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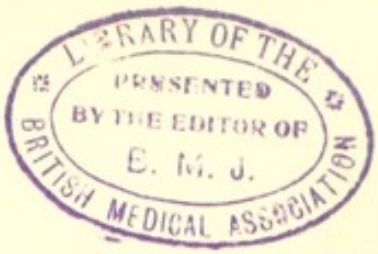
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A TEXT-BOOK

UPON THE

PATHOGENIC BACTERIA AND PROTOZOA

FOR STUDENTS OF MEDICINE AND PHYSICIANS

BY

JOSEPH McFARLAND, M. D., Sc. D.

Professor of Pathology and Bacteriology in the Medico-Chirurgical College, Philadelphia; Pathologist to the Philadelphia General Hospital and to the Medico-Chirurgical Hospital, Philadelphia; Fellow of the College of Physicians of Philadelphia

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PREFACE TO THE EIGHTH EDITION

It is a difficult thing to write a preface for an *Eighth Edition*. No part of the work was found to be so embarrassing or was subjected to greater procrastination.

What can be said that has not been said seven times already? Probably very little about the book; certainly very much about the feelings of the author.

He desires to express to those who have already made acquaintance with the book, and may with friendly feelings look into its new edition, his sincere satisfaction and appreciation of the hearty receptions that have been accorded his previous attempts. He also desires to thank his reviewers for some helpful criticisms.

So numerous are the additions, subtractions and alterations to which the seventh edition was submitted in the preparation of this *Eighth Edition*, that it might almost be said that the text had been rewritten. Indeed they were such that the type of the entire book has been reset. It now appear with slightly larger pages; in two sizes of type, and gives a general effect of contraction, though there is really an expansion of matter that would have covered more than fifty of the old-size pages.

The "Pathogenic Bacteria and Protozoa" is a medical work. It is hoped that it shall be found helpful to medical workers—students and practitioners of every class.

PHILADELPHIA, PA. THE AUTHOR.
November, 1915.

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PART I. GENERAL

HISTORICAL INTRODUCTION

Biology, chemistry, medicine, and surgery, in their evolution, contributed to a new branch of knowledge, Bacteriology, whose subsequent development has become of inestimable importance to each. Indeed, bacteriology illustrates the old adage, "The child is father of the man," for while it is in part the offspring of the medicine of the past, it has established itself as the dictator of the medicine of the present and future, especially so far as concerns the infectious diseases.

THE EVOLUTION OF BACTERIOLOGY

I. BIOLOGIC CONTRIBUTIONS; THE DOCTRINE OF SPONTANEOUS GENERATION

Among the early Greeks we find that Anaximander (43d Olympiad, 610 B. C.) of Miletus held the theory that animals were formed from moisture. Empedocles of Agrigentum (450 B. C.) attributed to spontaneous generation all the living beings which he found peopling the earth. Aristotle (384 B. C.) is not so general in his view of the subject, but asserts that "*sometimes* animals are formed in putrefying soil, sometimes in plants, and sometimes in the fluids of other animals."

Three centuries later, in his disquisition upon the Pythagorean philosophy, we find Ovid defending the same doctrine of spontaneous generation, while in the Georgics, Virgil gives directions for the artificial production of bees.

The doctrine of spontaneous generation of life was not only current among the ancients, but we find it persisting through the Middle Ages, and descending to our own generation. In 1542, in his treatise called "De Subtilitate," we find Cardan asserting that water engenders fishes, and that many animals spring from fermentation. Van Helmont gives special instructions for the artificial production of mice, and Kircher in his "Mundus Subterraneus" (chapter "De Panspermia Rerum") describes and *actually figures* certain animals which were produced under his own eyes by the transforming influence of water on fragments of stems from different plants.*

About 1671, Francesco Redi seems to have been the first to doubt that the maggots familiar in putrid meat arose *de novo:*

* See Tyndall: "Floating Matter in the Air."

"Watching meat in its passage from freshness to decay, prior to the appearance of maggots, he invariably observed flies buzzing around the meat and frequently alighting on it. The maggots, he thought, might be the half-developed progeny of these flies. Placing fresh meat in a jar covered with paper, he found that although the meat putrefied in the ordinary way, it never bred maggots, while meat in open jars soon swarmed with them. For the paper he substituted fine wire gauze, through which the odor of the meat could rise. Over it the flies buzzed, and on it they laid their eggs, but the meshes being too small to permit the eggs to fall through, no maggots generated in the meat; they were, on the contrary, hatched on the gauze. By a series of such experiments Redi destroyed the belief in the spontaneous generation of maggots in meat, and with it many related beliefs."

In 1683 Anthony van Leeuwenhoek, justly called the "Father of microscopy," demonstrated the continuity of arteries and veins through intervening capillaries, thus affording ocular proof of Harvey's discovery of the circulation of the blood; *discovered bacteria*, seeing them first in saliva, discovered the rotifers, and first saw the little globules in yeast which Latour and Schwann subsequently proved to be plants.

Leeuwenhoek involuntarily reopened the old controversy about spontaneous generation by bringing forward a new world, peopled by creatures of such extreme minuteness as to suggest not only a close relationship to the ultimate molecules of matter, but an easy transition from them.

In succeeding years the development of the compound microscope showed that putrescent infusions, both animal and vegetable, teemed with minute living organisms.

Abbé Lazzaro Spallanzani (1777) filled flasks with organic infusions, sealed their necks, and, after subjecting their contents to the temperature of boiling water, placed them under conditions favorable for the development of life, without, however, being able to produce it. Spallanzani's critics, however, objected to his experiment on the ground that air is essential to life, and that in his flasks the air was excluded by the hermetically sealed necks.

Schulze (1836) set this objection aside by filling a flask only half full of distilled water, to which animal and vegetable matters were added, boiling the contents to destroy the vitality of any organisms which might already exist in them, then sucking daily into the flask a certain amount of air which was passed through a series of bulbs containing concentrated sulphuric acid, in which it was supposed that whatever germs of life the air might contain would be destroyed. This flask was kept from May to August; air was passed through it daily, yet without the development of any infusorial life.

It must have been a remarkably germ-free atmosphere in which

Schulze worked, for, as was shown by those who repeated his experiment, under the conditions that he regarded as certainly excluding all life, germs can readily enter with the air.

In 1838 Ehrenberg devised a system of classifying the minute forms of life, a part of which, at least, is still recognized at the present time.

The term "infusorial life" having been used, it is well to remark that during all the early part of their recognized existence the bacteria were regarded as animal organisms and classed among the *infusoria*.

Tyndall, stimulated by the work of Pasteur, conclusively proved that the micro-organismal germs were in the dust suspended in the atmosphere, and not ubiquitous in distribution. His experiments were very ingenious and are of much interest. First preparing light wooden chambers, with a large glass window in the front and a smaller window in each side, he arranged a series of test-tubes in the bottom, half in and half out of the chamber, and a pipet, working through a rubber diaphragm, in the top, so that when desired the tubes, one by one, could be filled through it. Such chambers were allowed to stand until all the contained dust had settled, and then submitted to an optical test to determine the purity of the contained atmosphere by passing a powerful ray of light through the side windows. When viewed through the front, this ray was visible only so long as there were particles suspended in the atmosphere to reflect it. When the dust had completely settled and the light ray had become invisible because of the purity of the contained atmosphere, the tubes were cautiously filled with urine, beef-broth, and a variety of animal and vegetable broths, great care being taken that in the manipulation the pipet should not disturb the dust. Their contents were then boiled by submergence in a pan of hot brine placed beneath the chamber, in contact with the projecting ends of the tubes, and subsequently allowed to remain undisturbed for days, weeks, or months. In nearly every case life failed to develop in the infusions after the purity of the atmosphere was established.

II. CHEMIC CONTRIBUTIONS; FERMENTATION AND PUTREFACTION

As in the world of biology the generation of life was an all-absorbing problem, so in the world of chemistry the phenomena of fermentation and putrefaction were inexplicable so long as the nature of the ferments was not understood.

In the year 1837 Latour and Schwann succeeded in demonstrating that the minute oval bodies which had been observed in yeast since the time of Leeuwenhoek were living organisms—vegetable forms—capable of growth.

So long as yeast was looked upon as an inert substance it was impossible to understand how it could impart fermentation to other substances; but when it was shown by Latour that the essential

element of yeast was a growing plant, the phenomenon became a perfectly natural consequence of life. Not only the alcoholic, but also the acetic, lactic, and butyric fermentations have been shown to result from the energy of low forms of vegetable life, chiefly bacterial in nature. Prejudice, however, prevented many chemists from accepting this view of the subject, and Liebig strenuously adhered to his theory that fermentation was the result of the internal molecular movements which a body in the course of decomposition communicates to other matter whose elements are connected by a very feeble affinity.

Pasteur was the first to prove that fermentation is an ordinary chemic transformation of certain substances, taking place as the result of the action of living cells, and that the capacity to produce it resides in all animal and vegetable cells, though in varying degree.

In 1862 he published a paper "On the Organized Corpuscles Existing in the Atmosphere," in which he showed that many of the floating particles collected from the atmosphere of his laboratory were organized bodies. If these were planted in sterile infusions, abundant crops of micro-organisms were obtained. By the use of more refined methods he repeated the experiments of others, and showed clearly that "the cause which communicated life to his infusions came from the air, but was not evenly distributed through it."

Three years later he showed that the organized corpuscles which he had found in the air were the spores or seeds of minute plants, and that many of them possessed the property of withstanding the temperature of boiling water—a property which explained the peculiar results of many previous experimenters, who failed to prevent the development of life in boiled liquids inclosed in hermetically sealed flasks.

Chevreul and Pasteur, by having proved that animal solids do not putrefy or decompose if kept free from the access of germs, suggested to surgeons that putrefaction in wounds is due rather to the entrance of something from without than to changes within. The deadly nature of the discharges from putrescent wounds had been shown in a rough manner by Gaspard as early as 1822 by injecting some of the material into the veins of animals.

III. MEDICAL AND SURGICAL CONTRIBUTIONS; THE STUDY OF THE INFECTIOUS DISEASES

Probably the first writing in which a direct relationship between micro-organisms and disease is suggested is by Varro, who says: "It is also to be noticed, if there be any marshy places, that certain minute animals breed [there] which are invisible to the eye, and yet, getting into the system through mouth and nostrils, cause serious disorders (diseases which are difficult to treat)."

Surgical methods of treatment depending for their success upon

exclusion of the air, and of course, incidentally if unknowingly, exclusion of bacteria, seem to have been practised quite early. Theodoric, of Bologne, about 1260 taught that the action of the air upon wounds induced a pathologic condition predisposing to suppuration. He also treated wounds with hot wine fomentations. The wine was feebly antiseptic, kept the surface free from bacteria, and the treatment was, in consequence, a modification of what in later centuries formed antiseptic surgery.

Henri de Mondeville in 1306 went even further than Theodoric, whom he followed, and taught the necessity of bringing the edges of a wound together, covered it with an exclusive plaster compounded of turpentine, resin, and wax, and then applied the hot wine fomentation.

In 1546 Geronimo Fracastorius published at Venice a work "*De contagione et contagiosis morbis et curatione*," in which he divided infectious diseases into—

1. Those infecting by immediate contact (true contagions).
2. Those infecting through intermediate agents, such as fomites.
3. Those infecting at a distance or through the air. He mentions as belonging to this class phthisis, the pestilential fevers, and a certain kind of ophthalmia (conjunctivitis).

"In his account of the true nature of disease germs, or *seminaria contagionum*, . . . he describes them as particles too small to be apprehended by our senses, but as capable in appropriate media of reproduction, and in this way of infecting surrounding tissues.

"These pathogenic units Fracastorius supposed to be of the nature of colloidal systems, for if they were not viscous or glutinous by nature they could not be transmitted by fomites. Germs transmitting disease at a distance must be able to live in the air a certain length of time, and this condition he holds is possible only when the germs are gelatinous or colloidal systems, for only hard, inert, discrete particles could endure longer.

"Fracastorius conceived that the germs became pathogenic through the action of animal heat, and in order to produce disease it is not necessary that they should undergo dissolution, but only metabolic change."*

In 1671 Kircher wrote a book in which he expressed the opinion that puerperal fever, purpura, measles, and various other fevers were the result of a putrefaction caused by worms or animalcules. His opinions were thought by his contemporaries to be founded upon too little evidence, and were not received.

Plencig, of Vienna, became convinced that there was an undoubted connection between the microscopic animalcules exhibited by the microscope and the origin of disease, and advanced this opinion as early as 1762.

In 1704 John Colbach described "a new and secret method of

* "Brit. Med. Jour.," May 7, 1910, p. 1122.

treating wounds by which healing took place quickly, without inflammation or suppuration."

Boehm succeeded in 1838 in demonstrating the occurrence of yeast plants in the stools of cholera, and conjectured that the process of fermentation was concerned in the causation of that disease.

In 1840 Henle considered all the evidence that had been collected, and concluded that the cause of the infectious diseases was to be sought for in minute living organisms or fungi. He may be looked upon as the real propounder of the GERM THEORY OF DISEASE, for he not only collected facts and expressed opinions, but also investigated the subject ably. The requirements which he formulated in order that the theory might be proved were so severe that he was never able to attain to them with the crude methods at his disposal. They were so ably elaborated, however, that in after years they were again postulated by Koch, and it is only by strict conformity with them that the definite relationship between micro-organisms and disease has been determined.

Briefly summarized, these requirements are as follows:

1. A specific micro-organism must be constantly associated with the disease.

2. It must be isolated and studied apart from the disease.

3. When introduced into healthy animals it must produce the disease, and in the animal in which the disease has been experimentally produced the organism must be found under the original conditions.

In 1843 Dr. Oliver Wendell Holmes wrote a paper upon the "Contagiousness of Puerperal Fever."

In 1847 Semmelweiss, of Vienna, struck by the similarity between fatal wound infection with pyemia and puerperal fever, cast aside the popular theory that the latter affection was caused by the absorption into the blood of milk from the breasts, and announced his belief that the disease depended upon poisons carried by the fingers of physicians and students from the dissecting room to the woman in child-bed, and recommended washing the hands of the accoucheur with chlorin or chlorid of lime, in addition to the use of soap and water. He was laughed to scorn for his pains.

In 1849 J. K. Mitchell, in a brief work upon the "Cryptogamous Origin of Malarious and Epidemic Fevers," foreshadowed the germ theory of disease by collecting a large amount of evidence to show that malarial fevers were due to infection by fungi.

Pollender (1849) and Davaine (1850) succeeded in demonstrating the presence of the anthrax bacillus in the blood of animals suffering from and dead of that disease. Several years later (1863) Davaine, having made numerous inoculation experiments, demonstrated that this bacillus was the *materies morbi* of the disease. The bacillus of anthrax was probably the first bacterium shown to be specific for a

disease. Being a very large bacillus and a strongly vegetative organism, its growth was easily observed, while the disease was one readily communicated to animals.

Klebs, who was one of the pioneers of the germ theory, published, in 1872, a work upon septicemia and pyemia, in which he expressed himself convinced that the causes of these diseases must come from without the body. Billroth, however, strongly opposed such an idea, asserting that fungi had no especial importance either in the processes of disease or in those of decomposition, but that, existing everywhere in the air, they rapidly developed in the body as soon as through putrefaction a "Faulnisszymoid" (putrefactive ferment), or through inflammation a "Phlogistischezymoid" (inflammatory ferment), supplying the necessary feeding-grounds, was produced.

In 1873 Obermeier observed that actively motile, flexible spiral organisms were present in large numbers in the blood of patients in the febrile stages of relapsing fever.

In 1875 the number of scientific men who had entirely abandoned the doctrine of spontaneous generation and embraced the germ theory of disease was small, and most of those who accepted it were experimenters. A great majority of medical men either believed, like Billroth, that the presence of fungi where decomposition was in progress was an accidental result of their universal distribution, or, being still more conservative, adhered to the old notion that the bacteria, whose presence in putrescent wounds as well as in artificially prepared media was unquestionable, were spontaneously generated there.

Before many of the important bacteria had been discovered, and while ideas upon the relation of micro-organisms to disease were most crude, some practical measures were suggested that produced greater agitation and incited more observation and experimentation than anything suggested in surgery since the introduction of anesthetics—namely, *antisepsis*.

"It is to one of old Scotia's sons, Sir Joseph Lister, that the everlasting gratitude of the world is due for the knowledge we possess in regard to the relation existing between micro-organisms and inflammation and suppuration, and the power to render wounds aseptic through the action of germicidal substances."*

Lister, convinced that inflammation and suppuration were due to the entrance of germs from the air, instruments, fingers, etc., into wounds, suggested the employment of carbolic acid for the purpose of keeping sterile the hands of the operator, the skin of the patient, the surface of the wound, and the instruments used. He finally concluded every operation by a protective dressing to exclude the extrance of germs at a subsequent period.

Listerism, or "antisepsis," originated in 1875, and when Koch published his famous work on the "Wundinfectionskrankheiten"

* Agnew's "Surgery," vol. 1, chap. 11.

