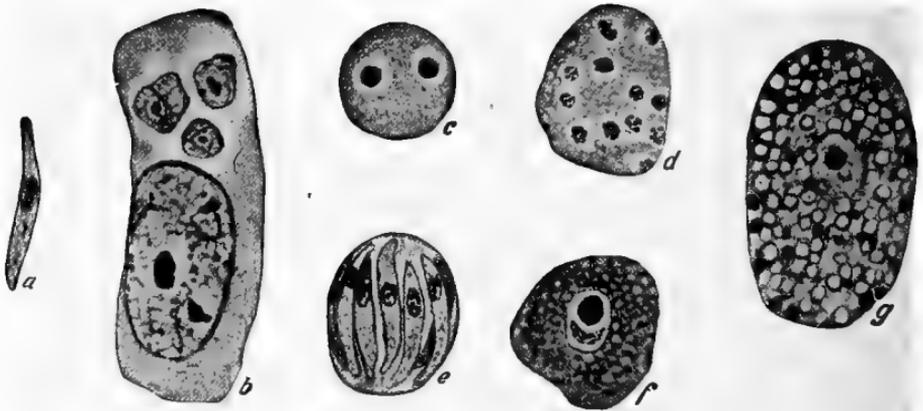


FIG. 189.—*Eimeria stiedae*. *a*, Young agamete (merozoite). *b*, Epithelial cell invaded by three young agametes. *c*, *d*, and *e*, Stages in the multiple division of the agamete. *f*, Young macrogametocyte. *g*, Full-grown macrogametocyte. (From Doflein after Hartmann.)

fertilized cell divides to form eight cells, sporozoites, which are enclosed within four oval spores (two in each) within the wall of the oöcyst. If this cyst is ingested by another rabbit the cycle of development starts anew.

Coccidiosis is a very common disease in rabbits. The animal suffers from severe diarrhea and loss of appetite, and becomes emaciated. Young rabbits often die of the disease. Diagnosis is readily made by finding the oöcysts in the feces. Children have been found to be infected with this organism. Cattle, horses, sheep and swine are also susceptible and serious epizootics of coccidiosis due to *E. stiedae* have been observed in cattle.

**Eimeria (Coccidium) Schubergi.**—This coccidium occurs in the intestine of a common myriapod (thousand-legged worm), *Lithobius forficatus*. It is the organism in which Schaudinn worked out the life-cycle now regarded as typical for Eimeriadae, and which corresponds very closely to that of *E. stiedæ*. (See Fig. 80, page 162).

**Hæmoproteus Columbæ.**—Celli and Sanfelice in 1891 observed this organism in the red blood cells of doves. It is widely distributed as a parasite of wild doves and has been found in Europe and in North and South America. The life-history of the parasite in the vertebrate host and its mode of transmission by flies of the genus *Lynchia* has been most fully studied by Aragoa.<sup>1</sup> In the circulating blood of doves the organism is most commonly seen as a large crescent-shaped structure occupying most of the interior of an erythrocyte and crowding the nucleus of the latter to one side or encircling it. The outline of the erythrocyte and the outline of its nucleus are not distorted. The parasites are definitely recognizable as females and males, macrogametocytes with granular, deeply staining cytoplasm and microgametocytes with a paler cytoplasm. When these are ingested by the fly along with its blood meal, the gametes arise, fertilization takes place and there is produced a creeping oökinete which apparently does not penetrate the intestinal wall in the fly or indeed undergo any further development there. It gains the blood stream of a new host, especially young nestlings, when the fly bites them. It is taken up by a leukocyte which comes to rest in the pulmonary capillaries of the young bird. Here the parasite produces a very large cyst and divides to form very numerous minute sporozoites. When the cyst bursts these sporozoites gain the blood stream, penetrate erythrocytes and grow to produce the gametocytes again. The asexual cycle of schizogony seems to be lacking.

This organism is important as a typical example of *Hæmoproteus*, as it is the one species of this genus in which the life cycle has been most completely studied.

<sup>1</sup> *Archiv f. Protistenkunde*, 1908, Bd. XII, S. 154-167.

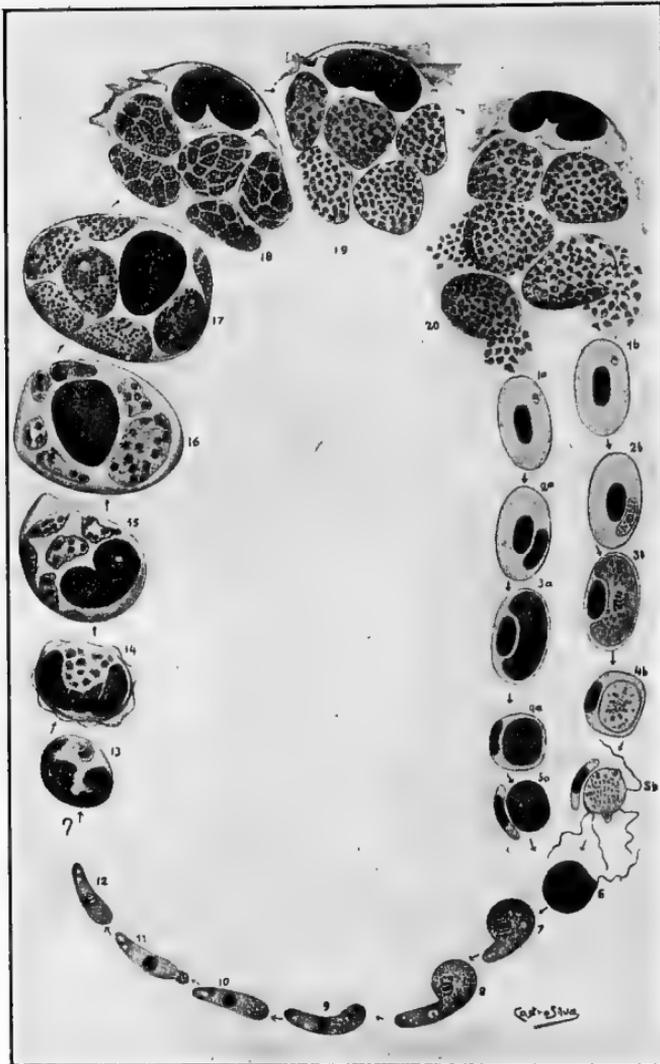


FIG. 190.—*Haemoproteus columbae*. 1a to 3a, Development of the female parasite in the blood of the dove; 1b to 3b, development of the male parasite in the blood of the dove; 4a, 4b, 5a, 5b, 6 to 12, development in the digestive tube of the fly (*Lynchia*); 13 to 20, development of the parasite inside leukocytes in the lung of the dove. (After Aragao.)

**Hæmoproteus (Halteridium) Danilewskyi.**—Grassi and Feletti<sup>1</sup> first clearly recognized this organism as a definite malarial parasite of birds. It is widely distributed and has been found in very many different birds, including sparrows, doves, owls, robins, blackbirds and crows. The life history is incompletely known. In the blood of the infected bird the organism first appears as a small oval or lance-shaped body within the cytoplasm of an erythrocyte. This enlarges, without distorting the outline or displacing the nucleus of the blood-cell, and stretches along one side of the cell. It curves about the nucleus and is enlarged at either end when fully developed. Two types, macrogametocytes and

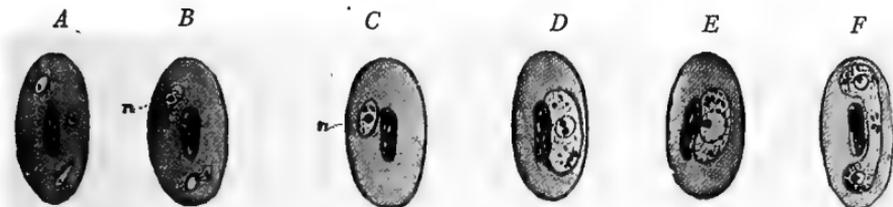


FIG. 191.—*Hæmoproteus danilewskyi*. A and B, Fresh triple infection of red blood cells. C, D and E, Growing parasites, the last two showing vesicular nuclei. F, Full-grown halteridium with two nuclei. (After Dofstein.)

microgametocytes, are easily recognizable in stained preparations. If blood containing these mature halteridia is diluted with citrated salt solution and studied under the microscope the further changes in the sexual cells may often be followed. Each gametocyte bursts the erythrocyte enclosing it and assumes a rounded outline. In the microgametocyte the protoplasmic granules exhibit violent agitation and several fine filamentous processes suddenly shoot out from its periphery and lash about. After a few moments these microgametes separate completely and rapidly swim away. Meanwhile, the macrogametocyte has escaped from its erythrocyte and come to rest in a rounded condition. A microgamete approaches and penetrates the macrogamete, and in a few minutes this fertilized sphere elongates into a curved spindle and actively creeps over the slide. It is then

<sup>1</sup> *Centralbl. f. Bakt.* 1891, Bd. IX, S. 403-409; 429-433; 460-467.

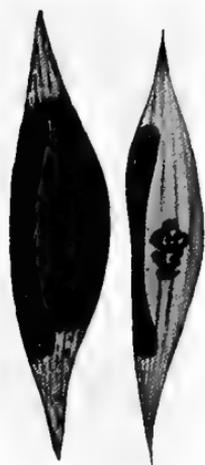


FIG. 192.—*Hæmoproteus (Leukocytozoön) ziemanni*. Macrogametocyte and microgametocyte (paler.) (From Doflein after Schaudinn.)

known as the oökinete. Further development has not been observed, but there can be little doubt that the further stages of sporogony and also the unobserved stages of schizogony in the bird are somewhat analogous to those of *H. columbæ* or to those of the plasmodia of human malaria. Whether the halteridia which occur in various species of birds are all of one species cannot be decided without further investigations.

### *Hæmoproteus (Leukocytozoön) Ziemanni*.—

This organism was doubtless seen by Danilewsky in 1890.<sup>1</sup> Ziemann in 1898 described it as a parasite in the blood of hawks. Its known life history is very incomplete, and even the nature of the blood cell containing it is somewhat doubtful. The youngest stage observed in the blood is a small oval parasite<sup>2</sup> situated at the side of the nucleus of the blood cell. The latter appears

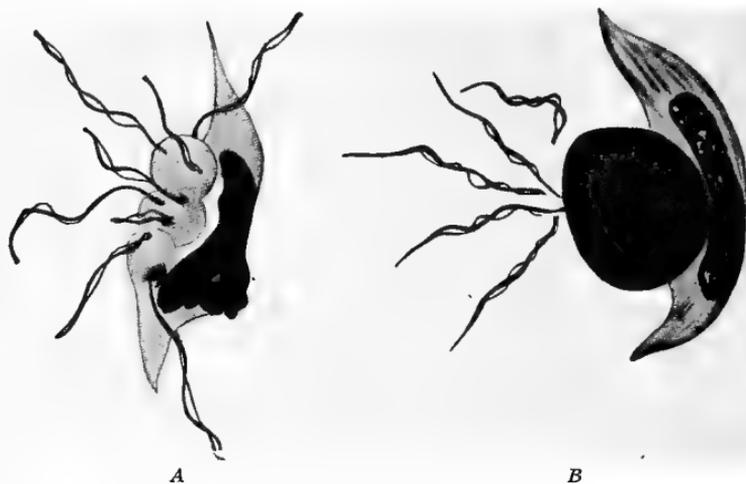


FIG. 193.—*Hæmoproteus (Leukocytozoön) ziemanni*. A, Formation of microgametes from the microgametocyte; B, Fertilization of the macrogamete by one of the microgametes swarming about it. (From Doflein after Schaudinn.)

<sup>1</sup> *Centrabl. f. Bakt.*, 1891, Bd. IX, S. 401, Fig. 1.

<sup>2</sup> The description here given is derived in part from unpublished observations by Novy and MacNeal. See *Proc. Soc. Exp. Biol. and Med.*, 1904-05, Vol. II, pp. 23-28; *American Medicine*, 1904, Vol. VIII, pp. 932-934.

to be an erythroblast, an immature red blood cell in which there is little or no hemoglobin. As the parasite enlarges, the host cell becomes swollen and its nucleus much flattened and distorted. The parasite itself grows long and rather slender and is differentiated

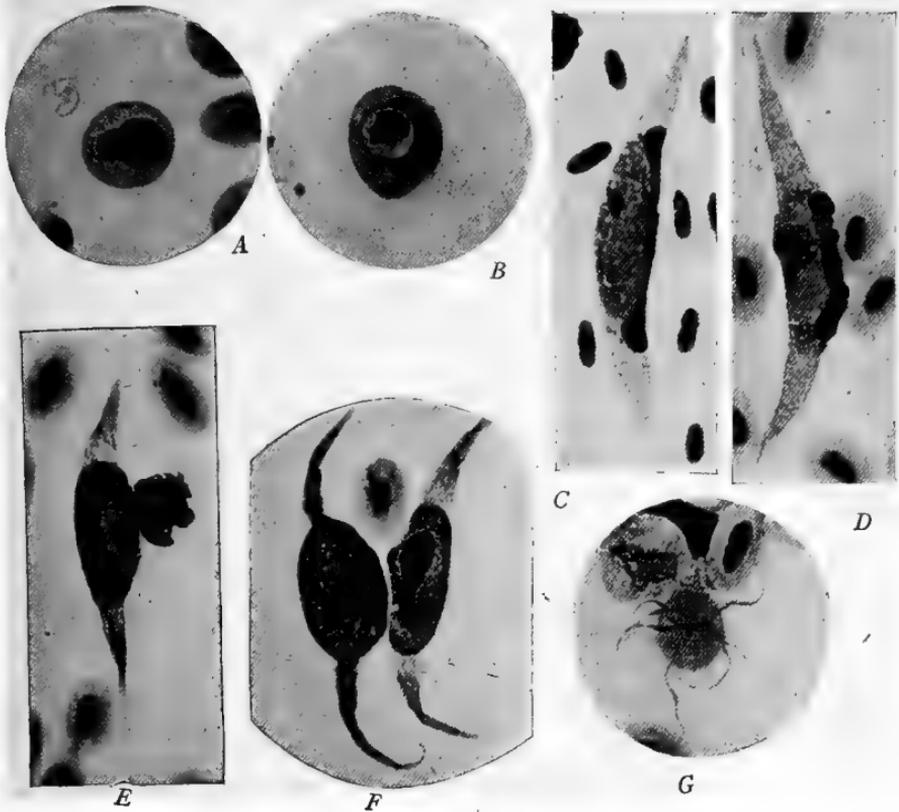


FIG. 194.—*Haemoproteus (Leukocytozoön) ziemanni* in the blood of an owl with a pure infection. A, Young parasite in an erythroblast. B, Growing parasite distorting the nucleus of the host-cell. C and D, Further stages of growth with marked distortion of the nucleus and of the outline of the host cell. E, Full-grown macrogametocyte. F, Macrogametocyte and microgametocyte in the same field. G, Formation of microgametes from the microgametocyte. (After microphotographs of Prof. F. G. Novy.)

to form either the male or the female gametocyte, readily distinguished by their appearance in stained preparations. Meanwhile, the host cell becomes very much elongated and pointed at the ends. The explanation of this peculiar distortion of the cell

the salivary glands, from which they escape into the human host when the mosquito bites. The cycle in *Anopheles* requires eight days at a temperature of  $28^{\circ}$  to  $30^{\circ}$  C. At temperatures below  $17^{\circ}$  C. the microgametes are not produced.