(Traumatic Infectious Diseases), in 1878, it spread slowly at first, but surely in the end, to all departments of surgery and obstetrics.

From time to time, as the need for them was realized, the genius of investigators provided new devices which materially aided in their work, and have made possible many discoveries that must otherwise have failed. Among them may be mentioned the improvement of the compound microscope, the use of sterilized culture fluids by Pasteur, the introduction of solid culture media and the isolation methods by Koch, the use of the cotton plug by Schröder and van Dusch, and the introduction of the anilin dyes by Weigert.

It is interesting to note that after the discovery of the anthrax bacillus by Pollender and Davaine, in 1849, there was a period of nearly twenty-five years during which no important pathogenic organisms were discovered, but during which technical methods were being elaborated, making possible a rapid succession of subsequent important discoveries.

Thus, in 1873, Obermeier discovered Spirillum obermeieri of relapsing fever.

In 1879 Hansen announced the discovery of bacilli in the cells of leprous nodules, and Neisser discovered the gonococcus

In 1880 the bacillus of typhoid fever was observed by Eberth and independently by Koch, Pasteur published his work upon "Chicken-cholera," and Sternberg described the pneumococcus, calling it *Micrococcus pasteuri*.

In 1882 Koch made himself immortal by his discovery of and work upon the tubercle bacillus, and in the same year Pasteur published a work upon "*Rouget du porc*," and Löffler and Shütz discovered the bacillus of glanders.

In 1884 Koch reported the discovery of the "comma bacillus," the cause of cholera, and in the same year Löffler isolated the diphtheria bacillus, and Nicolaier the tetanus bacillus.

In 1892 Canon and Pfeiffer discovered the bacillus of influenza.

In 1894 Yersin and Kitasato independently isolated the bacillus causing the bubonic plague, then prevalent at Hong-Kong.

A new era in bacteriology, and probably the most triumphant achievement of scientific medicine, was inaugurated in 1890, when Behring discovered the principles of the "blood-serum therapy." Since that time investigations have been largely along the lines of immunity, immunization, and the therapeutic serums, the names of Behring, Kitasato, Wernicke, Roux, Ehrlich, Metschnikoff, Bordet, Wassermann, Shiga, Madsen, and Arrhenius taking front rank.

The discovery of the Treponema pallidum, the specific organism of syphilis, was made in 1905 by Schaudinn and Hoffmann, long after clinical study of the disease had anticipated it to such an extent that when the discovery was finally made it was unnecessary to modify our ideas of the disease in any essential.

In the same year, 1905, Castellani discovered the Treponema pertenue, the cause of frambesia or yaws.

In 1911 Noguchi succeeded in obtaining pure cultures of the treponema.

In 1913 Flexner and Noguchi appear to have been successful in cultivating the virus of acute anterior poliomyelitis, *in vitro*.

During the time that so much investigation of the problems of infection was in progress the discoveries were by no means restricted to the bacteria and their products, as the reader might infer from the perusal of a chapter whose purpose is to explain the development of the department of science now known as Bacteriology. Other organisms of different—*i.e.*, animal—nature were also found in large numbers.

In 1875 Lösch discovered the Amœba coli; in 1878 Rivolta described the Coccidium cuniculi of the rabbit; in 1879 Lewis first saw Trypanosoma lewisi in the blood of the rat; in 1881 Laveran discovered Plasmodium malariæ in the blood of cases of human paludism; in 1885 Blanchard described the sarcocystis in muscle-fibers; in 1893 Councilman and Lafleur studied Amœba dysenteriæ in the stools and tissues of human dysentery; in 1903 Leishman and Donovan found the little body, Leishmania donovani, in the splenic juice of cases of kala-azar, and in 1903 Dutton and Forde, working independently, observed trypanosomes—the Trypanosoma gambiense of African lethargy—in the blood of human beings.

That the specific micro-organisms of many of the infectious diseases remained undiscovered was a source of perplexity so long as it was supposed that all living things must be visible to the eye aided by the microscope. To-day, thanks to the invention of the ultra-microscope, that shows the existence of things too small to be defined, and still more to the adaptation of the method of filtration to the study of the diseases in question, we realize that the "viruses" of disease may be visible or invisible and that they have no limitations of size. Just as bacteria readily find their way through paper filters, so the invisible and hence undescribed viruses—*i.e.*, micro-organisms—of yellow fever, pleuro-pneumonia of cattle, foot-and-mouth disease, rinderpest, hog-cholera, African horse-fever, infectious anemia or swamp sickness of horses, fowl plague, small-pox, cow-pox, sheep-pox, horse-pox, swine-pox, and goat-pox are at some or all stages able to pass through the Berkefeld or diatomaceous earth filters, and some of them through the much less porous unglazed porcelain or Chamberland filters. Thus there is opened a new world that is ultramicroscopic, but still teems with invisible living organisms.

CHAPTER I

STRUCTURE AND CLASSIFICATION OF THE MICRO-ORGANISMS

BACTERIA

When Leeuwenhoek with his improved microscope discovered the new world of micro-organisms, he supposed them, on account of the active movements they manifested, to be small animals, and described them as animalculæ. The early systematic writers, Ehrenberg and Dujardin, fell into the same error, and it was many years before biologists had arrived at even approximate accuracy in arranging them. Indeed, for a long time a great number baffled systematic writers, and no less an authority than Haeckel, in 1878, suggested that they form a group by themselves to be known as *Protista*. Such a grouping, however, was unsatisfactory alike to botanists and zoölogists, and, therefore, was used by few.

It was evident that structure could not be looked upon as a satisfactory differential character, for between the protozoa, or most simple animals, and the protophyta, or most simple plants, the structural differences were too minute to prevent overlapping. Motion and locomotion had to be abandoned, since it was common to both groups. Reproduction was likewise an unreliable means when taken by itself, for much the same means of multiplication were found to obtain in both groups. One great physiologic and metabolic difference was, however, noted: plants possess the power of nourishing themselves upon purely inorganic compounds, while animals are unable to do so and cannot live except upon complex molecular combinations synthesized by the plants. In this metabolic difference we find the present criterion for the separation of the living organisms into the two main groups. But this does not dispose of all of the difficulties, for there are certain small groups to which it does not apply. Thus, for example, the fungi which, when judged by other criteria, are undoubted plants, lack the power of inorganic synthesis, and so resemble animals.

Fortunately, the question is a purely academic one. Though seemingly at first sight a most fundamental one, it is, in reality, of trifling importance, for after a limited experience the student unhesitatingly assigns most of the known organisms to one or the other groups, and that occasional mistakes may be made, and organisms, like the spirochæta, appear sometimes in the group of plants among the bacteria, and in other writings in the group of animals among the protozoa, is a matter of small consequence so long

as the knowledge of the organisms themselves is in no particular diminished by the method of classifying them.

In discussing the matter Delage says, "The question is not so important as it appears. From one point of view and on purely theoretic grounds it does not exist, while from another standpoint it is insoluble. If one be asked to divide living things into two distinct groups, of which one contains only animals and the other only plants, the question is meaningless, for plants and animals are concepts which have no objective reality, and in nature they are only individuals. If in considering those forms which we regard as true animals and plants we look for their phylogenetic history and decide to place all of their allies in one or the other group, we are sure to reach no result; such attempts have always been fruitless."

"Huxley pointed out as early as 1876 the extremely close relationship between the lowest algæ and some of the flagellates, and it is the general opinion that no one feature separates the lowest plants from the lowest animals, and the difficulty—in many cases the impossibility—of distinguishing between them is clearly recognized.

"The point of view which demands a strict separation of animals and plants has, however, little utility save, perhaps, to determine the limits of a text-book or a monograph."*

The relative position of the pathogenic vegetable micro-organisms to the other vegetable organisms can be determined by reference to the following table. The wide separation of the bacteria in Group II. and all of the others, which appear in Group X., should be noted.

The various genera to which the pathogenic fungi belong are by no means closely related to one another, as can at once be seen by the following amplification of Group X. Eumycetes:

No entirely satisfactory grouping of the bacteria themselves has yet been achieved, the best characters to be used as the basis of classification being undecided. The best system for their provisional arrangement is probably that of Migula,† or the modification of it suggested by F. D. Chester,‡ in which the morphology, sporulation, and appendages of the bacteria all enter as important features.

Size.—Bacteria are so minute that a special unit has been adopted for their measurement. This is the *micron*, *micromillimeter* or μ, and is the one-thousandth part of a millimeter, equivalent to the one-twenty-five-thousandth ($\frac{1}{25000}$) of an inch.

There is no limit to the minuteness of micro-organisms. Visibility is no longer a criterion. There are micro-organisms that can be seen with low powers, others that can only be seen with high powers, and a few that probably cannot be seen with any power of

* Calkins', "The Protozoa," p. 23.

† "System der Bakterien," Jena, 1897–1900 (vols. I and II appearing at different times).

‡ "Preliminary Arrangement of the Species of the Genus Bacterium," "Ninth Annual Report of the Delaware College Agricultural Experiment Station," 1897, Newark, Delaware, U. S. A.

the microscope. These are called "invisible viruses." They are known to us through the biological quality of filtrates in which they are present because of their ability to pass through the pores of the filters. For this reason they are also called "filterable viruses." As they cannot be seen, we have no way of classifying them; they may be bacteria or protozoa, or neither or both.

TABLE I

THE PLANT KINGDOM

Cryptogamia (κρυπτος hidden, γαμος marriage). Plants without flowers or seeds, reproducing by spores.

These primary divisions, like the corresponding primary division of animals into vertebrata and invertebrata, are now falling into disuse.

Thallophyta—(θαλλος a young shoot, φυτον a plant). Plants without differentiation into root, stem, leaf, flower, etc.

Algæ

I. Phytosarcodina; myxothallophyta; myxomycetes (slime moulds).
II. Schizophyta (σχιζειν to cleave or split; φυτον plant). Plants reproducing by division.
Class 1. Schizomycetes (**Bacteria**).
Class 2. Schizophyceæ (blue-green algæ).

III. Flagellata } Flagellates; organisms claimed equally by botanists
IV. Dinoflagellata } and zoologists.
V. Zygophyceæ—conjugate algæ.
VI. Chlorophyceæ—green algæ.
VII. Charales—Stoneworts.
VIII. Phæophyceæ—brown seaweeds.
IX. Rhodophyceæ—red seaweeds.

Fungi—

X. Eumycetes—**fungi, moulds,** yeasts, smuts, rusts, mildews, etc. (See Table II.)
XI. Embryophyta asiphonogama.

Archegoniatæ—(ἀρχεγονος, the first of the race). Plants showing a regular alternation of two generations in the life history. The asexual generation multiplies by spores.

Bryophyta (βρυον mossy seaweed, φυτον plant). Liverworts and mosses.
Pteridophyta (πτερις fern, φυτον plant). Ferns, horse-tails, club-mosses, ground pine, etc.

Phanerogamia (φανερος visible, γαμος marriage). Plants having flowers and seeds.

XII. Embryophyta siphonogama.

Spermatophyta—(σπερμα seed, φυτον plant). Plants with true flowers and true seeds.

Gymnospermæ (γυμνός naked, σπερμα seed). Cycads, pines, spruces, cedars, ginkos, etc.
Angiospermæ (ἀγγεῖον a tube or vessel, σπερμα a seed).
Monocotyledons.
Dicotyledons.

TABLE II

X. Eumycetes (ευ good, μυκητος fungus). The true fungi: plants without chlorophyl.
Class 1. Phycomycetes (φυκος seaweed), alga-like fungi.
Order 1. Zygomycetes.
Sub-order—Mucorineæ.
Family—Mucoraceæ.
Genus—**Mucor.**
Order 2. Oömycetes.

Class 2. Hemiascomycetes.
Order 1. Hemiascales.
Family—Saccharomycetaceæ.
Genus—**Saccharomyces.**
" —**Blastomyces** (?).

Class 3. Euascomycetes.
Order 1. Euascales (contains 45 families).
Family—Aspergillaceæ.
Genus—**Aspergillus.**
" —**Penicillium.**

Class 4. Laboulbeniomycetes.
Order 1. Laboulbeniales.

Fungi imperfecti. This is a large supplementary group, of imperfectly known fungi not included in the tabulation. In it we find **Oidium.**

Class 5. Basidiomycetes.
Sub-class—Hemibasidii.
Order 1. Hemibasidiales.
Family—Ustilaginaceæ (smuts).
Sub-class—Eubasidii.
Order 1. Protobasidiomycetes.
Family—Uredineineæ (rusts).
Order 2. Autobasidiomycetes (mushrooms, toad-stools, etc.).

CLASSIFICATION OF THE BACTERIA

I. ORDER: EUBACTERIA (True Bacteria)

A. Sub-order: Haplobacteria (Lower Bacteria)

I. Family Coccaceæ. Cells globular, becoming slightly elongate before division. Division in one, two, or three directions of space. Formation of endospores very rare.
(A) Without flagella.
1. *Streptococcus.* Division in one direction of space, producing chains like strings of beads.
2. *Micrococcus.* Division in two directions of space, so that tetrads are often formed.
3. *Sarcina.* Division in three directions of space, leading to the formation of bale-like packages.
(B) With flagella.
1. *Planococcus.* Division in two directions of space, like micrococcus.
2. *Planosarcina.* Division in three directions, like sarcina.

II. Family Bacteriaceæ. Cells more or less elongate, cylindric, and straight. They never form spiral windings. Division in one direction of space only, transverse to the long axis of the cell.
(A) Without flagella.
1. *Bacterium.* Occasional endospores.
(B) With flagella.
2. *Bacillus.* Flagella arising from any part of the surface. Endospore-formation common.
3. *Pseudomonas.* Flagella attached only at the ends of the cell. Endospores very rare.

III. Family Spirillaceæ. Cells twisted spirally like a corkscrew, or representing sections of the spiral. Division only transverse to the long diameter.

1. *Spirosoma.* Rigid; without flagella.
2. *Microspira.* Rigid; having one, two, or three undulating flagella at the ends.
3. *Spirillum.* Rigid; having from five to twenty curved or undulating flagella at the ends.
4. *Spirochæta.** Serpentine and flexible. Flagella not observed; probably swim by means of an undulating membrane.

B. Sub-order: Trichobacteria (Higher Bacteria)

IV. Family Mycobacteriaceæ. Cells forming long or short cylindric filaments, often clavate-cuneate or irregular in form, and at times showing true or false branchings. No endospores, but formation of gonidia-like bodies due to segmentation of the cells. No flagella. Division at right angles to the axis of rod in filament. Filaments not surrounded by a sheath as in Chlamydobacteriaceæ.

1. *Mycobacterium.* Cells in their ordinary form, short cylindric rods often bent and irregularly cuneate. At times Y-shaped forms or longer filaments with true branchings may produce short coccoid elements, perhaps gonidia. (This genus includes the Corynebacterium of Lehmann-Neumann.) No flagella.
2. *Actinomyces.* Cells in their ordinary form as long branched filaments; growth coherent, dry or crumpled. Produce gonidia-like bodies. Cultures generally have a moldy appearance, due to the development of aërial hyphæ. No flagella.

V. Family Chlamydobacteriaceæ. Forms that vary in different stages of their development, but all characterized by a surrounding sheath about both branched and unbranched threads. Division transverse to the length of the filaments.

1. *Cladothrix.* Characterized by pseudo-dichotomous branchings. Division only transverse. Multiplication by the separation of whole branches. Transplantation by means of polar flagellated swarm-spores.
2. *Crenothrix.* Cells united to form unbranched threads which in the beginning divide transversely. Later the cells divide in all three directions of space. The products of final division become spheric, and serve as reproductive elements.
3. *Phragmidiothrix.* Cells at first united into unbranched threads. Divide in three directions of space. Late in the development, by the growth of certain of the cells through the delicate, closely approximated sheath, branched forms are produced.
4. *Thiothrix.* Unbranched cells inclosed in a delicate sheath. Non-motile. Division in one direction of space. Cells contain sulphur grains.

II. ORDER: THIOBACTERIA (Sulphur Bacteria)

I. Family Beggiatoaceæ. Cells united to form threads which are not surrounded by an inclosing sheath. The septa are scarcely visible. Divide in one direction of space only. Motility accomplished through the presence of an undulating membrane. Cells contain sulphur grains.

There are two families, numerous sub-families, and thirteen genera in this order. They are all micro-organisms of the water and soil, and have no interest for the medical student.

Structure.—*Nucleus.*—When subjected to the action of nuclear stains, large vague nuclear formations are usually observed in the bacterial cells.†

* The spirochæta and some closely related forms are now thought to be more properly classified among the protozoa than among the bacteria. They will, therefore, appear again in the tabulation of the protozoan organisms.

† For literature upon the nucleus of the bacteria, see the lengthy paper by Douglas and Distaso ("Centralbl. für Bakt.," etc., I. Abt. Orig., LXVI, p. 321).

Cytoplasm.—The cytoplasm, of which very little exists between the large nucleus and cell-wall, is sometimes granular, as in Bacillus megatherium, and sometimes contains fine granules of chlorophyl, sulphur, fat, or pigment.