Development of the estivo-autumnal parasite through the stages of schizogony has been obtained by Bass and Johns<sup>1</sup> in the test-tube, in a medium consisting of defibrinated blood to which 0.5 per cent glucose has been added. They were able to keep the organisms alive for ten days at a temperature of  $40^{\circ}$  C., during

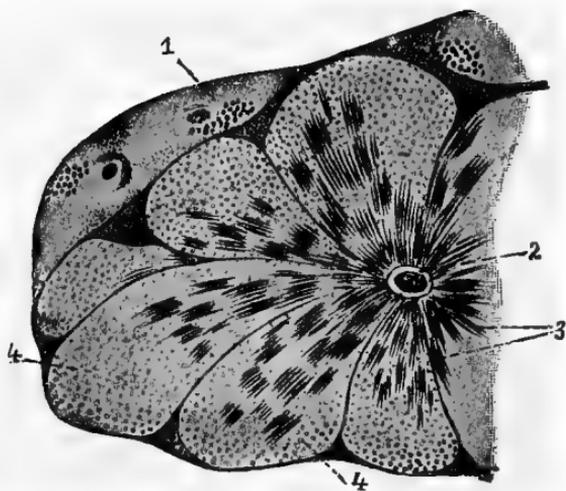


FIG. 205.—Section through salivary gland of *Anopheles* showing numerous sporozoites of *Plasmodium falciparum*. 1, Fat bodies; 2, gland duct; 3, sporozoites of *Plasmodium*; 4, Secretion in the gland cells. (From Doflein after Grassi.)

which period the developmental cycle was repeated four or five times. Their findings have been confirmed by other investigators. More recently Joukoff<sup>2</sup> has reported partial development in the test-tube, of the cycle of sporogony in the case of *Pl. falciparum*, and greater success with *Pl. malariae*.

**Plasmodium Vivax.**—The parasite of tertian malaria is distinctly different from the estivo-autumnal parasite. The young merozoite is 1 to  $2\mu$  in diameter and practically not to be distin-

<sup>1</sup> *Journ. Exp. Med.*, 1912, Vol. XVI, pp. 567-579.

<sup>2</sup> *Compt. Rend. Soc. Biol.*, 1913, Vol. LXXIV, pp. 136-138.

guished, but very early in its growth it becomes actively ameboid and extends irregular and slender processes into the protoplasm of its host cell. As the parasite enlarges, the erythrocyte, often but not always, becomes swollen, paler, and shows a coarse granu-

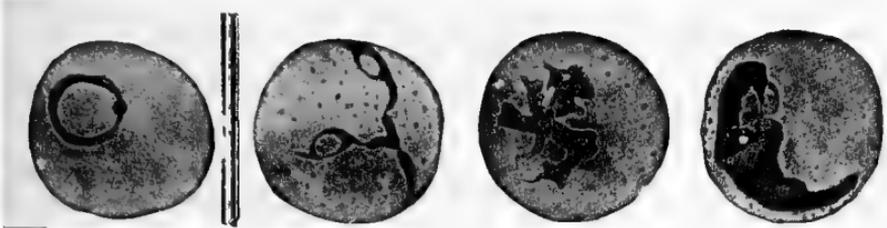


FIG. 206.—*Plasmodium vivax*. Stages of growth in the asexual cycle, commonly seen in the peripheral blood. Three of the cells show granules in the hemoglobin, the stippling of Schüffner.  $\times 2200$ . (After Doflein.)

lation, the stippling of Schueffner. The parasite often attains a diameter greater than that of the average blood cell before it segments. The segmentation gives rise to from 15 to 30 merozoites which enter new erythrocytes and begin the cycle anew.

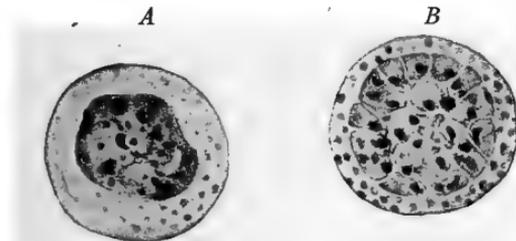


FIG. 207.—*Plasmodium vivax*. Multinucleated stage preceding division and the stage of multiple division (sporulation); found in the blood just before and during a chill.  $\times 2200$ . (After Doflein.)

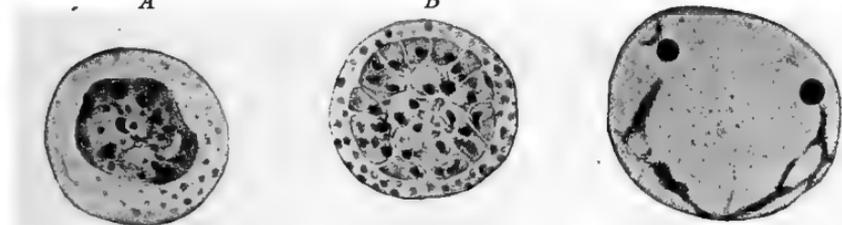


FIG. 208.—*Plasmodium vivax*. Double infection of a red blood cell which is considerably enlarged as a result; Schüffner's stippling slight.  $\times 2200$ . (After Doflein.)

This complete cycle of schizogony takes place in the peripheral circulation and requires almost exactly 48 hours.

The young parasites destined to become gametocytes exhibit relatively less ameboid movement. Their pigment exists as large granules, some of them even rod-shaped. The macrogametocyte attains a diameter of 15 to 25 $\mu$  and usually destroys its

erythrocyte and escapes from it entirely. The cytoplasm stains deeply with methylene blue. The microgametocyte is smaller with paler cytoplasm. The development of the parasite in the mosquito (*Anopheles*) is wholly analogous to that of *Pl. falciparum*, although there are some slight morphological differences observed. Development ceases at temperatures below 16° C.

**Plasmodium Malariae.**—The young quartan parasite is not characteristic, but in its growth it soon stretches as a band across the erythrocyte. Later it almost fills the cell and then segments, producing 6 to 14, most often 8, merozoites. The infected erythro-

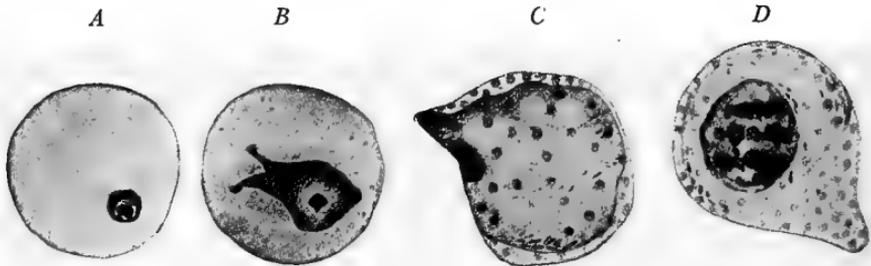


FIG. 209.—*Plasmodium vivax*. Stages in growth of the sexual cells (gametocytes). A and B, Young sexual cells distinguished from the agametes by the absence of vacuoles and the more regular outline. C, Full-grown macrogametocyte. D, Full-grown microgametocyte.  $\times 2200$ . (After Doflein.)

cyte is not enlarged or distorted nor does it become pale or show granulation. The gametocytes, when stained, are not very different in appearance from the asexual cells. In the living preparation they show much more active protoplasmic movement. The sexual cycle takes place in *Anopheles* and agrees very well with that of the other two malarial parasites, as far as it has been studied.

Malaria is probably the most important as well as the most well-known human disease due to protozoa. It is characterized by recurrent paroxysms of fever with afebrile intervals, progressive anemia and weakness, with the accumulation of a dark brown or black pigment in the spleen and liver. This pigment is produced by the parasites and set free into the blood when they

segment. The estivo-autumnal malaria caused by *Pl. falciparum* shows a somewhat irregular and not very characteristic fever curve, but usually there is fever every day (quotidian fever). The tertian fever due to infection with *Pl. vivax* is char-



FIG. 210.—*Plasmodium malariae*. Stages of the asexual cycle in the circulating blood. Note the absence of granulation from the hemoglobin and the uniform size of the red blood cells.  $\times 2200$ . (After Doflein.)

acterized by febrile attacks recurring at intervals of 48 hours and bearing a very definite relation to the asexual cycle of the parasite. The segmentation of the plasmodium is coincident with

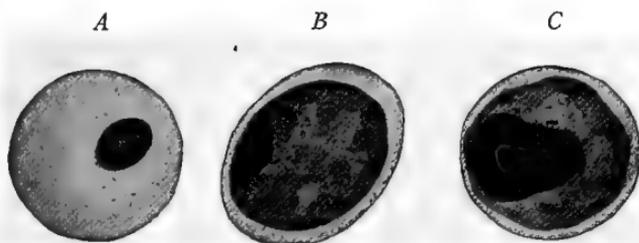


FIG. 211.—*Plasmodium malariae*. Sexual cells in the circulating blood. A, Young gametocyte. B, Full-grown macrogametocyte. C, Full-grown microgametocyte.  $\times 2200$ . (After Doflein.)

the chill and the rise in the patient's temperature. In quartan malaria due to infection with *Pl. malariae*, the fever recurs at intervals of 72 hours, again at the stage of segmentation in the asexual cycle of the parasite. Obviously an association of two

is unknown, but it may be due to the mechanical streaming of the blood acting upon the bladder-like cell which has been deprived of elasticity by the destructive action of the parasite. The further stages in the cycle of sporogony are unknown. An asexual multiplication probably occurs in some internal organs of the bird. Fantham has observed schizogony in the spleen of *Lagopus scoticus*, the red-game grouse of Scotland, infected with a similar parasite *Leukocytozoön iovati*.

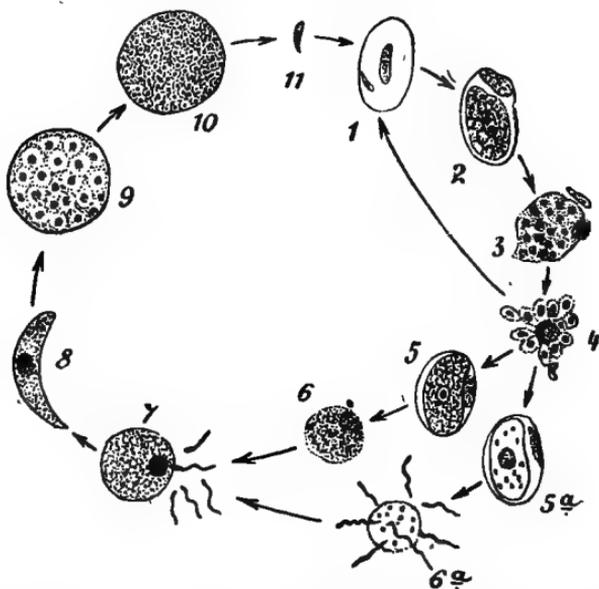


FIG. 195.—Diagram of the developmental cycle of *Proteosoma*. 1. Sporozoite entering an erythrocyte; 1, 2, 3 and 4, the cycle of schizogony; 5, macrogametocyte; 5a, microgametocyte; 6, macrogamete; 6a, formation of microgametes; 7-fertilization; 8, oökinete; 9, formation of sporoblasts (in mosquito); 10, formation of sporozoites; 11, sporozoite. (From Doflein after Schaudinn.)

**Proteosoma (Plasmodium) Præcox.**—Grassi and Feletti described this malarial parasite of birds and designated it as *Hæmamaeba præcox*.<sup>1</sup> The parasite is very common in the blood of small birds, such as sparrows, robins and larks, in all parts of the world. The cycle of schizogony is completed in the peripheral circulation. The small merozoite or agamete enters an erythro-

<sup>1</sup> *Centr. f. Bakt.*, 1891, Bd. IX, S. 407.

cyte and enlarges, retaining its oval or circular form. The nucleus of the host cell is pushed out of position but its form is not materially altered. The full-grown parasite segments, producing 10 to 30 merozoites and leaving behind a small residual body containing the accumulated pigment, thus completing the asexual



FIG. 196.—*Proteosoma praecox* in the blood of a field lark (*Glauda arvensis*). A, Young parasite in a blood cell. B, Half-grown parasite which has pushed aside the nucleus of the erythrocyte. C, Parasite with clump of pigment and many nuclei. The nucleus of the erythrocyte has been lost (uncommon). D, Division into eighteen merozoites. (From Doflein after Wasielewski.)

cycle, which may be repeated many times. After a time some of the growing parasites become differentiated to form macrogametocytes and microgametocytes, which are kidney-shaped and do not divide nor undergo further development in the vertebrate host. When the blood is drawn and diluted with citrated salt so-



FIG. 197.—Midgut of a culex mosquito, covered with oöcyts of *Proteosoma praecox*. V, Vasa malpighii. (From Doflein after Ross.)

lution, or taken in by a mosquito, four to eight microgametes are formed just as has been described for *H. columbæ*. They are very slender actively motile spindles without flagella. Fertilization of the macrogamete and the production of an oökinete takes place in the usual manner. The latter penetrates the intestinal epithelium of the mosquito (*Culex* sp.) and enlarges to produce

a spherical cyst filled with an enormous number of thread-like sporozoites. These escape into the body cavity of the mosquito as the cyst bursts, and are generally distributed throughout the body of the insect. They assemble, probably as a result of some chemical stimulus, in the salivary glands of the mosquito, whence they are injected into the wound as the insect bites, and at once invade erythrocytes to begin the cycle of schizogony.

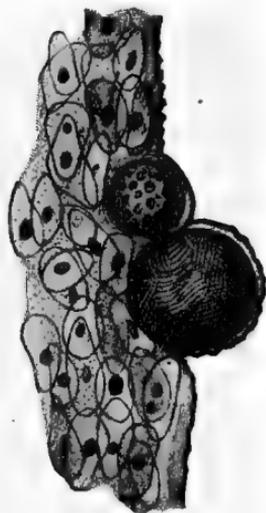


FIG. 198.—Oöcyst of *Proteosoma præcox*, developed in the intestine of *Aedes (Stegomyia) calopus*, showing numerous sporozoites. (From Do-  
stein after Neumann.)

The discovery of the sexual cycle of proteosoma in the mosquito and the conclusive proof that this form of bird malaria is transmitted by a mosquito stands to the everlasting credit of Ronald Ross. His brilliant discovery made in India in 1898, pointed the way to the solution of the whole problem of the transmission of the malarial diseases and their practical restriction.

Proteosoma is a favorable parasite for class study, as it is readily transmitted from bird to bird (sparrows or canaries) by injection of infected blood, and the parasites often become very numerous in the blood. There seems to be no good reason for placing this organism in a separate genus from the human malarial parasites.