Capsule.—Each cell is surrounded by a distinct cell-wall, which in some species shows the cellulose reaction with iodin.

The cell-walls of certain bacteria at times undergo a peculiar gelatinous change or permit the exudation of gelatinous material from the cytoplasm, and appear surrounded by a halo or capsule. Such capsules are seen about the pneumococcus as found in blood or sputum, Friedländer's bacillus, as seen in sputum, Bacillus aërogenes capsulatus in blood or tissue, and many other organisms. Friedländer pointed out that the capsule of his pneumonia bacillus, as found in the lung tissue or in the "prune-juice" sputum, was very distinct, though it could not be demonstrated at all when the organisms grew in gelatin.

Polar Granules.—By carefully staining an appropriate organism, certain peculiarities of structure can sometimes be shown. Thus, some bacilli contain distinct "polar granules" (metachromatic or Babes-Ernst granules)—rounded or oval bodies—situated at the ends of the cell. Their significance is unknown. They have been supposed to bear some relationship to the biologic activity of the organism, especially its pathogenesis, but this is uncertain, and Gauss* and Schumburg† believe that they vary with the reaction of the culture-media upon which the bacteria grow and have nothing to do with virulence. The diphtheria bacillus and the cholera spirillum stain very irregularly in fresh cultures, as if the tingeable substance were not uniformly distributed throughout the cytoplasm. Vacuolated bacteria and bacteria that will not stain, or stain very irregularly, may usually be regarded as degenerated organisms (involution forms) which, because of *plasmolysis*, or solution, can no longer stain uniformly.

Flagella.—Many bacteria possess delicate straight or wavy filaments, called *flagella*, which appear to be organs of locomotion.

Messea‡ has suggested that the bacteria be classified, according to the arrangement of the flagella, into:

I. Gymnobacteria (forms without flagella).
II. Trichobacteria (forms with flagella).
    1. Monotricha (with a single flagellum at one end).
    2. Lophotricha (with a bundle of flagella at one end).
    3. Amphitricha (with a flagellum at each end).
    4. Peritricha (flagella around the body, springing from all parts of its surface).

* "Centralbl. f. Bakt.," etc., Feb. 5, 1902, XXXI, No. 3, p. 106.
† Ibid., June 3, 1902, XXXI, No. 14, p. 604.
‡ "Rivista d'igiene e sanata publica," 1890, II.

This arrangement is, however, less satisfactory than that of Migula already given.

Motility.—The greater number of the bacteria supplied with flagella are actively motile, the locomotory power no doubt being the lashing flagella. The rod and spiral micro-organisms are most plentifully supplied with flagella; only a few of the spheric forms have them.

The presence of flagella, however, does not invariably imply motility, as they may also serve to stimulate the passage of currents of nutrient fluid past the organism, and so favor its nutrition. The flagellate bacteria are more numerous among the saprophytic than the pathogenic forms.

Bacillus megatherium has a distinct but limited ameboid movement.

The dancing movement of some of the spheric bacteria seems to be the well-known Brownian movement, which is a physical phenomenon. It is sometimes difficult to determine whether an organism viewed under the microscope is really motile or whether it is only vibrating. One can usually determine by observing that in the latter case it does not change its relative position to surrounding objects.

In some cases the colonies of actively motile bacteria, such as the proteus bacilli, show definite migratory tendencies upon 5 per cent. gelatin. The active movement of the bacteria composing the colony causes its shape constantly to change, so that it bears a faint resemblance to an ameba, and moves about from place to place upon the surface of the gelatin.

Reproduction.—Fission.—Bacteria multiply by binary division (fission). A bacterium about to divide appears larger than normal, and, if a spheric organism, more or less ovoid. By appropriate staining karyokinetic changes may be observed in the nuclei. When the conditions of nutrition are good, fission progresses with astonishing rapidity. Buchner and others have determined the length of a generation to be from fifteen to forty minutes.

The results of binary division, if rapidly repeated, are almost appalling. "Cohn calculated that a single germ could produce by simple fission two of its kind in an hour; in the second hour these would be multiplied to four, and in three days they would, if their surroundings were ideally favorable, form a mass which can scarcely be reckoned in numbers." "Fortunately for us," says Woodhead, "they can seldom get food enough to carry on this appalling rate of development, and a great number die both for want of food and because of the presence of other conditions unfavorable to their existence."

Sporulation.—When the conditions for rapid multiplication by fission are no longer good, many of the organisms guard against extinction by the formation of *spores*.

Endospores, or spores developed within the cells, are generally

formed in the elongated bacteria—bacillus and spirillum—but Zopf has observed similar bodies in micrococci. Escherich also claims to have found undoubted spores in a sarcina.

Spores may be either round or oval. As a rule, each organism produces a single spore, which is situated either at its center or at its end. When, as sometimes happens, the diameter of the spore is greater than that of the bacillus, it causes a peculiar barrel shape bulging of the organism, described as *clostridium*. When the distending spore is at the end, a "Trommelschläger," or "drumstick," is formed. End-spores are almost characteristic of anaerobic bacilli. When the formation of a spore is about to commence, a small bright point appears in the cytoplasm, and increases in size until its diameter is nearly or quite as great as that of the bacterium. A dark, highly refracting capsule is finally formed about it. As soon as the spore arrives at perfection the bacterium seems to die, as if its vitality were exhausted.

The spores differ from the bacteria in that their capsules prevent evaporation and enable them to withstand drying and the application of a considerable degree of heat. Very few adult bacteria are able to resist temperatures above 70°C. Spores are, however,

Fig. 1.—Diagram illustrating sporulation: *a*, Bacillus inclosing a small oval spore; *b*, drumstick bacillus, with the spore at the end; *c*, clostridium; *d*, free spores; *e* and *f*, bacilli escaping from spores.

uninjured by such temperatures, and can even successfully resist the temperature of boiling water (100°C.) for a short time. The extreme desiccation caused by a protracted exposure to a dry temperature of 150°C. will invariably destroy them, as will also steam under pressure. Not only can the spores successfully resist a considerable degree of heat, but they are also unaffected by cold of almost any intensity. Von Szekely* found anthrax spores capable of germination after eighteen years and six months in some dried-up old gelatin cultures found in his laboratory.

Arthrospores.—The formation of arthrospores is less clear, and seems to be the conversion of the entire organism into a spore or permanent form. Arthrospores have been observed particularly among the micrococci, where certain individuals become enlarged beyond the normal, and surrounded by a capsule.

Though the cell-wall of the adult bacterium is easily penetrated by solutions of the anilin dyes, it is difficult to stain spores, which are distinctly more resistant to the action of chemic agents than the bacteria themselves.

* "Zeitschr. für Hygiene," 1903, XLIV, 3.

Germination of Spores.—When a spore is about to germinate, the contents, which have been clear and transparent, become granular, the body increases slightly in size, the capsule becomes less distinct, and in the course of time splits open to allow the escape of a young organism. The direction in which the capsule ruptures varies in different species. Bacillus subtilis escapes from the side of the spore; Bacillus anthracis from the end. This difference can be made use of as an aid in differentiating otherwise similar organisms.

So soon as the young bacillus escapes it begins to increase in size, develops a characteristic capsule, and presently begins the propagation of its species by fission.

Morphology.—The three principal forms of bacteria are spheres (cocci), rods (bacilli), and screws (spirilla).

Cocci.—The spheric bacteria, from a fancied resemblance to little berries, are called *cocci* or *micrococci*. When they divide, and the resulting organisms remain attached to one another, a

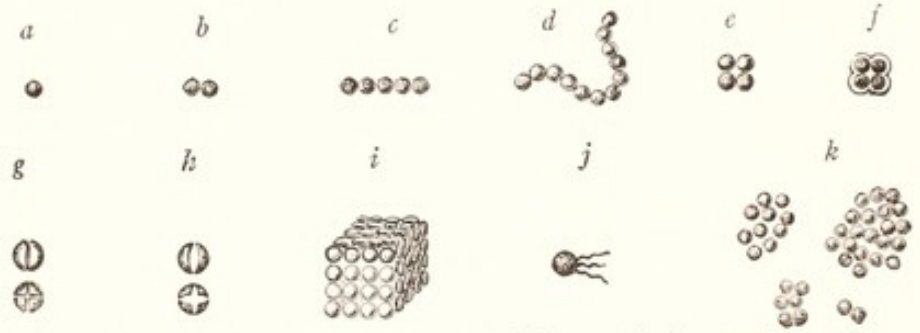


Fig. 2.—Diagram illustrating the morphology of the cocci: *a*, Coccus or micrococcus; *b*, diplococcus; *c*, *d*, streptococci; *e*, *f*, tetracocci or merismopedia; *g*, *h*, modes of division of cocci; *i*, sarcina; *j*, coccus with flagella; *k*, staphylococci.

diplococcus is produced. Diplococci may consist of two attached spheres, though each half commonly shows flattening of the contiguous surfaces. In a few cases, as the gonococcus, the approximated surfaces may be slightly concave, causing the organism to resemble the German biscuit called a "Semmel." When a second binary division occurs, and four resulting individuals remain attached to one another, without disturbing the arrangement of the first two, a tetrad, or *tetracoccus*, is formed. To the entire groups of cocci dividing in two directions of space so as to produce fours, eights, twelves, etc., on the same plane, the name *merismopedia* has been given. Migula uses the term *micrococcus* for the unflagellated tetrads, and *planococcus* for the flagellated forms.

If division takes place in three directions of space, so as to produce a cubic "package" of cocci, the resulting aggregation is described as a *sarcina*. This form resembles a dice or a miniature bale of cotton. Few sarcinæ have flagella, similar flagellated organisms being called by Migula *planosarcina*.

If division always take place in the same direction, so that the

cocci remain attached to one another like a string of beads, the organism is described as a *streptococcus*.

Cocci commonly occur in irregular groups having a fancied resemblance to bunches of grapes. Such are called *staphylococci*, and most organisms not finding a place in the varieties already described are so classed.

Cocci associated in globular or lobulated clusters, incased in a resisting gelatinous, homogeneous mass have been described by Billroth as *ascococcus*. Cocci solitary or in chains, surrounded by an incasement of almost cartilaginous consistence, have been called *leuconostoc*.

Fig. 3.—Diagram illustrating the morphology of the bacilli: *a*, *b*, *c*, Various forms of bacilli; *d*, *e*, bacilli with flagella; *f*, chain of bacilli, individuals distinct; *g*, chain of bacilli, individuals not separated.

Bacilli.—Better known, if not more important, bacteria consist of elongate or "rod-shaped forms," and bear the name *bacillus* (a rod). These present considerable variation of form. Some are ellipsoid, some long and slender. Some have rounded ends, as Bacillus subtilis; others have square ends, as B. anthracis. Some are large, some exceedingly small. Some always occur singly, never uniting to form threads or chains; others are nearly always so conjoined.

The bacilli divide by transverse fission only, so that the only peculiarity of arrangement is the formation of threads or chains. In the older writings, short, stout bacilli were described under the generic term *bacterium*. Migula now employs the term to include only bacillary forms without flagella. A *pseudomonas* is a bacillary

Fig. 4.—Diagram illustrating the morphology of the spirilla: *a*, *b*, *c*, Spirilla.

form with polar flagella. Some of the flexile bacilli have sinuous movements resembling the swimming of a snake or an eel, and are sometimes described as *vibrio;* but this name also has passed into disuse, except in France.

Spirilla.—If a rod-shaped bacterium is spirally twisted and resembles a corkscrew, it is called *spirillum*. The rigid forms without flagella are known as *spirosoma;* rigid forms with flagella, *spirilla* and *microspira*.

A spiral organism of ribbon shape is called *spiromonas*, while a similar oganism of spindle shape is called a *spirulina*. One species of spiral bacteria in whose cytoplasm sulphur granules have been detected has been called *ophidiomonas*.

Spiral organisms with undulating membranes are known as *spirochæta*, but these and the similar genus *treponema* are now regarded as more correctly placed among the protozoan organisms.

THE HIGHER BACTERIA

The **Higher Bacteria** form a group intermediate between the Schizomycetes, or true bacteria, and the Hyphomycetes, or molds. In the classification of Migula and Chester they include the Myco-

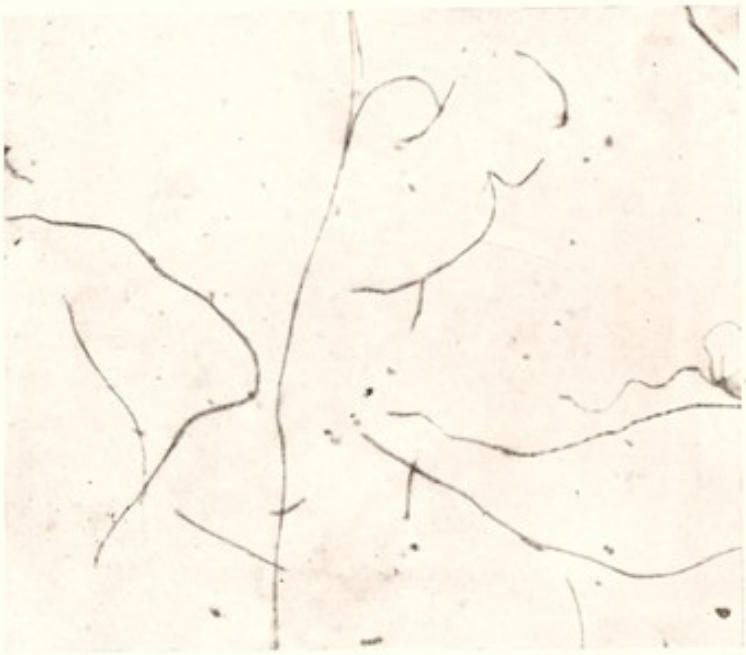


Fig. 5.—Cladothrix, showing false branching. (From Hiss and Zinsser, "Text-Book of Bacteriology," D. Appleton & Co., publishers.)

bacteriaceæ and the Chlamydobacteriaceæ. Some, like Petruschky, believe them to be more closely related to the true molds than to the bacteria. They are characterized by filamentous forms with real or apparent branchings. The filaments are usually regularly divided transversely, so as to appear as if composed of bacilli. The free ends only seem to be endowed with reproductive functions, and develop peculiar elements that differentiate the higher from the other bacteria whose cells are all equally free and independent.

Leptothrix.—These comprise long threads which do not branch. They are not always easily separated from chains of bacilli. They rarely appear to play a pathogenic rôle, though those inhabiting the mouth occasionally secure a foothold upon the edges of the tonsillar crypts, where they grow, with the formation of persistent white patches. This form of leptothrix mycosis is chronic and diffi-

cult to treat. The leptothrix is a very difficult organism to secure in culture. The attempts of Vignal* and of Arustamoff† were successful, but upon the usual culture-media the organisms grew very sparingly.

Cladothrix.—These also produce long thread-like filaments, but they occasionally show what is described as false branching; that is, branches seem to originate from the threads, but no distinct connection between the thread and the apparent branch obtains. None of the cladothrices is known to be pathogenic. They are frequent organisms of the atmospheric dust, and not infrequently appear as "weeds" in culture-media. The colonies grow to about a centimeter in diameter, are usually white in color, irregularly rounded,

Fig. 6.—Streptothrix enteola. Film preparation from peptone-beef-broth culture, fourteen days at 37°C. × 1000. (Foulerton.)

sharp at the edges, more or less concentric, dry and powdery (not velvety) or scaly on the surface. They commonly liquefy gelatin and blood-serum.

Streptothrix.—These organisms certainly branch. They also form endospores. Many of them can be cultivated. Not a few are found under circumstances suggesting pathogenic action. For a long time there has been a disposition to regard Bacillus tuberculosis as a form of streptothrix, since old cultures show branching involution forms. The old genus actinomyces is also included by a number of writers among the streptothrices, so that the Actinomyces bovis of Bollinger is called Streptothrix actinomyces, the Actinomyces maduræ, Streptothrix maduræ, and the organism found by Nocard in the disease known as "*farcin du bœuf*," Streptothrix farcinica.

* "Annales de physiologie," 1886.

† "Kolle and Wassermann, "Handbuch der Pathogenen Mikroorganismen," 1903, II, p. 851; Wratsch, 1889.

There seems, however, no adequate ground for this arrangement, and the old genus Actinomyces should be kept. Eppinger found a streptothrix in the pus of a cerebral abscess, and Petruschky, Berestneff, Flexner, Norris, and Larkin have found streptothrices in cases of pulmonary disease simulating tuberculosis. The organisms described by these writers were not identical, so that there are probably several different species. They usually grow well upon ordinary media and upon solid media form whitish, glistening, well-circumscribed colonies attaining a diameter of several millimeters. As they grow old they turn yellowish or brownish. They liquefy gelatin. Some of the cultures were not harmful to the laboratory animals, others caused suppuration.

Actinomyces.—The chief characterization of the organisms of this group is a clavate expansion of the terminal ends of radiating filaments. These are seen in sections of diseased tissues containing the organisms, but rarely are well shown in the artificial cultures. For further particulars of these organisms see Actinomyces bovis, etc.

THE YEASTS, OR BLASTOMYCETES

The organisms of this group are sharply separated from the bacteria by their larger size, elliptic form, and by multiplication by gemmation or budding.

Fig. 7.—Blastomycetes dermatitidis. Budding forms and mycelial growth from glucose agar. (Irons and Graham, in "Journal of Infectious Diseases".)

Each organism is surrounded by a sharply defined, doubly contoured, highly refracting, transparent cellulose envelope. Commonly each cell contains one or more distinct vacuoles. When multiplication is in progress, smaller and larger buds are formed.

The yeasts, of which Saccharomyces cerevisiæ may be taken as the type, are active fermentative organisms, quickly splitting the sugars into CO_2 and alcohol, and are largely cultivated and used in the manufacture of fermented liquors and bread. They grow well in fermentable culture-media and most of them also grow upon the ordinary laboratory culture-media. Many varieties, some of which produce red or black pigment, some no pigment at all, are known. They play very little part in the pathogenic processes. Burse has observed a case of generalized fatal infection caused by an yeast that he calls Saccharomyces hominis. Gilchrist, Curtis, Ophüls, and others have seen localized human infections by blastomycetes. (See Blastomycetic dermatitis.)

THE OÏDIA

These organisms seem to occupy a place intermediate between the yeasts and the molds—the blastomycetes and the hyphomycetes. In certain stages they appear as oval cells which multiply by gem-

Fig. 8.—Oïdium, showing the various vegetative and reproductive elements. × 350. (Grawitz.)

mation, but instead of becoming separated, hang together. At a later stage of development they grow into long filamentous formations suggesting the mycelia of molds, but being less regular. Certain cells also develop as reproductive organs.

They are common micro-organisms of the air and appear as frequent causes of contamination in culture-media, upon all forms of which they grow readily, producing liquefaction where possible. They engage in but few pathogenic processes, the most familiar being that brought about by Oïdium albicans, which causes the common disease of childhood known as thrush (*q. v.*).

THE MOLDS

In this group it is customary to place a miscellaneous collection of organisms having in common the formation of a well-marked

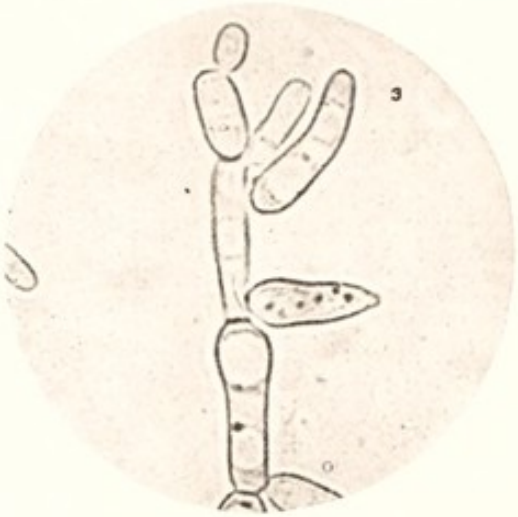


Fig. 9.—Oïdium. (Kolle and Wassermann.)

mycelium, but being so diversified in other respects as to place them in widely separated groups in the systematic arrangement of the

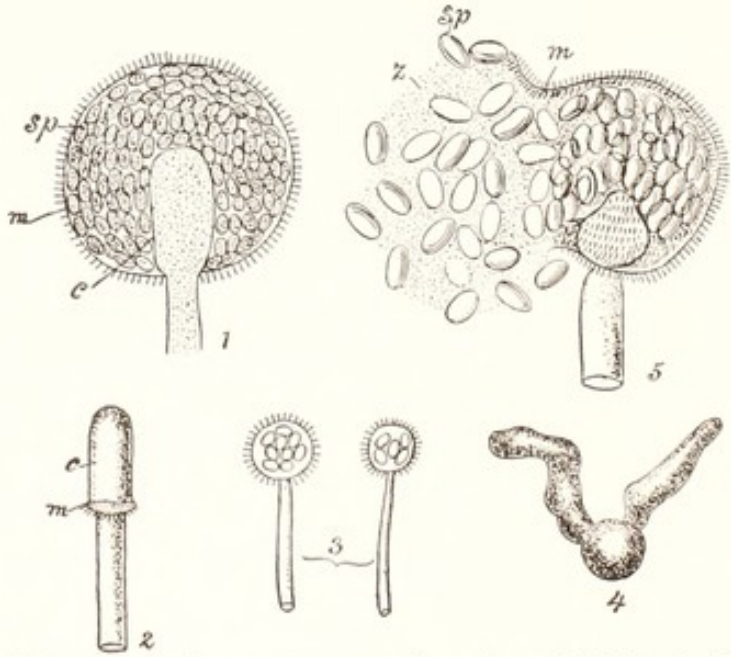


Fig. 10.—Mucor mucedo: 1, A sporangium in optical longitudinal section; *c*, columella; *m*, wall of sporangium; *sp*, spores; 2, a ruptured sporangium with only the columella (*c*) and a small portion of the wall (*m*) remaining; 3, two smaller sporangia with only a few spores and no columella; 4, germinating spores; 5, ruptured sporangium of Mucor mucilaginus with deliquescing wall (*m*) and swollen interstitial substance (*z*); *sp*, spores. (After Brefeld.)

fungi. Some are correctly placed among the "Imperfect fungi," some among the Ascomycetes, and some among the Phycomy-

cetes. They are all active enzymic agents and produce fermentative and putrefactive changes.

1. *Achorion.*—The organisms of this genus are characterized by a more or less branched hypha, 3 to 5μ in diameter, which break up after a time into rounded or cuboidal spores. The Achorion schönleini is highly pathogenic and will be described in the section upon Favus.

2. *Tricophyton and Microsporon.*—These names are applied somewhat loosely to organisms affecting skin and hair follicles of men and animals. They form tangled slender mycelia with many spores of varying size. They occasion "ringworm," barber's itch, pityriasis, and tinea. Further description of the organisms will be found in the section upon Ringworm.

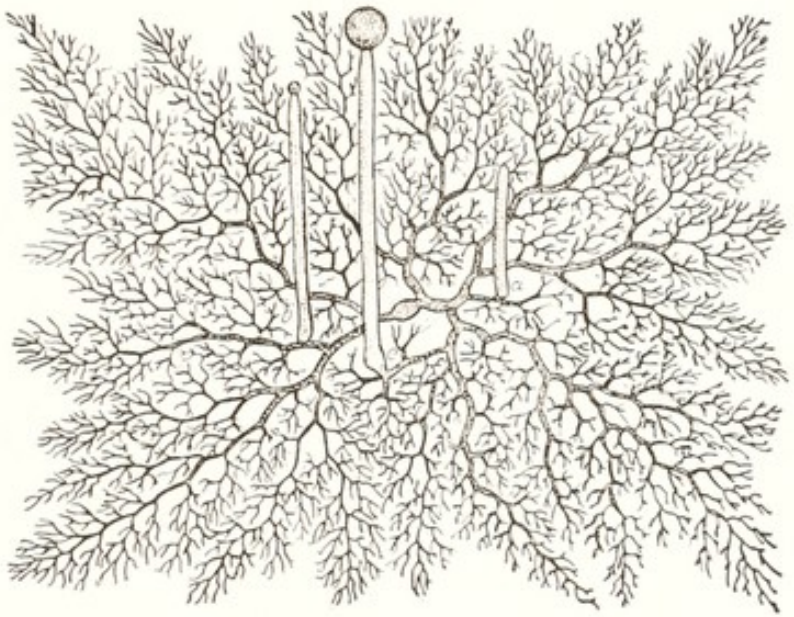


Fig. 11.—Mucor mucedo. Single-celled mycelium with three hyphæ and one developed sporangium. (After Kny, from Tavel.)

3. *Mucor.*—The mucors, or "black molds," belong to the phycomycetes. They form a thick, tangled mycelium, in and above which the rounded black sporangia can be seen with the naked eye. The mycelium becomes divided at the time of reproduction. Multiplication takes place asexually through conidia-spores which develop within sporangia, and sexually by the conjugation of specialized terminal septate branches of the mycelium, which conjugate with similar cells, belonging to other colonies, to form zygospores.

The sporangia form upon the ends of aërial hypha and consist of a smooth spherical capsule within which the spores develop, to become liberated only when the membrane ruptures. The colonies, each of which is unisexual, may be described as + and −. Colonies of the + type will not conjugate; colonies of the − type will not conjugate, but when terminal filaments of + and − come together, conjugation occurs and zygospore formation takes place.

Mucors are not infrequent organisms of the atmosphere and occasionally appear as contaminations upon solid culture-media. About 130 species are known. Of these, Mucor corymbifer, Mucor rhizopodiformis, Mucor ramosus, Mucor pusillus, Mucor septatus, and Mucor conoides are said by Plaut* to be pathogenic when introduced into laboratory animals. Mucor corymbifer has been known to produce inflammation of the external auditory meatus in man.† General mucor mycosis in man has also been observed by Paltauf‡ to result from the presence of the same organism.

4. *Aspergillus and Eurotium.*—The organisms of this genus are included among the Ascomycetes. They are common organisms of

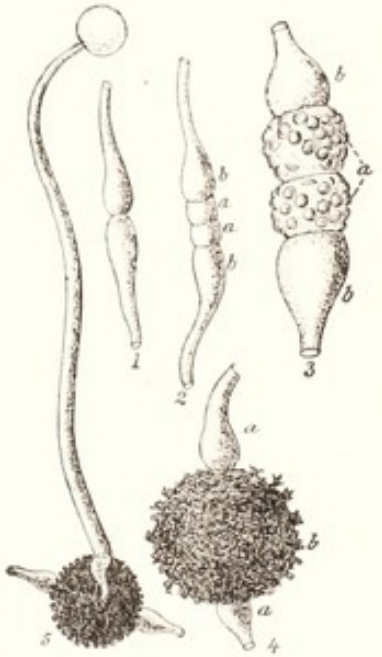


Fig. 12.—Mucor mucedo. Different stages in the formation and germination of the zygospore: 1, Two conjugating branches in contact; 2, septation of the conjugating cells (*a*) from the suspensors (*b*); 3, more advanced stage in the development of the conjugating cells (*a*); 4, ripe zygospore (*b*) between the suspensors (*a*); 5, germinating zygospore with a germ-tube bearing a sporangium. (After Brefeld.)

the air and frequent contaminations of solid culture-media. To secure them an agar-agar plate can be exposed to the atmosphere of the laboratory for a short time, then covered and stood aside for a day or two, when tangled mycelial growths with rapidly spreading hyphæ will usually be discovered. The recognition is easily made when the sporangia appear. These are well shown in the accompanying illustration. The mycelium is divided into many cells. Reproduction is asexual and takes place through conidia spores. The fruit hyphæ, which are aërial, terminate in rounded extremities which are known as columella, from which many radiating sterigmata arise, each terminating in a series of rounded spores. A sexual

* Kolle and Wassermann, "Die Pathogenen Mikroorganismen," 1903, 1, 552.
† Hückel-Lösch in Flügge, "Die Mikroorganismen." ‡ Ibid.

form of reproduction also takes place through the production of ascospores. Many species are known, only a few of which are pathogenic.

Aspergillus malignum has been found by von Lindt in the auditory meatus of man.

Aspergillus nidulans occasionally infects cattle. It is pathogenic for laboratory animals, usually causing death in sixty hours. The kidneys are found enlarged to twice their normal size, and show small whitish dots and stripes of cell infiltration containing the fungi.

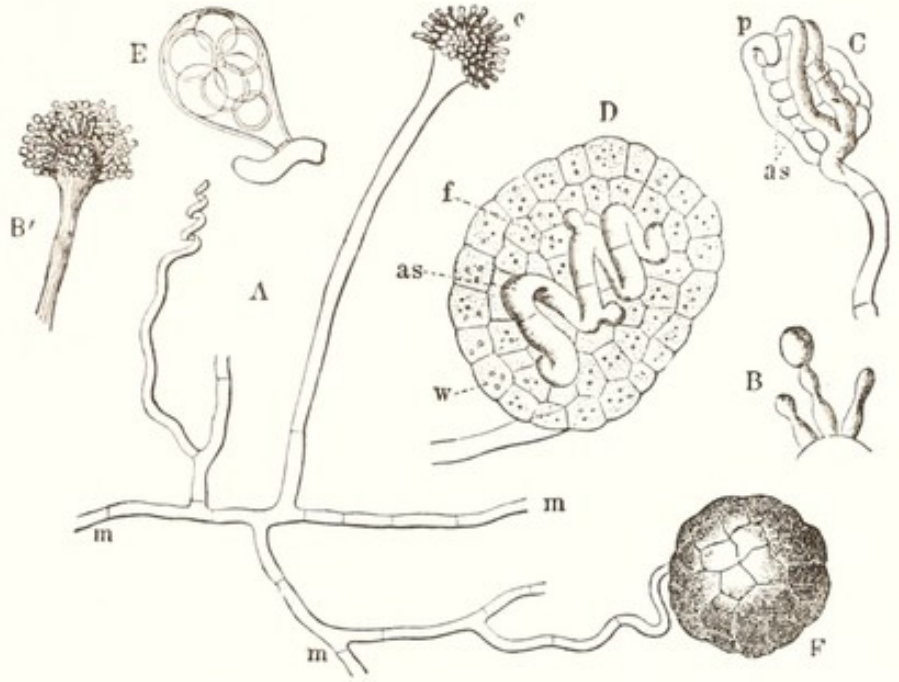


Fig. 13.—Aspergillus glaucus: A, A portion of the mycelium *m*, with a conidiaphore *c*, and a young perithrecium *F*, magnified 100 diameters; B and B′, conidiaphore with conidia; B, individual sterigma greatly magnified; C, early stage of the development of the fructifying organ; D, young perithrecium in longitudinal section; *w*, the future wall of the contents; *as*, the screw, magnified 250 diameters; E, an ascus with spores from a perithrecium, magnified 600 diameters. (duBary.)

The heart muscle, diaphragm, and spleen may also be involved. The liver usually escapes. It takes a large number of spores to infect.

Aspergillus fumigatus.—This is a widespread and not infrequently pathogenic form. Its most common lesion is a pneumomycosis, in which the lung is riddled with small inflammatory necrotic and cavernous areas containing the molds. The same condition has occasionally been observed in human beings, Sticker having collected 39 cases.*

Leber and others have observed keratitis following corneal infection by this organism.

Aspergillus flavus is also pathogenic.

* Nothnagel's Spezielle Path. u. Therap., XIV, 1900.

Aspergillus subfuscus is also pathogenic and highly virulent.

Aspergillus niger.—Pathogenic and found at times in inflammation of the external auditory meatus.

5. *Penicillium.*—These are common green molds, widely disseminated throughout the atmosphere and frequent sources of contamination of the culture-media in the laboratory. Moist bread exposed to the atmosphere soon becomes covered with them. They are included in the group of *fungi imperfecti*, and are characterized by a luxuriant tangled septate mycelium, with aërial fruit hyphæ, ending in conidiophores, each of which divides into two or three sterigmata, the tip of which forms a chain of rounded spores. The whole germinal organ thus comes to resemble a whisk-broom or, as

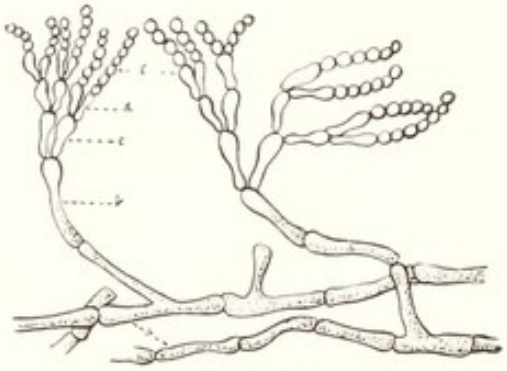


Fig. 14.—Penicillium. (Eyre.)

Hiss describes it, a skeleton hand, in which the conidiophore corresponds to the wrist; the sterigmata, to the metacarpal bones; the chains of spores, to the phalanges.

None of the penicillia is known to be pathogenic either for man or animals.

Penicillium crustaceum (glaucum) is the most common source of contamination of the laboratory media.

Penicillium minimum, which may be identical with the preceding, was once found in the human ear by Sievenmann.

THE PROTOZOA

The *protozoa* are unicellular animal organisms as differentiated from the *metazoa* which are multicellular animal organisms. The restriction, implied by the term unicellular is, however, too narrow, for there are colonial protozoa that consist of many cells, yet share other protozoan characters.

For the purposes of this work, however, all protozoa are to be regarded as unicellular and the individuals independent of one another.

Classification.—Many schemes have been devised for systematically arranging the protozoa, that which follows being an abbreviation of the standard classification, made to correspond with the requirements of this work that deals only with the pathogenic forms.

CLASSIFICATION OF THE PATHOGENIC PROTOZOA

Phylum PROTOZOA (πρῶτος first, ζῶον animal). Unicellular animal organisms.