#### **Plasmodium Falciparum (Præcox).—**

Laveran in 1880 discovered the first malarial parasite in the blood of man and correctly interpreted his observations. The distinctions between the three species was recognized by Golgi, and the life history of the parasites and especially their relation to mosquitoes and insects in general has been most thoroughly studied by Grassi.<sup>1</sup> *Pl. falciparum* is the parasite of estivo-autumnal or pernicious malaria of man. The young organism is 1 to 1.5 $\mu$  in diameter. It penetrates a red blood cell and enlarges. A vacuole appears in the

<sup>1</sup> Grassi: *Die Malaria*, IIte Auflage, Jena, 1901.

center, giving the parasite the appearance of a signet ring, the setting being represented by the nucleus or chromatin granule

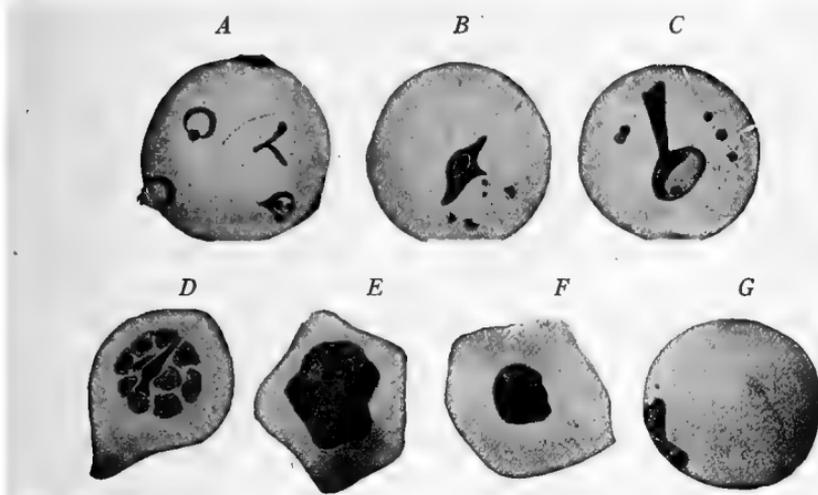


FIG. 199.—*Plasmodium falciparum*, forms in the asexual cycle (schizogony). A, Multiple infection of an erythrocyte, showing signet rings and parasites attached to the external surface. B and C, Growing parasites with Mauer's granules in the erythrocytes. D, Growing parasite without granulation of the hemoglobin. E, Half-grown parasite showing pigment. F, and G, Multiple division (sporulation), rarely seen in the peripheral blood. (After Doflein.)

which stains violet red with the Romanowsky stains. The parasite attains a diameter of about  $6\mu$ , when it segments to produce 7 to 16 merozoites or agametes which enter new erythrocytes and repeat the cycle. The larger stages of this cycle of schizogony are rarely seen in the peripheral circulation, and the segmentation of the parasite occurs in the capillaries of the internal organs. The cycle probably requires 48 hours for its completion. The erythrocyte is not enlarged by the growth of the parasite within it but tends rather to become smaller. Maurer has observed an irregular



FIG. 200.—Section through a capillary in the brain, showing numerous dividing forms of the non-pigmented type of *Pl. falciparum*. (Stained preparation.) (From Doflein after Mannaberg.)

granulation of the erythrocytes. Why the cells containing the larger forms should remain in the internal capillaries of the body is not definitely known.

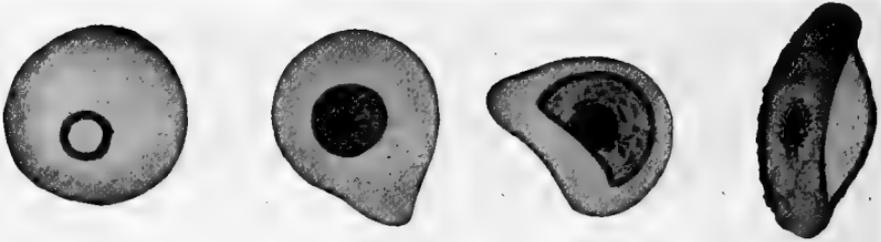


FIG. 201.—*Plasmodium falciparum*. Stages in the development of the gametocytes (crescents).  $\times 2200$ . (After Doflein.)

The gametocytes develop by the growth of ordinary merozoites, which become crescentic early in their development and differen-

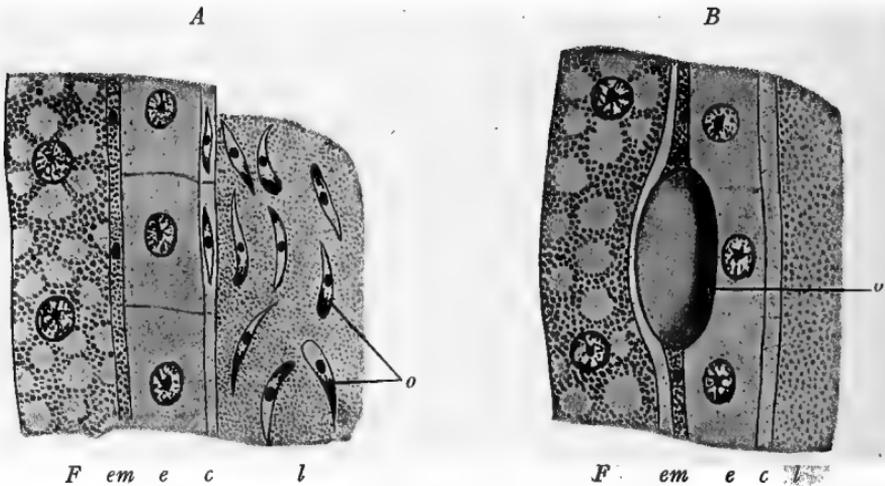


FIG. 202.—Sections through the stomach wall of *Anopheles* showing stages in the development of *Pl. falciparum*. *A*, Fixed a few hours after the infective feeding, showing oökinetes within the lumen and two in the cuticula of the epithelium. *B*, Fixed a few days after the infective feeding, showing the partly grown oöcyst in the stomach wall. *F*, Fat surrounding the stomach; *em*, tunica elastico-muscularis; *e*, epithelium; *c*, cuticula; *l*, lumen of stomach. (From Doflein after Grassi).

tiated into deeply staining macrogametocytes and pale-staining microgametocytes. These are produced especially in the bone marrow and they circulate in the peripheral blood. Further

development takes place when the blood is taken into the stomach of a mosquito of the genus *Anopheles*. Here the microgametes, slender actively motile threads, are given off by the microgametocyte and fertilize the macrogametes, producing oökinetes, which actively penetrate the epithelium. In the wall of the mosquito's stomach each oökinete gives rise to a rapidly growing cyst and within this an enormous number of very slender sporozoites are developed. The ripe cyst bursts into the body cavity and the sporozoites become generally distributed throughout the body of the insect and later assemble in the secreting cells of



FIG. 203.—Digestive tract of *Anopheles*, the stomach of which is covered with numerous oöcysts of *Pl. falciparum*, viewed from the left side. *c*, Cloaca; *s*, stomach; *o*, oöcysts of *Plasmodium*; *mt*, malpighian tubules; *sb*, sucking bladders; *sg*, salivary gland. (From Doflein, modified after Ross and Grassi.)



FIG. 204.—*Plasmodium falciparum*. Ripe sporozoites arranged about residual bodies within the oöcyst, cut in various directions (7 to 8 days after infection of the mosquito). (From Doflein after Grassi.)

or more crops of parasites reaching maturity at different times may give rise to a variety of fever curves.

The diagnosis of malaria is most conclusively established by recognizing the parasites in the blood of the patient. One should examine a fresh drop of blood, unstained, under the microscope, and also thin films of blood stained with some one of the Romanowsky stains. The parasites may be very scarce in old cases and especially in those patients who have been treated.

The mosquitoes which transmit human malaria were first recognized by Ross and have been most thoroughly studied by Grassi. The mosquito is capable of causing malaria only after it has fed upon a person harboring the parasite in his blood.<sup>1</sup> The members of the genus *Culex*, the most common mosquitoes, do not permit the development of the plasmodia within them, but this occurs, so far as is known, only in certain species of the genus *Anopheles*. *A. maculipennis* in Europe and *A. quadrimaculatus* in America appear to be the most important species. They are easily recognized by the four small black spots on each wing due to a relative accumulation of pigmented scales in these situations. The members of the genus *Anopheles* are readily distinguishable from *Culex* by the form and arrangement of their eggs, the form and position of the larvæ and by the general form and structure of the adult insect, as well as its posture when at rest.