Class **Rhizopoda** (ῥίζα root, πῶδος foot). Having soft plasmic bodies with or without external protecting shells. The contour subject to change through the formation of extensions known as pseudopods. These may be blunt, rounded, or lobose, filamentous, or anastomosing. The nutrition is holozoic or holophytic.

Order GYMNAMŒBA (γυμνός naked). Rhizopoda without external shells or coverings.

Genus Amœba (ἀμοίβα to change).
Genus Entamœba.
Genus Chlamydophrys.
Genus Leydenia.

Class **Mastigophora** (μάστιγος whips, φόρος to bear). Organisms of well-defined form, naked or surrounded by a well-defined membrane. Nutrition is holozoic, holophytic, parasitic, or saprophytic. Mouth, contractile vesicle, and nucleus usually present.

Order FLAGELLATA (Latin, *flagellare*, to beat). Small organisms with a well-defined mononucleate body, at the anterior end or both ends of which are one or more flagella. Actively motile. May become encysted. Nutrition is holozoic, holophytic, parasitic, or saprophytic.

Family *Cercomonidæ*. Body pyriform with several anterior flagella and an undulating membrane.

Genus Cercomonas.
Genus Trichomonas.
Genus Monas.
Genus Plagiomonas.

Family *Lambliadæ*. Body pyriform, very much attenuated behind. Ventral surface shows a reniform depression, about the posterior part of which there are six flagella. There are also two flagella at the posterior extremity.

Genus Lamblia (Megastomum).

Family *Trypanosomidæ*. Body delicately fusiform. Contains a nucleus, a blepharoplast or centrosome, and an undulating membrane. A single wavy flagellum arises in the posterior part of the body close to the centrosome, passes along the edge of the undulating membrane to the anterior extremity, where it continues free for some distance. Nutrition parasitic. Reproduces by division.

Genus Trypanosoma.
Genus Leishmania.
Genus Babesia.

Family *Spirochætidæ*. Organisms very long and spirally twisted. Nucleus indistinct. Multiplication probably by longitudinal division only. Nutrition is parasitic or saprophytic.

Genus Spirochæta. Body flattened, with a very narrow undulating membrane.

Genus Treponema. Body not flattened. No undulating membrane. Extremities sharp pointed and terminating in short flagella.

Class **Sporozoa** (σπόρος a spore, ζῶον an animal). Organisms unprovided with cilia or flagella in the adult stage. Always endoparasites in the cells, tissues, or cavities of other animals. Nutrition is parasitic and osmotic. Reproduction always by spore-formation, the sporozoïtes either being produced by the parent or indirectly from spores, into which the parent divides.

Subclass Telosporidia. Spore-formation ends the individual life, the entire organism being transformed to spores.

Order GREGARINIDA. Possess distinct membrane with myonemes during adult life; locomotion mainly by contraction. Young stages alone (cephalonts) are intracellular parasites, the adults (sporonts) being found in the digestive tract or the body cavities. Sporulation takes place after or without conjugation, but within a cyst that is never formed, while the parasite is intracellular.

Order Coccidiida. Spherical or ovoid in form, without a free and motile adult stage. Never ameboid. Sporulation takes place within cysts formed while the organism is an intracellular parasite.
Genus Coccidium.
Genus Eimeria.
Order Hæmosporidiida. Sporozoa of small size living in the blood-corpuscles or plasma of vertebrates. The adult form is mobile and in some cases provided with myonemes. Reproduction by endogenous or asexual sporulation, while in the host or by exogenous sporulation after conjugation.
Genus Plasmodium.
Subclass Neosporidia. Organisms that form sporocysts throughout life, the entire cell not being used up in the formation of the spores.
Order Sarcosporidia. The initial stage of the life history is passed in the muscle cells of vertebrates. Form is elongate, tubular, oval, or even spherical. Cysts have a double membrane, in which reniform or falciform sporozoïtes are formed.
Genus Sarcocystis.
Genus Miescheria.
Genus Balbiania.
Subclass Haplosporidia. Spores provided with large round nuclei. No polar capsules.
Genus Rhinosporidium.
Class **Infusoria** (Latin, *infusus*, to pour into. The organisms were given this name because they were first found in infusions exposed to the air). Protozoa in which the motor apparatus is in the form of cilia, either simple or united into membranes, membranelles, or cirri. The cilia may be permanent or limited to the embryonic stages. There are two kinds of nuclei, macronucleus and micronucleus. Reproduction is effected by simple transverse division or by budding. Nutrition is holozoic or parasitic.
Subclass Ciliata. Mouth and anus usually present. The contractile vacuole often connected with a complicated system of canals.
Order Holotrichida. The cilia are similar and distributed all over the body, with a tendency to lengthen at the mouth. Trichocysts are always present, either over the whole body or in special regions.
Genus Colpoda.
Genus Chilodon.
Order Heterotrichida. Organisms possessing a uniform covering of cilia over the entire body, and an adoral zone consisting of short cilia fused together into membranelles.
Suborder Polytrichina. Uniform covering of cilia.
Family *Bursaridæ*. The body is usually short and pocketlike, but may be elongated. The chief characteristic is the peristome, which is not a furrow, but a broad triangular area deeply insunk, and ending in a point at the mouth. The adoral zone is usually confined to the left peristome edge or it may cross over to the right anterior edge.
Genus Balantidium.

Structure.—From the table it will at once be evident that the protozoa form an extremely varied group, and that no kind of descriptive treatment can be looked upon as adequate that does not consider individuals.

Cytoplasm.—In some of the smaller protozoa, and in certain stages of others, the cytoplasm appears almost hyaline and structureless. In most cases, however, it appears granular, and in the larger organisms, such as ameba, it presents the appearance which some described as granular, others, as frothy. The accepted theory of structure teaches that the protoplasm is honeycombed or frothy, and that it is

filled with endless chambers in which its enzymes and other active substances, etc., are stored up and its functions carried on.

In addition to these chambers, which are minute and of uniform size, there are larger spaces called vacuoles, some of which are the result of temporary conditions—accumulations of digested but not yet assimilated food, etc.; but others, seen in ameba and in the ciliata, are large, permanent, and characterized by rhythmical contractions through which they disappear from one part of the body substance to appear in another. These are known as "contractile vacuoles," and are supposed to subserve the useful purpose of assisting in maintaining cytoplasmic currents and so distributing the nourishing juices.

The cytoplasm also contains remnants of undigested or indigestible foods which constitute the paraplasm or deuteroplasm. In a

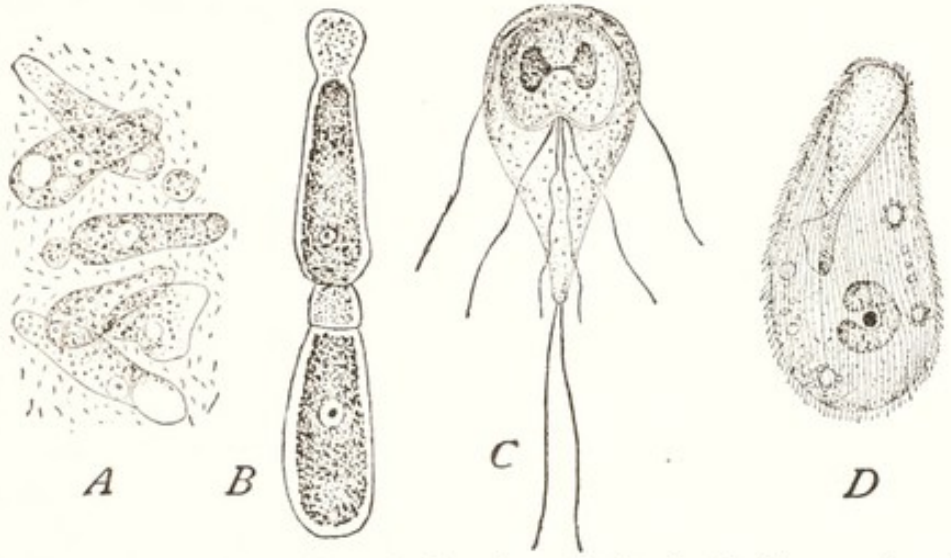


Fig. 15.—Internal parasites: *A*, Amœba coli, Lösch; *B*, Monocystis agilis, Leuck., a gregarine; *C*, Megastoma entericum, Grassi, a flagellate; *D*, Balantidium coli, Ehr., a ciliate.

few cases granules of chlorophyl are also to be found in organisms otherwise resembling animals too closely to be confused with plants.

The cytoplasm may be soft and uniform in quality, or there may be a surface differentiation into ectosarc, or body covering, and endosarc, body substance. In the rhizopoda there is little difference between the two, though certain fresh-water ameba cover themselves with minute grains of mineral substance, but in most of the mastigophora and infusoria corticata the ectosarc is characterized by a peculiar rigidity that gives the animal a definite and permanent form. From the surface covering or ectosarc coarse threads or fine hair-like appendages—flagella and cilia—often project. In many of the infusoria the ectosarc contains trichocysts from which nettling or stinging threads are thrown out when the organisms are irritated.

The body substance may show no morphologic differentiation in rhizopoda, but in the corticata there may not only be a permanent

form, but there may be adaptations, such as an oral aperture, sometimes infundibular in shape and communicating with the soft endosarc through a blind tube. An anal aperture may also be present.

In the higher infusoria the ectosarc may also be continued posteriorly to form a stalk, by which the organism attaches itself (Vorticella). Such stalks are contractile.

Nucleus.—In certain protozoa of very simple and indefinite structure—spirochæta and treponema—no distinct well-contoured nucleus can be observed.

In the rhizopoda the nucleus is a distinct organ surrounded by a nuclear membrane and containing the usual chromatin and linin.

The greater number of mastigophora possess two distinct bodies, either a nucleus and a centrosome or a major and minor nucleus. This is well shown in trypanosoma.

The infusoria vary greatly in the character of the nuclei. As a rule, there are two indefinite nuclei, the macronucleus and the micronucleus. Both seem to be essential organs, and in the phenomena supervening upon conjugation both participate. The nuclei of the protozoa are, therefore, extremely diversified, and vary from the most simple collections of granules of nuclear substance to large well-formed fantastically shaped composite organs.

Movement.—Some kind of movement is to be observed at some period in the life of almost every protozoan.

In rhizopoda with the soft ectosarc the movement consists of flowing currents by which lobose projections of the body substance appear now here, now there, in the form of pseudopodia, or else a continuous flowing, by which the upper surface continually coming forward in a thin layer coincides with the progress of the animal, which continually rolls over and over as it were.

In mastigophora the movement of the more rigid bodies is effected through the presence of longer or shorter, flexile or rigid, coarse threads or "whips." These usually project anteriorly—trypanosoma—and by means of a spiral movement draw the cell along with a propeller-like action; symmetrically arranged flagella may operate more like oars.

The sporozoa usually manifest very little movement, yet their sporozoïtes are motile, and the spermatozoïtes are also motile and commonly flagellated.

The infusoria are actively motile through abundant fine hair-like formations known as cilia. These, multitudinous as they are, vibrate synchronously with an oar-like movement, propelling the organisms forward or backward or making them revolve with great rapidity. Independent cilia not infrequently encircle the oral aperture, causing a vortex, in which the minute structures upon which the creatures feed are caught and carried into the body.

Size.—The protozoa show very great variation in size. Some of

the sporozoa form minute parasites of the red blood-corpuscles or other cells of the vertebrates. The treponema is so small that it can slowly find its way through the pores of a Berkefeld filter.

On the other hand, the sarcoporidium is so large that one of its cysts, composed of a single organism, can be seen with the naked eye. Certain protozoa that play no part in morbid processes—myxosporidia—and so do not come within the scope of this work, may be several centimeters in diameter.

Reproduction.—The reproduction of the protozoa takes place both asexually and sexually. It may be that there are no strictly asexual protozoa, nearly all forms having been shown upon intimate acquaintance to be subject to occasional conjugation. Conjugation may result in the loss of individual identity or the conjugated individuals may again separate.

Whether the reproduction takes place asexually without conjugation or sexually after conjugation, it always occurs by division, which may be simple and binary or complex and multiple.

Wherever a distinct nucleus can be found, the multiplication of the protozoa is preceded by some kind of mitotic change. The more complex the structure of the nucleus, the more complicated and perfect the mitosis.

The elongate protozoa divide lengthwise, which is sometimes contrary to expectation, as in the cases of treponema and spirochæta.

The multitudinous sporozoïtes into which the zygotes of the sporozoa divide are commonly the result of anterior division into intermediate bodies known as oöcysts, oökinetes, sporocysts, etc. The nuclear substance is first divided so as to be uniformly distributed among these, then further divided so that some of it reaches each sporozoïte.

In the process of sporulation the entire parent may be used up, as in the coccidium and plasmodium or the parent may continue to live and later form additional sporozoïtes, as in sarcocystis.

Encystment.—Nearly all of the protozoa are capable at times of encysting themselves, *i.e.*, surrounding themselves with dense capsules by which life may be preserved for some time amid such unfavorable surroundings as excessive cold, excessive dryness, and absence of food. Sometimes the encysted stage is the spore stage (coccidium), sometimes it is the adult stage (ameba). Under these circumstances we find an analogy with the sporulation of the bacteria which is not for purposes of multiplication, but for self-preservation. The encysted protozoa are less hardy, however, than the bacterial and other plant spores, and succumb to comparatively slight elevations of temperature.

CHAPTER II

BIOLOGY OF MICRO-ORGANISMS

THE distribution of micro-organisms is well-nigh universal. They and their spores pervade the atmosphere we breathe, the water we drink, the food we eat, and luxuriate in the soil beneath our feet.

They are not, however, ubiquitous, but correspond in distribution with that of the matter upon which they live and the conditions they can endure. Tyndall* found the atmosphere of high Alpine altitudes free from them, and likewise that the glacier ice contained none; but wherever man, animals, or plants live, die, and decompose, they are sure to be.

Their presence in the air generally depends upon their previous existence in the soil, its pulverization, and distribution by currents of the atmosphere. Koch has shown that the upper stratum of the soil is exceedingly rich in bacteria, but that their numbers decrease as the soil is penetrated, until below a depth of one meter there are very few. Remembering that micro-organisms live chiefly upon organic matter, this is readily understandable, as most of the organic matter is upon the surface of the soil. Where, as in the case of porous soil or the presence of cesspools and dung-heaps, the decomposing materials are allowed to penetrate to a considerable depth, micro-organisms may occur much farther below the surface; yet they are rarely found at any great depth, because the majority of them require free oxygen for successful existence.

The water of stagnant pools always teems with micro-organisms; that of deep wells rarely contains many unless it is polluted from the surface of the earth.

It has been suggested by Soyka that currents of air passing over the surface of liquids might take up organisms, but, although he seemed to show it experimentally, it is not generally believed. Where bacteria are growing in colonies they seem to remain undisturbed by currents of air unless the surface of the colony becomes roughened or broken.

Most of the organisms carried about by the air are what are called saprophytes, and are harmless.

Oxygen.—As all micro-organisms must have oxygen in order to live, the greater number of them grow best when freely exposed to the air. Some will not grow at all where uncombined oxygen is present, but secure all they need by severing it from its chemic combinations. These peculiarities divide bacteria into the

* "Floating Matter in the Air."

Aërobes, which grow in the presence of uncombined oxygen, and *Anaërobes*, which do not grow in the presence of uncombined oxygen.

As, however, some of the aërobic forms grow almost as well without free oxygen as with it, they are known as *optional* (facultative) *anaërobes*.

As examples of strictly aërobic bacteria Bacillus subtilis, Bacillus aërophilus, Bacillus tuberculosis, and Bacillus diphtheriæ may be given. These will not grow if oxygen is denied them. The cocci of suppuration, the bacillus of typhoid fever, and the spirillum of cholera grow almost equally well with or without free oxygen, and hence belong to the optional anaërobes. The bacilli of tetanus and of malignant edema and the non-pathogenic Bacillus butyricus, Bacillus muscoides, and Bacillus polypiformis, will not develop at all where any free oxygen is present, and hence are strictly anaërobic.

The higher bacteria, oïdia, molds and protozoa, are for the most part aërobes and optional anaërobes. Treponema pallidum seems to be a strictly anaërobic protozoan.

Food.—The bacteria grow best where diffusible albumins are present, the ammonium salts being less fitted to support them than their organic compounds. Proskauer and Beck* have succeeded in growing the tubercle bacillus in a mixture containing ammonium carbonate 0.35 per cent., potassium phosphate 0.15 per cent., magnesium sulphate 0.25 per cent., and glycerin 1.5 per cent. Some of the water microbes can live in distilled water to which the smallest amount of organic matter has been added; others require so concentrated a medium that only blood-serum can be used for their cultivation. The statement that certain forms of bacteria can flourish in clean distilled water seems to be untrue, as in this medium the organisms soon die and disintegrate. If, however, in making the transfer, a drop of culture material is carried into the water with the bacteria, the distilled water ceases to be such, and becomes a diluted bouillon fitted to support bacterial life for a time. Sometimes a species with a preference for a particular culture medium can gradually be accustomed to another, though immediate transplantation causes the death of the organism. Sometimes the addition of such substances as glucose and glycerin has a peculiarly favorable influence, the latter, for example, enabling the tubercle bacillus to grow upon agar-agar.

The yeasts grow best upon media containing sugars, but can also be cultivated upon media containing diffusible protein and non-fermentable carbohydrates and glycerin.

The molds flourish upon almost all kinds of organic matter, but perhaps attain their most rapid development upon media containing fermentable carbohydrates.

* "Zeitschrift für Hygiene," etc., Aug. 10, 1894, vol. XVIII, No. 1.

The saprophytic and parasitic protozoa live by osmosis and absorb through the ectosarc such substances as are capable of assimilation and nutrition. These forms are cultivable only upon media containing the same or approximately the same proteins as those to which they have been accustomed. Thus, to cultivate trypanosoma, blood-serum must be added to the media.

The larger protozoa live upon smaller animal and vegetable organisms, which they ingest entire. Such can only be artificially cultivated provided the attempt be made under conditions of symbiosis with some other and smaller organism that may constitute the food.

Moisture.—A certain amount of water is indispensable to the growth of bacteria. The amount can be exceedingly small, however, Bacillus prodigiosus being able to develop successfully upon crackers and dried bread. Artificial culture-media should not be too concentrated; at least 80 per cent. of water should be present.

The molds and oïdia grow well upon bread that contains very little moisture. Protozoa usually require fluid media. Pond-water protozoa can only grow in water, not in concentrated culture-media.

Reaction.—Should the pabulum supplied contain an excess of either alkali or acid, the growth of the micro-organisms is inhibited. Most true bacteria grow best in a neutral or feebly alkaline medium. There are exceptions to this rule, however, for Bacillus butyricus and Sarcina ventriculi can grow well in strong acids, and Micrococcus urea can tolerate excessive alkalinity. Acid media are excellent for the cultivation of molds. Neutral or feebly alkaline media serve best for the cultivable protozoa.

Light.—Most organisms are not influenced by the presence or absence of ordinary diffused daylight. The direct rays of the sun, and to a less degree the rays of the electric arc-light, retard and in numerous instances kill bacteria. In a careful study of this subject Weinzirl* found that when bacteria were placed upon glass or paper, and exposed to the direct rays of the sun, without any covering, most non-spore-bearing bacteria, including Bacillus tuberculosis, B. diphtheriæ, B. typhosus, S. choleræ asiaticæ, B. coli, B. prodigiosus, and others are killed in from two to ten minutes. Certain colors are distinctly inhibitory to the growth, blue being especially prejudicial.

Treskinskaja† found that sunlight had a marked destructive effect upon the tubercle bacillus, and varied according to altitude. By direct sunlight at the sea-level they were destroyed in five hours: at an altitude of 1560 meters, in three hours. In winter the time of destruction was about two hours longer than in summer. In diffused daylight the time required for destruction was about twice as long

* "Centralbl. f. Bakt. u. Parasitenk. Ref.," XLVII, Nos. 22–24, p. 681.
† "Jour. Infectious Diseases," 1907, vol. IV, Supplement, No. 3, p. 128.

as in direct sunlight. His experiments were performed with pure cultures dried in a thin layer upon glass.

Certain chromogenic bacteria produce colors only when exposed to the ordinary light of the room. Bacillus mycoides roseus produces its red pigment only in the dark. The virulence of many pathogenic bacteria is gradually attenuated if they are kept in the light.

Molds and yeasts grow best in the dark, so that in general it can be said that the vegetable micro-organisms, belonging to the fungi and having no chlorophyl, need no light and are injured rather than benefited by it.

The pathogenic protozoa have not been particularly studied with reference to light. Non-pathogenic water protozoa love the light and die in the dark.

Electricity, X-rays, etc.—Powerful currents of electricity passed through cultures have been found to kill the organisms and change the reaction of the culture-medium; rapidly reversed currents of high intensity, to destroy the pathogenesis of the bacteria and transform their toxic products into neutralizing bodies (antitoxin?). Attention has been called to this subject by Smirnow, d'Arsonval and Charin, Bolton and Pease, Bonome and Viola, and others.

An interesting contribution upon the "Effect of Direct, Alternating, Tesla Currents and X-rays on Bacteria" was made by Zeit,* whose conclusions are as follows:

1. A continuous current of 260 to 320 milliampères passed through bouillon cultures kills bacteria of low thermal death-points in ten minutes by the production of heat (98.5°C). The antiseptics produced by electrolysis during this time are not sufficient to prevent the growth of even non-spore-bearing bacteria. The effect is a purely physical one.

2. A continuous current of 48 milliampères passed through bouillon cultures for from two to three hours does not kill even non-resistant forms of bacteria. The temperature produced by such a current does not rise above 37°C., and the electrolytic products are antiseptic, but not germicidal.

3. A continuous current of 100 milliampères passed through bouillon cultures for seventy-five minutes kills all non-resistant forms of bacteria even if the temperature is artificially kept below 37°C. The effect is due to the formation of germicidal electrolytic products in the culture. Anthrax spores are killed in two hours. Subtilis spores were still alive after the current was passed for three hours.

4. A continuous current passed through bouillon cultures of bacteria produces a strongly acid reaction at the positive pole, due to the liberation of chlorin which combines with oxygen to form hypochlorous acid. The strongly alkaline reaction of the bouillon culture at the negative pole is due to the formation of sodium hydroxid and the liberation of hydrogen in gas bubbles. With a current of 100 milliampères for two hours it required 8.82 milligrams of H_2SO_4 to neutralize 1 cc. of the culture fluid at the negative pole, and all the most resistant forms of bacteria were destroyed at the positive pole, including anthrax and subtilis spores. At the negative pole anthrax spores were killed also, but subtilis spores remained alive for four hours.

5. The continuous current alone, by means of Du Bois-Reymond's method of non-polarizing electrodes, and exclusion of chemic effects by ions in Kruger's sense, is neither bactericidal nor antiseptic. The apparent antiseptic effect on suspension of bacteria is due to electric osmosis. The continuous electric current has no bactericidal nor antiseptic properties, but can destroy bacteria

* "Jour. Amer. Med. Assoc.," Nov. 30, 1901.

only by its physical effects (heat) or chemic effects (the production of bactericidal substances by electrolysis).

6. A magnetic field, either within a helix of wire or between the poles of a powerful electromagnet, has no antiseptic or bactericidal effects whatever.

7. Alternating currents of a 3-inch Ruhmkorff coil passed through bouillon cultures for ten hours favor growth and pigment production.

8. High-frequency, high potential currents—Tesla currents—have neither antiseptic nor bactericidal properties when passed around a bacterial suspension within a solenoid. When exposed to the brush discharges, ozone is produced and kills the bacteria.

9. Bouillon and hydrocele-fluid cultures in test-tubes of non-resistant forms of bacteria could not be killed by Röntgen rays after forty-eight hours' exposure at a distance of 20 mm. from the tube.

10. Suspensions of bacteria in agar plates and exposed for four hours to the rays, according to Rieder's plan, were not killed.

11. Tubercular sputum exposed to the Röntgen rays for six hours, at a distance of 20 mm. from the tube, caused acute miliary tuberculosis of all the guinea-pigs inoculated with it.

12. Röntgen rays have no direct bactericidal properties. The clinical results must be explained by other factors, possibly the production of ozone, hypochlorous acid, extensive necrosis of the deeper layers of the skin, and phagocytosis. The action of the *x*-rays upon bacteria has been investigated by Bonome and Gros,* Pott,† and others. When the cultures are exposed to their action for prolonged periods, their vitality and virulence seem to be slightly diminished. They are not killed by the *x*-rays.

Movement.—Rest seems to be the condition best adapted for micro-organismal development. Slow-flowing movements do not have much inhibitory action, but violent agitation, as by shaking a culture in a machine, may hinder or prevent it. This explains why rapidly flowing streams, whose currents are interrupted by falls and rapids, should, other things being equal, furnish a better drinking-water than a deep, still-flowing river.

Galli-Valerio‡ has shown, however, that agitation does not inhibit the growth of the anthrax, typhoid or colon bacilli or the pneumococcus, but sometimes facilitates it.

Association.—**Symbiosis** is the vital association of different species of micro-organisms by which mutual benefit to one or the other is brought about. **Antibiosis** is an association detrimental to one of the associated organisms. Bacterial growth is greatly modified by the association of different species. Coley found the streptococcus more active when combined with Bacillus prodigiosus; Pawlowski, that mixed cultures of Bacillus anthracis and Bacillus prodigiosus were less virulent than pure cultures of anthrax; Meunier, § that when the influenza bacillus of Pfeiffer is inoculated upon blood agar together with Staphylococcus aureus its growth is favored by a change which the staphylococci bring about in the hemoglobin.

A similar advantageous association has been pointed out by Sanarelli, who found that Bacillus icteroides grows best and retains

* "Giornal. med. del Regis Esercito," an 45, u. 6.

† "Lancet," 1897, vol. II, No. 21.

‡ "Centralbl. f. Bakt.," etc., Sept. 23, 1904, Orig., XXXVII, p. 151.

§ Société de Biologie, Séance du 11 Juin, 1898, "La Semaine médicale," June 15, 1898.

its vitality longest when grown in company with certain of the molds.

Rarely, the presence of one species of micro-organism entirely eradicates another. Hankin* found that Micrococcus ghadialli destroyed the typhoid and colon bacilli, and suggested the use of this coccus to purify waters polluted with typhoid.

An interesting experimental study of the bacterial antagonisms with special reference to Bacillus typhosus, that the student should read, is by W. D. Frost, and appears in the "Journal of Infectious Diseases," 1904, 1, p. 509.

Temperature.—According to Fränkel, bacteria will rarely grow below 16° and above 40°C., but Flügge has shown that Bacillus subtilis will grow very slowly at 6°C.; at 12.5°C. fission does not take place oftener than every four or five hours; at 25°C. fission occurs every three-quarters of an hour, and at 30°C. about every half-hour.

The temperature at which micro-organisms grow best is known as the *optimum*, the lowest temperature at which they continue active as the *minimum*, the highest that can be endured the *maximum*.

A few forms of bacteria grow at very high temperatures (60°–70°C.), and are described as *thermophilic*. They are found in manure piles and in hot springs. Tsiklinsky† has described two varieties of actinomyces and a mold that he cultivated from earth and found able to grow well at 48° to 68°C., though not at all at the temperature of the room.

Most bacteria are killed by temperatures above 60° to 75°C., but their spores can resist boiling water for some minutes, though killed by dry heat if exposed to 150°C. for an hour or to 175°C. for from five to ten minutes.

The resistance of low forms of life to low temperatures is most astonishing. Some adult bacteria and most spores seem capable of resisting almost any degree of cold. Ravenel‡ exposed anthrax spores to the action of liquid air for three hours; diphtheria bacilli, for thirty minutes; typhoid bacilli, for sixty minutes; and Bacillus prodigiosus, for sixty minutes, the temperature of the cultures being reduced to about —140°C., yet in no case was the vegetative capability of all of the bacteria destroyed, and when transferred to fresh culture bouillon they grew normally. His researches corroborate those of Pictet and Yung and others.

To say that bacteria are not injured by cold is a mistake, as Sedgwick and Winslow§ have found that when typhoid bacilli are frozen, the greater number of them are destroyed, and that subsequent

* "Brit. Med. Jour.," Aug. 14, 1897, p. 418.
† "Russ. Archiv f. Path.," etc., June, 1898, Bd. v.
‡ "The Medical News," June 10, 1899.
§ "Centralbl. f. Bakt. u. Parasitenk.," etc., May 26, 1900, Bd. XXVII, Nos. 18, 19, p. 684.

development of the frozen cultures takes place from the few surviving organisms.

Bacteria usually grow best at the temperature of a comfortably heated room (17°C.), and are not affected by its occasional slight variations. Some, chiefly the pathogenic forms, are not cultivable except at the temperature of the body (37°C.); others, like the tubercle bacillus, grow best at a temperature a little above that of the normal body.

The temperature endurance of the molds resembles that of the bacteria. The mycelia are killed at temperatures of 60°C. and over, but their spores endure 100°C. The yeasts and oïdia, that have no resisting spores, are killed at about 60°C. The protozoa are still more sensitive to heat variations than the plant organisms and are killed by less extreme variations. Here again, however, the encysted protozoa endure greater variations than the active organisms.

Effect of Chemic Agents.—The presence of chemic agents, especially certain of the mineral salts, in an otherwise perfectly suitable medium may completely inhibit the development of bacteria, and if added to grown cultures in greater concentration, destroy them. Such substances are spoken of as antiseptics in the former, germicides in the latter case. Bichlorid of mercury and carbolic acid are the most familiar examples of germicides.

Though these agents are supposed to operate in definite concentrations with almost unvarying result, Trambusti* found it possible to produce a tolerance to a certain amount of bichlorid of mercury by cultivating Friedländer's bacillus upon culture-media containing gradually increasing amounts of the salt, until from 1–15,000, which inhibit ordinary cultures, it could accommodate itself to 1–2000.

The various chemic agents act in different ways upon the micro-organisms. Thus, they may combine with the protoplasm to make a new and no longer vital compound; or, they may coagulate or dissolve or dehydrate or oxidize the protoplasm to a destructive extent.

The addition of chemic agents to solutions containing micro-organisms also changes the osmotic pressure. When an active organism is living in its normal environment, it contains within its plasm a greater concentration of solutes than are to be found in the surrounding fluid. Under these circumstances the pressure on the inside of the ectosarc or other cell membrane is greater than that on the outer side, and the cell is in a state of *turgor*. If now salts are added so that the solutes on the outside exceed those on the inside, water is drawn out and the protoplasm is made to shrink or condense. According to the degree of this change the organism will be embarrassed, made impotent, or destroyed.

On the other hand, when micro-organisms have enjoyed a concentrated medium like blood-serum and are suddenly transferred to

* "Lo Sperimentale," 1893–94.

distilled water, so much water may be suddenly drawn into their protoplasm that they swell up and may burst and go to pieces. This is particularly true of the delicate protozoa like the trypanosoma.

Metabolism.—According to their activities, micro-organisms are classed as—

Zymogens, when they cause fermentation.
Saprogens, when they cause putrefaction.
Chromogens, when they produce colors.
Photogens, when they phosphoresce.
Aërogens, when they evolve gas.
Pathogens, when they cause disease.

The metabolic activities of micro-organisms occasion many well-known changes in nature. Thus, it is through their energies that by fermentative and putrefactive changes organic matter is gradually transformed from complex to simple compounds. It is by the energy of bacteria that foul waters are gradually purified, and while it is true that the presence of large numbers of bacteria in water detracts from its potability, the very bacteria that cause its condemnation ultimately effect its purification by exhausting the organic matter it contains in their own nutrition. In the treatment of sewage by the "septic tank" method, the organic matter contained in the water is consumed through the agency of anaërobic and aërobic bacteria, until the water once more becomes clear and pure, the bacteria dying out as the nutrition becomes exhausted.

The promptness with which bacteria attack organic matter is seen in the changes brought about in foods, some of which are ruined in flavor or quality, though others are thought to be improved. Thus, the flavor of butter, sausage, and cheese, the aroma of wines, and many other important gustatory characteristics of our foods depend solely upon the activity of bacteria or other micro-organisms.

Many of these activities are harmless, and, indeed, advantageous, though the fact that they are not infrequently accompanied by chemic changes, some of which are poisonous, makes it necessary to watch and time their operations lest acridity, acidity, insipidity, or toxicity of the food replace the desired effect.

Briefly considered, the best known phenomena resulting from micro-organismal energy are as follows:

Fermentation.—Fermentation is catalysis of carbon compounds caused by catalysts or ferments resulting from micro-organismal metabolism. The alcoholic fermentation, which is a familiar phenomenon to the layman as well as to the brewer and chemist, depends upon the activity of an yeast-plant, one of the saccharomyces fungi by which the sugar is broken up into alcohol and carbon dioxid, with some glycerin and other by-products. The following equation shows the chief changes produced:

$$\underset{\text{Sugar}}{C_6H_{12}O_6} = \underset{\text{Alcohol}}{2C_2H_5OH} + \underset{\text{Carbon dioxid}}{2CO_2}$$

There are also several bacteria which produce the acetic fermentation, though it is generally attributed to Bacillus aceticus. There are two equations to express this fermentation:

$$\text{I. } \underset{\text{Alcohol}}{CH_3CH_2OH} + \underset{\text{Oxygen}}{O} = \underset{\text{Aldehyd}}{CH_3CHO} + \underset{\text{Water}}{H_2O}$$

$$\text{II. } \underset{\text{Aldehyd}}{CH_3CHO} + \underset{\text{Oxygen}}{O} = \underset{\text{Acetic acid}}{CH_3COOH}$$

A number of different bacilli seem capable of converting milk-sugar into lactic acid, though Bacillus acidi lactici is the best known and most active acid producer. The butyric fermentation generally due to Bacillus butyricus may also be caused by other bacilli. (For an exact description of the chemistry of the fermentations reference must be made to special text-books.*) The lactic acid and butyric acid fermentation, have the following equations:

$$\text{I. } \underbrace{C_{12}H_{22}O_{11}}_{\text{Lactose or milk sugar}} + H_2O = \underbrace{C_6H_{12}O_6}_{\text{Galactose}} + \underbrace{C_6H_{12}O_6}_{\text{Dextrose}}$$

$$\text{II. } \underbrace{C_6H_{12}O_6}_{\text{Galactose}} = \underbrace{2C_3H_6O_3}_{\text{Lactic acid}}$$

$$\text{III. } \underbrace{C_6H_{12}O_6}_{\text{Galactose}} = \underbrace{C_4H_8O_2}_{\text{Butyric acid}} + CO_2 + 2H_2$$

Putrefaction.—Putrefaction is a catalysis of proteins resulting from the activity of micro-organismal catalysts or enzymes. It is associated with the evolution of a vile odor. The first step in the process seems to be the transformation of the albumins into peptones, then the splitting up of the peptones into gases, amino-acids, bases, and salts. In the process innocuous albumins are frequently changed to toxalbumins, and sometimes to peculiar putrefactive alkaloids known as *ptomains*.