The restriction and prevention of malaria is founded upon the knowledge of its nature and its mode of spread. The measures include (1) the destruction of malarial parasites in man by thorough treatment of the disease with quinine, (2) destruction of mosquitoes and mosquito larvæ and the drainage, oiling or screening of their breeding places, and (3) exclusion of mosquitoes from contact with infected persons and also from contact with healthy persons, by the use of screens. The thorough application of these measures has demonstrated the possibility of effectively controlling this disease even in the tropics.

<sup>1</sup> Fermi and Lumbau: *Centrbl. f. Bakt.*, 1912, Bd. LXV, pp. 105-112.

Culex

Anopheles

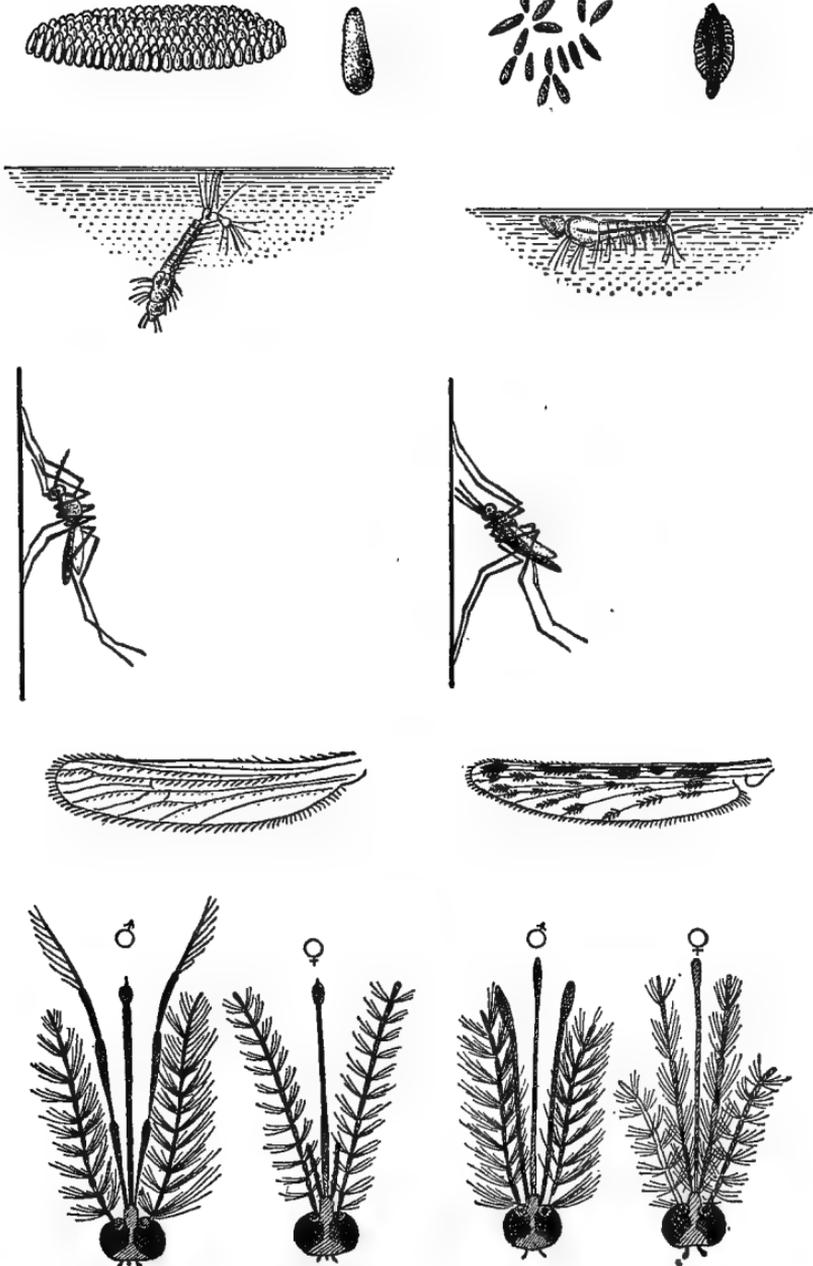


FIG. 212.—Comparison of Culex and Anopheles. Eggs, larvæ (note position), position of insects at rest, wings, heads showing antennæ and palpi. (From Jordan after Kollé and Hetsch.)

**Plasmodium Kochi.**—This is a malarial parasite which causes a mild fever in monkeys. It is not transmissible to man. Other species of malarial parasites have been recognized in these animals.

**Babesia<sup>1</sup> Bigemina.**—Smith and Kilborne discovered this organism in the red blood-corpuscles of cattle suffering from Texas fever. The parasite is pear-shaped, 2 to 4 $\mu$  long and 1.5 to 2 $\mu$  wide and usually occurs in pairs within the erythrocytes. The cytoplasm is quite clear without granules or pigment and contains one or two chromatin bodies. Minute ameboid forms are also found. Multiplication apparently takes place by longitudinal

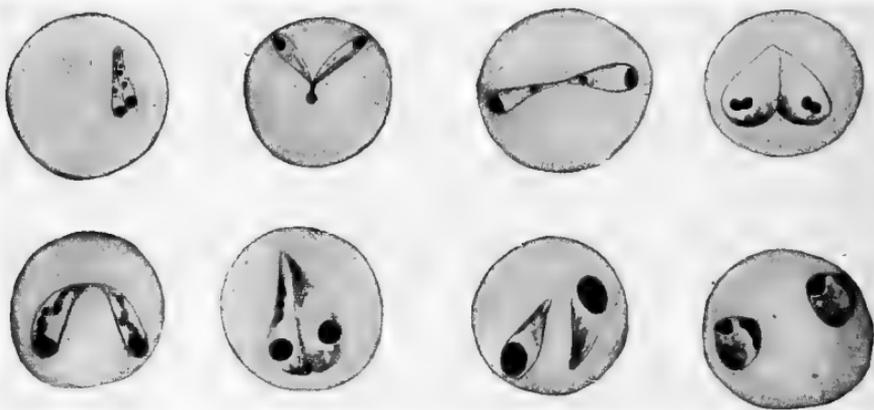


FIG. 213.—*Babesia bigemina*. Characteristic forms in the peripheral blood of cattle.  $\times 2000$ . (After Doflein.)

division of the pear-shaped forms as well as by multiple division of the ameboid forms. Macrogametocytes and microgametocytes have been recognized. The transmission of the parasite from animal to animal is effected by the cattle tick, *Boöphilus bovis*, (*Rhipicephalus annulatus*) as was conclusively demonstrated by Smith and Kilborne, the first instance in which such a relation was proved for any blood-sucking invertebrate. The details of the life cycle in the tick are unknown. It is certain however that the infection is conveyed to the next generation of ticks

<sup>1</sup> The generic name *Pyrosoma* bestowed by Smith and Kilborne in 1893 is incorrect, because this is the name of a genus of marine animals belonging to the Tunicata. *Babesia* proposed by Starcovici in 1893 has the next claim to priority.

through the eggs and that these young ticks are capable of infecting cattle. Renewed investigation of the parasite is much to be desired.

Texas fever is a very important disease of cattle in the southern United States and a similar disease occurs in Europe, Africa and South America. Young cattle usually survive the disease and become immune. Older cattle imported into the endemic area contract Texas fever and usually die of it. Immunity may be conferred by injecting blood which contains a small number of parasites, taken from an animal which has passed the acute stage of the disease. Restriction of the Texas-fever area in the United States is slowly progressing as a result of systematic eradication of the tick.

**Babesia Canis.**—This organism occurs in the blood of dogs suffering from the so-called malignant jaundice, and has been carefully studied by modern methods by Nuttall and Graham-Smith and later by Breinl<sup>1</sup> and Hindle. In morphology and life history it agrees with *B. bigemina* as far as these have been worked out, but *B. canis* is incapable of infecting cattle. The infection is transmitted to dogs by several different species of ticks.