Vaughan and Novy define a *ptomain* as "*a chemical compound, basic in character, formed by the action of bacteria on organic matter.*" The chemistry of these bodies is very complex, and for a satisfactory description of them Vaughan and Novy's book† is excellent.

Ptomains probably play but a small part in pathologic conditions. They are formed almost exclusively outside of the living body, and only become a source of danger when ingested with the food. It is supposed that cases of ice-cream and cheese poisoning are usually due to *tyrotoxicon*, a ptomain produced by the putrefaction of the protein substances of the milk before it is frozen into ice-cream or made into cheese. The safeguard is to freeze the milk only when perfectly fresh and avoid mixing the milk, cream, sugar, and flavoring substances, and allowing the mixture to stand for some time beforehand.

* See "Enzymes and Their Applications," by Jean Effront, translated by S. C. Prescott, New York, 1902; "Micro-organisms and Fermentation," by Alfred Jörgensen, translated by A. K. Miller and A. E. Lennholm, London 1900; and the many writings of Christian Hansen.

† "Ptomaines and Leucomaines," 1888; "Cellular Toxins," 1902.

The occasional cases of "Fleischvergiftung," "meat-poisoning," or "Botulismus," are due to the development of toxic ptomains in consequence of the growth of certain bacteria (Bacillus botulinus) in the meat. Kaensche* has carefully investigated the subject, and given a synoptic table containing all the described bacteria of this class. His researches show that there are at least three different bacilli whose growth causes the meat to become poisonous.

With the increase of knowledge upon the toxic character of the bacteria themselves, the importance of the toxic ptomains has diminished, until at present we have come to regard them as very rare causes of disease.

Production of Gases.—Various gases are given off during decomposition and fermentation, among them being CO_2, H_2S, NH_4, H, CH_4. Gases produced by aërobic bacteria usually fly off from the surface of the culture unnoticed, but if the bacterium be anaërobic and develop the lower part of a tube of solid culture media, a visible bubble of gas is usually formed about the colonies. Such gas bubbles are almost invariably present in cultures of the bacilli of tetanus and malignant edema.

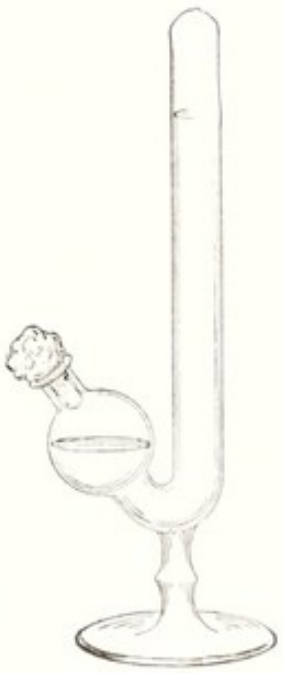


Fig. 16.—Smith's fermentation tube.

To quantitatively determine the gas-production, some form of the Smith fermentation-tube is most convenient. The tube is filled with bouillon containing some sugar, sterilized as usual, inoculated, and stood aside to grow. As the gases form, the bubbles ascend and accumulate in the closed arm. In estimating quantitatively, one must be careful that the tube is not so constructed as to allow the gas to escape as well as to ascend into the main reservoir.

For the determination of the nature of the gases produced, Theobald Smith has recommended the following method:

"The bulb is completely filled with a 2 per cent. solution of sodium hydroxid (NaOH) and tightly closed with the thumb. The fluid is shaken thoroughly with the gas and allowed to flow back and forth from the bulb to the closed branch, and the reverse several times to insure intimate contact of the CO_2 with the alkali. Lastly, before removing the thumb all the gas is allowed to collect in the closed branch so that none may escape when the thumb is removed. If CO_2 be present, a partial vacuum in the closed branch causes the fluid to rise suddenly when the thumb is removed. After allowing the layer of foam to subside somewhat the space occupied by gas is again measured, and the difference between this amount and that measured before shaking with the sodium hydroxid solution gives the proportion of CO_2 absorbed. The explosive character of the residue is determined as follows: The cotton plug is replaced and the gas from the closed branch is allowed to flow into the bulb and mix with the air there present. The plug is then removed and a lighted match inserted into

* "Zeitschrift für Hygiene," etc., June 25, 1896, Bd. XXII, Heft 1.

the mouth of the bulb. The intensity of the explosion varies with the amount of air present in the bulb. The relative proportion of gases resulting from the fermentation is frequently of importance for the differential diagnosis of related bacteria. Smith has designated this relation of $\frac{H}{CO_2}$ as the 'gas formula.' The colon bacillus has a gas formula corresponding to $\frac{H}{CO_2} = \frac{2}{1}$, Other aerogenic bacilli sometimes show a formula $\frac{H}{CO_2} = \frac{1}{2}$.,,

Liquefaction of Gelatin.—As certain organisms grow in gelatin, the medium becomes partly or entirely liquefied. This peculiarity is apparently independent of any other property of the organisms, and is manifested alike by pathogenic and non-pathogenic forms. The liquefaction is supposed to be dependent upon a form of peptonization. Bitter* and Sternberg† have shown that if from a culture in which liquefaction has taken place the bacteria be removed by filtration, the filtrate will retain the power of liquefying gelatin, showing the property is not resident in the bacteria, but in some substance in solution in their excreted products. These products were described as "tryptic enzymes" by Fermi,‡ who found that heat destroyed them. Mineral acids seem to check their power to act upon gelatin. Formalin renders the gelatin insoluble. Some of the bacteria liquefy the gelatin in such a peculiar and characteristic manner as to make the appearance a valuable guide for the differentiation of species.

Production of Acids and Alkalies.—Under the head of "Fermentation" the formation of acetic, lactic, and butyric acids has been discussed. Formic, propionic, baldrianic, palmitic, and margaric acids also result from microbic metabolism. As the acidity progresses, it impedes, and ultimately completely inhibits, the activity of the organisms. The cultivation of the bacteria in milk to which litmus or lacmoid has been added is a convenient method for detecting changes of reaction. Rosolic acid solutions may also be used, the acid converting the red into an orange color. Neutral red is also much employed for this purpose, the acids turning it yellow.

The quantitative estimation of changes in reaction can be best made by titration, and the fermentation-tube culture can be employed for the purpose. The contents of the bulb and branch should be shaken together, a measured quantity withdrawn, and titration with $\frac{n}{20}$ sodium hydroxid, or $\frac{n}{20}$ hydrochloric acid, performed.

The alkali most frequently formed by bacterial growth is ammonium, which is set free from its combinations, and either flies off as a gas or forms new combinations with acids simultaneously formed. Some bacteria produce acids only, some alkalies only, others both

* "Archiv für Hygiene," 1886, Heft 2.
† "Medical News," 1887, No. 14.
‡ "Centralbl. f. Bakt.," etc., 1891, Bd. x, p. 401.

acids and alkalies. Both acids and the alkalies, when in excess, serve to check the further activity of the micro-organisms.

Chromogenesis.—Bacteria that produce colored colonies or impart color to the medium in which they grow are called *chromogenic;* those producing no color, *non-chromogenic*. Most chromogenic bacteria are saprophytic and non-pathogenic. Some of the pathogenic forms, as Staphylococcus pyogenes aureus, are, however, color producers. It seems more likely that certain chromogenetic substances unite with constituents of the culture medium to produce the colors than that the bacteria form the actual pigments; but, as Galeotti* has shown, there are two kinds of pigment, one being soluble, readily saturating the culture medium, as the pyocyanin and fluorescin of Bacillus pyocyaneus, the other insoluble, not tingeing the solid culture media, but retained in the colonies, like the pigment of Bacillus prodigiosus. The pigments are found in greatest intensity near the surface of a bacterial mass. The coloring matter never occupies the cytoplasm of the bacteria (except Bacillus prodigiosus, in whose cells occasional pigment-granules may be seen), but occurs as an intercellular deposit.

Almost all known colors are formed by different bacteria. One bacterium will sometimes elaborate two or more colors; thus, Bacillus pyocyaneus produces pyocyanin and fluorescin, both being soluble pigments—one blue, the other green. Gessard† has shown that when Bacillus pyocyaneus is cultivated upon white of egg, it produces only the green fluorescent pigment, but if cultivated in pure peptone solution it produces only the blue pyocyanin. His experiments prove the very interesting fact that for the production of fluorescin it is necessary that the culture medium contain a definite amount of a phosphatic salt. Sometimes, an organism produces two pigments, one is soluble, the other insoluble, so that the colony will appear one color, the medium upon which it grows another. The author once found an interesting coccus,‡ with this peculiarity, upon the conjunctiva. It formed a brilliant yellow colony upon the surface of agar-agar, but colored the agar-agar itself a beautiful violet. In this case the yellow pigment was insoluble, the violet pigment soluble and diffusible through the jelly. Some organisms will only produce pigments in the light; others, as Bacillus mycoides roseus, only in the dark. Some produce them only at the room temperature, but, though growing luxuriantly in the incubator, refuse to produce pigments at so high a temperature. Thus, Bacillus prodigiosus produces a brilliant red color when growing at the temperature of the room, but is colorless when grown in the incubator. The reaction of the culture medium is also of much importance in this connection. Thus, Bacillus prodigiosus produces an intense

* "Lo Sperimentale," 1892, XLVI, Fasc. III, p. 261.

† "Ann. de l'Inst. Pasteur," 1892, pp. 810–823.

‡ See Norris and Oliver, "System of Diseases of the Eye," vol. II, p. 489, and "University Medical Magazine," Philadelphia, Sept., 1895.

scarlet-red color upon alkaline and neutral media, but is colorless or pinkish upon slightly acid media. Some of the pigments—perhaps most of them—are formed only in the presence of oxygen.

Production of Odors.—Gases, such as H_2S and NH_4, and acids, butyric and acetic acids, have sufficiently characteristic odors. There are, however, a considerable number of pungent odors which seem to arise from independent odoriferous principles. Many of them are extremely unpleasant, as that of the tetanus bacillus. The odors seem to be peculiar individual characteristics of the organisms.

Production of Phosphorescence.—Cultures of Bacillus phosphorescens and numerous other organisms are distinctly phosphorescent. So much light is sometimes given out by gelatin cultures of these bacteria as to enable one to see the face of a watch in a dark room. Gorham found the photogenesis most marked when the organisms are grown in alkaline media at *room* temperature. Most of the phosphorescent bacteria are found in sea-water, and are best cultivated in sea-water gelatin. Some are familiar to butchers through the phosphorescence they cause on the surface of stale meats.

Production of Aromatics.—Phenol, kresol, hydrochinone, hydroparacumaric acid, and paroxyphenylic-acetic acid are by no means uncommon products of bacteria. The most important is *indol*, which was at one time thought to be peculiar to the cholera spirillum, but is now known to be produced by many other bacteria. The best method of testing for it is that of Salkowski,* known as the nitrosoindol reaction. To perform it, 10 cc. of the fluid to be tested receive an addition of 10 drops of concentrated sulphuric acid. The mixture is shaken in a test-tube. A few cubic centimeters of a 0.02 per cent. solution of potassium nitrite are then allowed to flow down the side of the tube. If indol is present, a purple-red color develops at the junction of the two fluids.† McFarland and Small‡ have found that the intensity of this color corresponds to the quantity of indol present, and that quantitative tests can be made by means of a comparative color test series.

The Formation of Nitrates.—A process of fundamental importance is carried on by certain lowly bacteria of the soil. Since plants are unable to assimilate the free nitrogen of the air, but must obtain this element from the soil in the form of some soluble compound, and since there is a relatively limited amount of combined nitrogen in the world, it becomes of the last importance that the supplies which are continually withdrawn from the soil should be replaced by the nitrogen liberated in the decay of organic material. This nitrogen, after a series of putrefactive changes have occurred, appears as ammonia. The odor of this gas is often plainly perceptible

* "Zeitschrift. f. physiol. Chemie," VIII, p. 417.
† See Grubs and Francis, "Bull. of the Hyg. Laboratory," 1902, No. 7.
‡ "Trans. of the American Public Health Association," 1905.

about manure heaps. In this form nitrogen is poorly adapted for use by plants, and moreover may be easily dissipated. An extensive further process of oxidation is carried on by the nitrifying bacteria, whereby nitrates are ultimately formed. These are eminently adapted for use by plants, and so the soil is rendered continuously capable of supporting vegetation.

Nitrosomonas and Nitrosococcus convert ammonia into nitrous acid, and Nitrobacter oxidizes the latter to form nitric acid.

These genera are well nigh universal in the soil. They do not grow on the ordinary culture media, but require special solutions, free from the diffusive albumins—free, indeed, from organic compounds of any sort. Their supplies of carbon are obtained by the dissociation of carbon dioxid. It is highly noteworthy that they are thus able to flourish without food more complex than ammonia, a fact which is without parallel among organisms devoid of chlorophyl.

Reduction of Nitrates.—A considerable number of bacteria are able to reduce nitrogen compounds in the soil or in culture media, prepared for them, into ammonia. To the horticulturist this matter is of much interest. Winogradsky* has described specific nitrifying bacilli which he found in soil, and asserts that the presence of ordinary bacteria in the soil causes no formation of nitrites so long as the special bacilli are withheld.

Reduction of nitrates can be determined experimentally by the use of a *nitrate broth*, made by dissolving in 1000 cc. of water 1 gram of peptone and 0.2 gram of potassium nitrate. The ingredients are dissolved, filtered, then filled into tubes, and sterilized. The tubes are inoculated and the results noted. As nitrites and ammonia are, however, commonly present in the air and are taken up by fluids, it is always well to control the test by an uninoculated tube tested with the reagents in the same manner as the culture.

Two solutions are employed† for testing the culture:

I. Naphthylamin, 0.1 gram,
Distilled water, 20.0 grams,
} Boil, cool, filter, and add 156 cc. of dilute (1:16) hydric acetate.

II. Sulphanilic acid, 0.5 gram.
Hydric acetate, diluted, 150.0 cc.

Keep the solutions in glass-stoppered bottles and mix equal parts for use at the time of employment.

About 3 cc. of the culture and an equal quantity of the uninoculated culture fluid are placed in test-tubes and about 2 cc. of the test fluid slowly added to each. The development of a red color indicates the presence of nitrites, the intensity of the color being in proportion to the quantity of nitrites present. If a very slight pinkish or reddish color in the uninoculated culture fluid and a deeper red in the culture develop, it shows that a small amount of nitrites

* "Ann. de l'Inst. Pasteur," 1891; "La Semaine médicale," 1892.
† "Journal of the American Public Health Association," 1888, p. 92.

was already present, but that more have been produced by the growth of the bacteria.

The presence of ammonia in either fluid is easily determined by the immediate development of a yellow color or precipitate when a few drops of Nessler's solution* are added.

Failure to determine either ammonia or nitrites may not mean that the nitrates were not reduced, but that they were reduced to N. It is, therefore, necessary to test the solutions for nitrates, which is done by the use of phenolsulphonic acid and sodium hydroxid, which in the presence of nitrates give a yellow color.

Combination of Nitrogen.—Not only do bacteria destroy or reduce nitrogen compounds, but some of them are also able to assimilate nitrogen from the air and so combine it as to be useful for the nourishment of vegetable and animal life. The most interesting organisms of this kind are found upon the roots of the leguminous plants, peas, clover, etc., and have been studied by Beyerinck.† It seems to be by the entrance of these bacteria into their roots that the plants are able to assimilate nitrogen from the atmosphere and enrich sterile ground. Every agriculturist knows how sterile soil is improved by turning under one or two crops of clover with the plough.

Peptonization of Milk.—Numerous bacteria possess the power of digesting—peptonizing—the casein of milk. The process varies with different bacteria, some digesting the casein without any apparent change in the milk, some producing coagulation, some gelatinization of the fluid. In some cases the digestion of the casein is so complete as to transform the milk into a transparent watery fluid.

Milk invariably contains large numbers of bacteria, that enter it from the dust of the dairy, many of them possessing this power and ultimately spoiling the milk. In the process of peptonization the milk may become bitter, but need not change its original reaction.