**Gregarina Blattarum.**—This organism lives as a parasite in the intestine of the common cockroach *Periplaneta orientalis*, and is therefore liable to be found in human food, and at times in specimens from human cases submitted to microscopic study, probably because of accidental presence of cockroaches in the containers employed. The vegetative cells are elongated, often attached together. The spore cyst results from the union of two cells and the subsequent repeated division of the fertilized cell to produce an enormous number of spores. These spores are discharged from the cyst when it enters a fluid medium: When fully developed, each spore contains eight sporozoites.

**Nosema Bombycis**—This organism was discovered by Naegeli in 1857. It is an example of the Neosporidia and is of peculiar interest as the cause of pèbrine, the disease of silkworms studied

<sup>1</sup> *Ann. Trop. Med. and Parasitol.*, Vol. II, pp. 233-248.

by Pasteur in 1866-1870, and largely eradicated by application of the methods devised by him as a result of his investigations. The spore of *N. bombycis* is  $1.5$  to  $2\mu$  wide by  $3\mu$  long. If treated with nitric acid it swells and reaches a length of  $6\mu$  and extends a slender thread which may be  $10\mu$  long. The spore is ingested

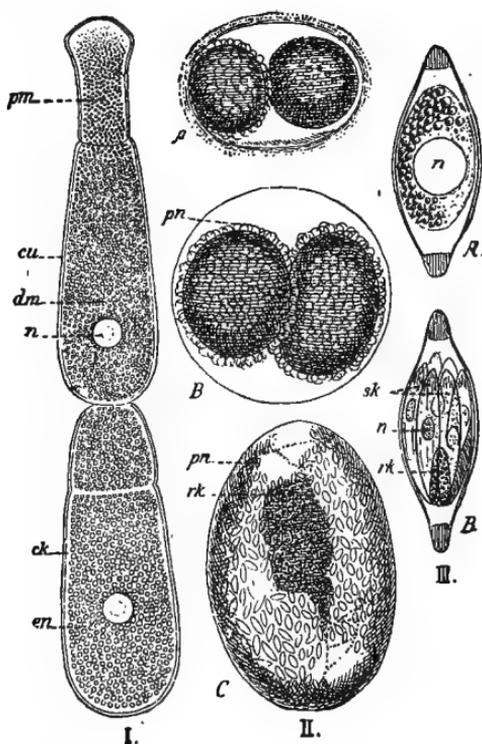


FIG. 214.—*Gregarina blattarum*. I, Two individuals stuck together. II, Cysts with conjugated cells and developing spores. IIIA, Unripe spore with undivided contents. IIIB, Ripe spore with eight sporozoites; *ck*, ectoplasm; *en*, endoplasm; *cu*, cuticula; *pm*, protomerit; *dm*, deuteromerit; *n*, nucleus; *pn*, spores; *rk*, residual body; *sk*, sporozoites. (From Doflein after R. Hertwig.)

by the silkworm and in its intestine the ameboid parasite escapes and penetrates the epithelium. It may pass to any part of the host to undergo its further development. Multiplication of the small rounded agamete results in the formation of long chains of oval bodies inside a cell of the host. From these the

spores are again produced. Pébrine is a disease of the greatest importance to the silkworm industry. It is effectively restricted

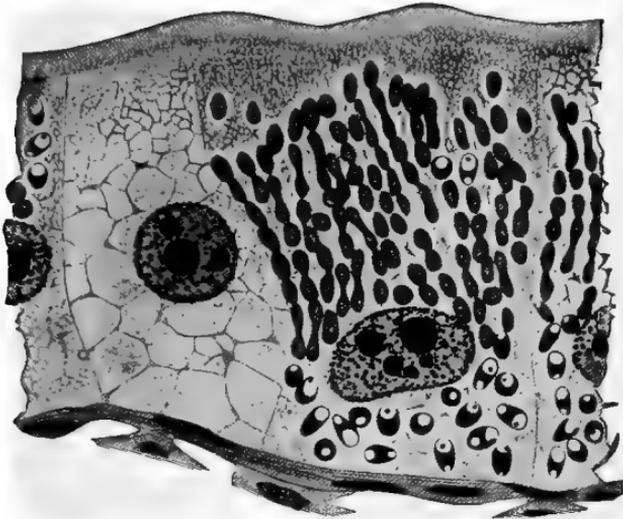


FIG. 215.—*Nosema bombycis*. Section of intestinal epithelium of silkworm showing spores of *Nosema* and also the peculiar multiplication resembling the growth of a mold. (From *Doflein* after *Stempel*.) (See also Fig. 83, p. 165.)

by a careful microscopic examination of all the silkworm eggs and the exclusion and destruction of all those in which the parasite exists (Pasteur's method).

## CHAPTER XXX

### CILIOPHORA

**Paramæcium Caudatum.**—This is the most common infusorian met with in stagnant water. Its length varies from 120 to 325 $\mu$ . The cell is spindle-shaped with a deep oral groove which takes a spiral course on one side of the body. The surface is thickly set with active cilia. Food particles are swept into the oral groove, enter the cytoplasm at its bottom and circulate in the cell within food vacuoles. Near the center of the cell is a large macronucleus and near it a smaller micronucleus. Multiplication takes place by simple longitudinal or oblique division.

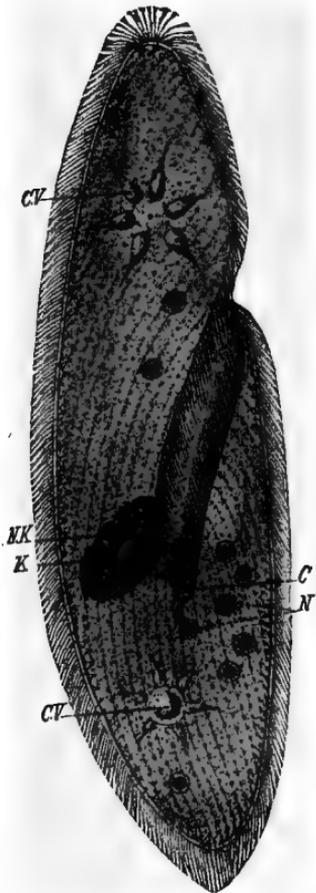


FIG. 216.—*Paramecium caudatum*. K, Macronucleus; NK, micronucleus; C, gullet; N, food vacuoles; CV, contractile vacuoles. (After Doflein.)

Conjugation is isogamic. The similar conjugating cells adhere to each other, the micronuclei divide twice and three of the four nuclei thus produced disintegrate, as does also the macronucleus. The remaining micronucleus divides into two and one of these passes into the other conjugating cell in exchange for a similar element. The newly acquired element unites with the element which remained behind to form the new nucleus. The new nucleus divides three times in suc-

cession to form eight nuclei, of which four enlarge to become macronuclei, one remains as a micronucleus and three disintegrate and disappear. The one micronucleus then divides by mitosis and the cell divides to form two paramæcia, each containing one micronucleus and two macronuclei. The next division gives rise to cells containing the normal number of nuclei, one micronucleus and one macronucleus.

The paramæcia are large saprophytic organisms, easily kept under cultivation in the laboratory, and they have been very extensively studied. Many conceptions founded upon these studies are considered to have a broad bearing upon the physiology of all living cells. For example Jennings<sup>1</sup> has found that conjugation serves two purposes, (1) to provide chemical stimulation of cell division and (2) to insure variety in the descendants. The variety in the descendants is a result of the exchange of nuclear material. Calkins<sup>2</sup> has discovered a specialization of function in paramæcium in respect to conjugation and concludes that in some of the descendants of an ex-conjugant the ability to conjugate is in abeyance, thus suggesting a resemblance to the somatic cells of a metazoön, while other descendants retain this function and are therefore analogous to the germ cells of a metazoön.

Three other species of paramæcium are recognized, namely, *P. aurelia*, *P. bursaria* and *P. putrinum*.

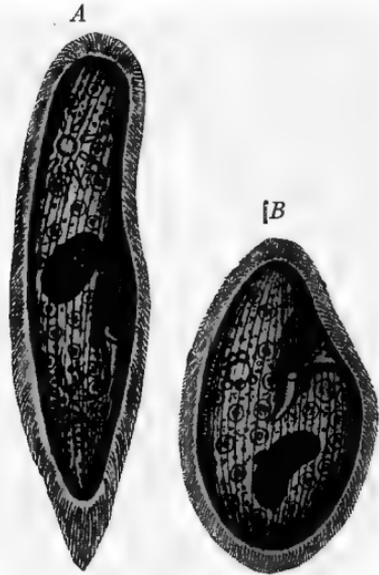


FIG. 217.—Paramæcia drawn at the same magnification. A, *Paramæcium caudatum*. B, *Paramæcium putrinum*. (From Doflein after Schewiakoff).

<sup>1</sup> Harvey Lectures, 1911-12, pp. 256-276.

<sup>2</sup> Proc. Soc. Exp. Biol. and Med., 1913, Vol. X, pp. 65-67.

**Opalina Ranarum.**—This is a common parasite in the intestine (cloaca) of the frog. It reaches a large size, 600 to 800 $\mu$  in diameter, is flattened and somewhat irregular in outline. The ectoplasm is striated and there are very many nuclei in the interior of the cell. In the springtime, as the frogs enter water to spawn, the parasites divide rapidly and give rise to cysts 20 to 40 $\mu$  in diameter. These escape into the slime and are ingested by the growing tadpoles. In the cloaca the cells escape from the cysts. They are differentiated into male and female gametes and

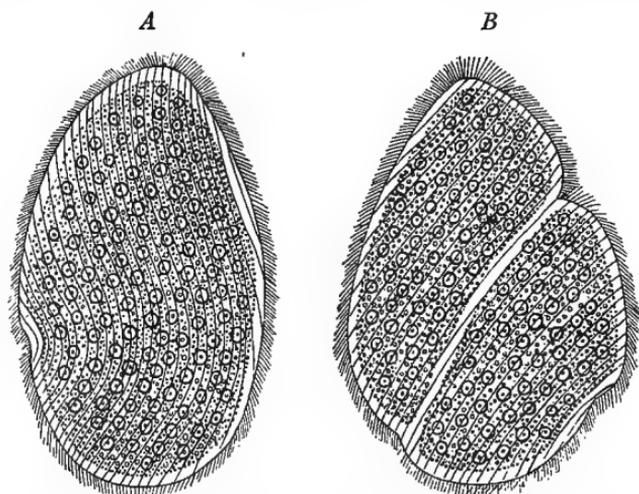


FIG. 218.—*Opalina ranarum*, showing the numerous vesicular nuclei. A, Ordinary form. B, Dividing form. (From Doflein after Zeller.)

fuse to form one cell which grows and multiplies in the developing frog.

**Balantidium Coli.**—This parasite of the human intestine was described by Malmsten in 1857. Its normal habitat seems to be in the large intestine of swine, where it is commonly found in large numbers. The cell is a short oval, 50 to 70 $\mu$  wide and 70 to 100 $\mu$  long, rarely larger. Its surface is covered with active cilia, and there is a short oral groove at the anterior end. The cytoplasm contains drops of fat and food vacuoles, often red blood cells and leukocytes of the host. The principal nucleus

is kidney-shaped and the accessory nucleus lies in contact with it. Multiplication takes place by simple transverse fission. Conjugation and cyst formation have been observed.

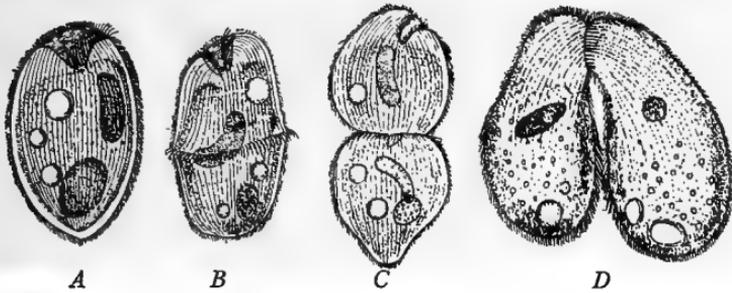


FIG. 219.—*Balantidium coli*. A, Fully developed individual, showing the nucleus above at the right and a food particle below. B and C, Division stages. D, Conjugation. (From Doflein after Leuckart.)

*Bal. coli* is sometimes found in man in cases of intestinal disorder with diarrhea. Its possible causal relation to the patho-

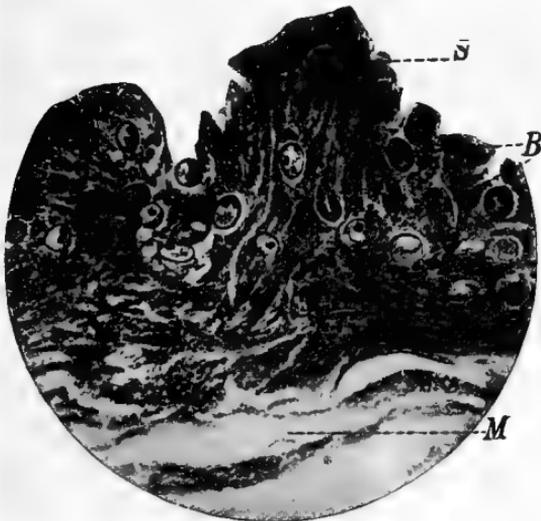


FIG. 220.—Section through the intestinal wall in a case of enteritis due to *Balantidium*. S, Serosa; M, Muscularis; B, Balantidia. (From Doflein after Solowjew.)

logical condition is not conclusively ascertained. In some instances the cells of *Balanlidium* have been found deeply situ-

ated in inflamed intestinal wall. Brooks<sup>1</sup> observed *Bal. coli* in several cases of dysentery in Orangoutangs in the New York Zoological Park and Brumpt<sup>2</sup> has been able to transfer balantidium infection from monkey to swine and back to monkey. Still there is perhaps some question as to the identity of the parasites found in man and in hogs.

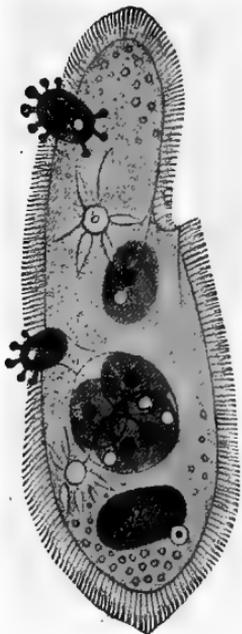


FIG. 221.—*Sphaerophrya pusilla* within a paramaecium. At one place there are four parasites and a fifth is escaping. Higher up, one of the parasites is just penetrating the host, and a single parasite is seen near the center of the paramaecium. (From Doflein after Bütschli.)

**Balantidium Minutum.**—Schaudinn in 1899 observed this organism in the human feces. It is smaller than *Bal. coli*, the greatest measurements being  $20 \times 30\mu$ , and the oral groove extends more than half way back along the side of the cell. It probably occurs rarely in the human small intestine. Other species of balantidium have been described.

**Sphaerophrya Pusilla.**—This organism is of peculiar interest because it lives as a parasite within another protozoön, the paramaecium. The cell of *Sph. pusilla* is spherical, 20 to  $40\mu$  in diameter, and provided with sucking tentacles and cilia when outside the body of the host.

<sup>1</sup> Proc. N. Y. Path. Soc., 1903, Vol. III, pp. 28-39.

<sup>2</sup> Compt. Rend. Soc. Biol., 1909, Vol. LXVII, pp. 103-105.

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