The phenomena of coagulation and digestion of milk can be made practical use of to aid in the separation of similar species of bacteria. Thus, the colon bacillus coagulates milk, but the typhoid bacillus does not.

Production of Disease.—Micro-organisms that produce disease are known as *pathogenic;* those that do not, as *non-pathogenic*. Between the two groups there is no sharp line of separation, for true pathogens may be cultivated under such adverse conditions that their virulence may be entirely lost, while those ordinarily harmless may be made virulent by certain manipulations. In order to determine that a micro-organism is possessed of pathogenic powers, the committee of bacteriologists of the American Public

* Nessler's solution consists of potassium iodid, 5 grams, dissolved in hot water, 5 cc. Add mercuric chlorid, 2.5 grams, dissolved in 10 cc. of water, then to the mixture add potassium hydrate, 16 grams, dissolved in water, 40 cc. and dilute the whole to 1000 cc.

† "Centralbl. f. Bakt.," etc., Bd. VII, p. 338.

Health Association* recommends that: (1) When a given form grows only at or below 18° to 20°C., inoculation of about 1 per cent. of the body-weight with a liquid culture seven days old should be made into the dorsal lymph-sac of a frog. (2) When a species grows at 25°C. and upward, an inoculation should be made into the peritoneal cavity of the most susceptible (in general) of warm-blooded animals—*i.e.*, the mouse, either the white or the ordinary house mouse. The inoculation should consist of about 1 per cent. of the body-weight of the mouse of a four- to eight-hour standard bouillon culture, or a broth or water suspension of one platinum loop from solid cultures. When such intraperitoneal injection fails, it is unlikely that other methods of inoculation will be successful in causing the death of the mouse. If the inoculations of the frog and mouse both prove negative, the committee think it unnecessary to insist upon any further tests of pathogenesis as being requisite for work in species differentiation.

Production of Enzymes.—Some of these have already been mentioned as causing fermentation and putrefaction, coagulating milk, dissolving gelatin, etc. There are, however, others which have interesting and important actions upon both animal and vegetable substances.

Emmerich and Löw† observed that in old cultures of Bacillus pyocyaneus the bacteria become transformed into a gelatinous mass, and were led to experiment with old and degenerating cultures condensed to $\frac{1}{10}$ volume in a vacuum apparatus. The bacteriolytic powers were then found to be much increased, and they were subsequently able to precipitate from the concentrated culture an enzyme, which they called *pyocyanase*. The authors reached the rather hasty conclusions that the cessation of growth of bacteria in cultures depends upon the generation of enzymes; that the enzymes destroy the dead bacteria; that the enzymes will kill and dissolve living bacteria and destroy toxins, and, therefore, are useful for the treatment of infectious diseases, and that antitoxins are simply accumulated enzymes which the immunized animals have received during treatment, and which, appearing in the serum, produce the effects so well known.

It is probable that many of the toxic effects of bacteria and their cultures depend upon enzymic substances, the nature of which we do not yet understand.

* "Jour. Amer. Public Health Assoc.," Jan., 1898.
† "Zeitschrift für Hygiene," 1899.

CHAPTER III

INFECTION

INFECTION is the successful invasion of an organism by micro-parasites. Unfortunately custom has sanctioned the use of the word in other and sometimes confusing senses, thus, a table or knife upon which micro-organisms are known to be or are even supposed to be; the mouth and intestine, which naturally harbor bacteria of various forms, or a splinter penetrating the skin and carrying harmless bacteria into the deeper tissues, are all said by the surgeon to be "*infected*," when, in fact, it would be more correct to describe them as *infective*.

The term infection should imply an abnormal state resulting from the deleterious action of the parasite upon the host. The colon bacillus is a harmless commensal of the intestine of every human being, and of most of the lower animals. The intestine is not "infected," but *infested* with it, and it is only when abnormal or unnatural conditions arise that infection can take place. This form of association of certain bacteria with certain parts of the body to which they do no harm, but into which they may rapidly invade when appropriate conditions arise, is described by Adami as *sub-infection*. The possibility of infection is always there, though it is but rarely that conditions arise under which it can be accomplished.

There are two inseparable factors to be considered in all infections: the organism *infecting* and the organism *infected*. The first is the *parasite*, the second, the *host*. Infectivity and infectability may depend upon peculiarities of either parasite or host. Organisms that have lived together as commensals, that is, in a state of neutral relationship for an almost indefinite period, may suddenly cease their customary association, because of newly acquired power of invasion on the one hand, or diminished vital resistance on the other, and infection take place where it had previously been impossible.

Bacteria are commonly called *saprophytic* when they live in nature apart from other living organisms, and *parasitic* when they live in or upon them. Saprophytic bacteria when accidentally transplanted from their natural environment to the body of some animal, for example, may or may not be capable of continuing life under the new conditions. In the greater number of cases they die, but sometimes the new environment seems better than the old, and they multiply rapidly, invade the tissues in all directions, eliminate their met-

abolic products into the juices, and occasion varying morbid conditions.

The parasitic bacteria live in habitual association with higher organisms. Sometimes, and indeed most commonly, it is a harmless association, like that of certain cocci upon the skin, but occasionally it results in the destruction of the tissues and the death of the host, as in tuberculosis, leprosy, etc.

The group of pathogenic organisms has no well-defined limits, for it is frequently observed that micro-organisms well known under other conditions, and not known to have been engaged in pathogenic processes, turn up unexpectedly as the cause of some morbid condition. Indeed, although we are acquainted with a large number of organisms that have never been observed in connection with disease, we are scarcely justified in concluding that they are incapable of producing injury should proper conditions arise.

SOURCES OF INFECTION

The sources of infection may be *exogenous* or *endogenous;* that is, they may arise through the admission to the tissues of micro-organisms from sources entirely apart from the individual infected, or through the admission of some of those parasitic and usually harmless organisms constantly associated with him.

Exogenous infections arise through accidental contact with infective agents belonging to the external world.

A polluted *atmosphere* may carry into the respiratory passages micro-organisms capable of colonizing there. From the respiratory passages, minute drops of secretion may be coughed or sneezed into the atmosphere to be inhaled by neighboring persons and infect them. Such "drop infection" has been studied in reference to tuberculosis and diphtheria, and doubtless explains the transmission of whooping-cough, pneumonia, and other respiratory disturbances. Polluted *water* or *food* may carry into the intestine micro-organisms whose temporary residence may entirely change the functional and structural integrity of the parts, as in typhoid fever, cholera and dysentery.

Wounds inflicted by the teeth of animals, by weapons, by implements, or by objects of various kinds, carry into the tissues micro-organisms whose operations, local or general, may variously affect the organism to its detriment. Examples are to be found in rabies, tetanus, anthrax, malignant and gaseous œdema, suppuration, etc.

Fomites, or objects made infective through contact with individuals suffering from smallpox, scarlatina, and other contagious or actively infectious diseases, become the means through which the specific micro-organisms may be conveyed to the well with resulting infection.

Contact with *unclean objects* of various kinds—spoons, knives, cups,

blow-pipes, catheters, syringes, dental instruments, etc.—may serve to transfer disease-producing organisms from one person to another who might otherwise never come in contact with them.

Attention should be called to the facility with which the diseases of childhood may be spread through the thoughtless or ignorant custom of many adults and children of using handkerchiefs, napkins, forks, cups, spoons, etc., in common; in having wash-rags, towels, hair-brushes and combs in common; cultivating the habit of putting lead-pencils, etc., in the mouth, and then passing them on to others who will do the same, and to many other relations of every-day life by which infectious agents may be spread. Scarlatina, measles, mumps, acute anterior poliomyelitis, ophthalmia, tuberculosis, ringworm, fevers, syphilis, etc., may all be spread through such means.

Suctorial insects seem occasionally to act as the medium by which micro-organisms withdrawn in blood from one person may be introduced into other persons so that they become infected. The flea thus brings about the spread of plague; the mosquito, of malaria; the tsetse fly, of trypanosomiasis; the tick, of relapsing fever, the louse of typhus fever, etc.

Endogenous infections arise through the activity of micro-organisms habitual to the body. They indicate morbid conditions of the body by which the *defensive mechanisms are disturbed*, so that organisms harmless under normal conditions become invasive.

All normal animals are presumably born free of parasitic micro-organisms, but it is impossible for them to remain so because of the universal distribution of micro-organismal life. The air, the water, the soil, and the food, as well as the associates of the young animal, all act as means by which micro-organisms, and especially bacteria, are brought to the surface and cavities of its body, and but a short time elapses after birth before it harbors the customary commensal and parasitic forms.

BACTERIAL TENANTS OF THE NORMAL HUMAN BODY

The Skin and Adjacent Mucous Membranes.—The slightly moist warm surface of the skin is well adapted to bacterial life, and its unavoidable contact with surrounding objects determines that a variety of organisms shall adhere to it. Of these, we can differentiate between forms whose presence is unexpected and temporary; others whose presence may be expected; and still others whose presence is invariable.

Elaborate investigations upon the bacterial flora of the skin have been made by Unna;* Mittman,† who studied the finger-nails, under which he found no less than seventy-eight different species; Maggiora,‡

* "Monatshefte für prakt. Dermatol.," 1888, VII, p. 817; 1889, VIII, pp. 293, 562; 1889, IX, p. 49; 1890, X, p. 485; 1890, XI, p. 471; 1891, XII, p. 249.

† "Archiv. f. path. Anat. u. Phys. u. f. klin. Med.," 1888, CXIII, p. 203.

‡ "Giornale della R. Societá d'Igiena," 1889, Fasc. 5, p. 335.

who isolated twenty-nine forms from the skin of the foot; and Preindelsberger,* who found eighty species of bacteria on the hands. Undoubtedly many of these organisms were accidentally present, and were at least only semi-parasitic. Not a few were met but once and were in no sense bacteria of the skin. The skin may also be temporarily contaminated with bacteria from other portions of the patient's body, as, for instance, from his intestine; thus Winslow† has found the colon bacillus upon the hands of ten out of one hundred and eleven persons examined. Wigura‡ also examined the hands of forty persons in hospitals, finding tubercle bacilli in two out of ten persons from phthisical wards, colon bacilli six times and typhoid bacilli once on the hands of nine attendants in the typhoid wards. He found streptococci and staphylococci many times. Welch§ and Robb and Ghriskey‖ seem to have been the first to make a clear differentiation between the accidentally present bacteria and the permanently parasitic organisms of the skin, and to show that certain cocci, producing white and yellow colonies upon agar-agar, were invariable in occurrence and penetrated to the lowest epidermal layers.

These cocci, of which Welch describes the most common as Staphylococcus epidermidis albus, are universally and invariably present upon the human skin, and must be regarded as habitual parasites.

Where the skin is peculiar in its moisture and greasiness, however, additional forms are found. Thus, in preputial smegma, in the axillæ, and sometimes about the lips and nostrils, a bacillary organism, Bacillus smegmatis, is invariable, and the recent work by Schaudinn and Hoffmann** has shown that the skin of the genitalia harbors a spiral organism which they call Spirochæta refringens.

In the *external auditory meatus* a coccus, Micrococcus cereus flavus, is almost always to be found in the waxy secretion.

Upon the *conjunctiva* as many accidental organisms may be found as shall have been caught by its moist surface, though the researches of Hildebrand and Bernheim and others seem to show that the tears have some antiseptic power and prevent the organisms from growing, so that in health there are very few permanent residents of the sac, certain cocci seeming to be the only constant forms.

The **mouth** has been carefully studied bacteriologically by Miller,†† who found six organisms—Leptothrix innominata, Bacillus buccalis

* "Samml. medic. Schriften," herausg. von der "Wiener klin. Wochenschrift," 1891; XXII, Wien, "Rev. Jahresbericht über die Fortschritten in der Lehre von den pathogenen Mikroorganismen," 1891, VII, p. 619.

† "Jour. Med. Research," vol. X, p. 463.

‡ "Wratsch." 1895, No. 14.

§ "Transactions of the Congress of American Physicians and Surgeons," 1891, II, p. 1.

‖ "Bulletin of the Johns Hopkins Hospital," 1892, III, p. 37.

** "Deutsche med. Woch.," May 5, 1905.

†† "Micro-organisms of the Human Mouth," Phila., 1890.

maximus, Leptothrix buccalis maxima, Iodococcus vaginatus, Spirillum sputigenum and Spirochæta dentinum (denticola)—in every mouth. Practically the same conclusions were reached by Vincentini.* These organisms are peculiar in that they will not grow in artificial culture. In addition to this permanent flora, Miller cultivated fifty-two other species, some of which were harmless, some well-known pathogens.

From the mouth these organisms may be traced into the pharynx and esophagus.

In studying the micro-organisms of dental caries Goodby† found a large number of organisms which he divided into three groups: *A*. Those that produce acids, including Streptococcus brevis, Bacillus necrodentalis (Goodby), Sarcina alba, Sarcina lutea, Sarcina aurantiaca, Staphylococcus pyogenes aureus, and Staphylococcus pyogenes salivarius (Biondi). *B*. Those that liquefy blood-serum: Bacillus mesentericus rubra, B. mesentericus vulgatus, B. mesentericus fuscus, Bacillus fuscus, a yellow bacillus, probably B. gingivæ pyogenes (Miller), and Bacillus liquefacium motilis. *C*. Those that produce pigment, including the same organisms as group *B*. In carious dentine two organisms, Streptococcus brevis and Bacillus necrodentalis, were invariably present.

The extinction of the great number of bacteria entering the mouth is referred by most bacteriologists to a bactericidal action of the saliva.

The **stomach** seems to retain very few of the many bacteria that must enter it, its persistently acid contents being inimical to their development. Certain sarcina, especially Sarcina ventriculi, may be found without any considerable departure from the normal state. In carcinoma and other forms of pyloric obstruction with dilatation, the bacterial flora increases, and in achlorhydria micro-organisms of fermentation make their appearance. They are, however, accidental and not permanent tenants of the organ.

In carcinoma of the stomach a bacillus, probably one of the lactic acid groups, early makes its appearance and is of some diagnostic importance. It is called after its discoverer the Oppler-Boas bacillus,‡ also on account of angulations found in its threads, Bacillus geniculatus. It is a large bacillus, tending to form long threads easily seen without an oil-immersion lens. It is probably non-motile, does not form spores, stains by Gram's method, and is said by Emory§ to divide longitudinally as well as transversely. This, as he says, will, if proved to be correct, be a most important means of identifying the species. Cultures are easily made in media acidified with lactic acid.

The **intestine** receives such micro-organisms as have survived whatever destructive influences the gastric juices may have exerted, and

* "Bacteria of the Sputa and Cryptogamic Flora of the Mouth," London, 1897.
† Transactions of the Odontological Society, June, 1899.
‡ "Deutsche med. Wochenschrift," 1905, No. 5.
§ "Bacteriology and Hematology," p. 114.

its alkaline contents, rich in proteins and carbohydrates in solution, are eminently appropriate for bacterial life. The flora of the intestine is, therefore, increased in number and variety of organisms as we descend from its beginning to its end. In the small intestine there may be no bacteria in the upper part of the jejunum, but in most cases Bacillus lactis aërogenes and bacilli of the colon groups are found. These increase in number as the iliocecal valve is reached. The cecum shows large numbers of colon bacilli. The rectum contains, in addition, many putrefactive organisms, such as Bacillus putrificus, Bacillus proteus vulgaris, members of the Bacillus subtilis group, and acid-producing organisms, such as Bacillus acidophilus.

An interesting and thorough study of these organisms of the bowel and their distribution has been made by Kohlbrugge.* The total number of permanent residents is not known. During infancy the predominating organism seems to be Bacillus lactis aerogenes; during adult life, Bacillus coli. Streptococci, especially Streptococcus coli gracilis, are also very common, if not invariable, inhabitants of the intestine. The total bacteria that finally appear in the feces, according to the studies of Strasburger† and Steele,‡ may reach the enormous figure of 38 per cent. of the total bulk.

MacNeal, Latzer, and Kerr,§ in an elaborate work upon the "Fecal Bacteria of Healthy Men," found that they furnished 46.3 per cent. of the total fecal nitrogen.

Rettger‖ found the Bacillus enteritidis sporogenes regularly present in the human feces and believes it to be responsible for some of the putrefactive processes that occur there.

The **vagina,** on account of its acid secretions, harbors but few bacteria. In a study of the vaginal secretions of 40 pregnant women who had not been subjected to digital examinations, douches, or baths, Bergholm** found but few organisms of limited variety.

The **uterus** harbors no bacteria in health, and but few in disease. The intervening acidity of the vagina makes it difficult for bacteria from the surface to penetrate so deeply, and the tenacious alkaline mucus of the cervix is an additional barrier to their progress. Careful studies of the bacteriology of the uterine secretions have been made by Gottschalk and Immerwahr†† and Döderlein and Winterintz.‡‡

The **urethra** harbors a few cocci which enter the meatus from the surface and remain local in distribution.

The normal **bladder** is free from bacteria.

The **nose** constantly receives enormous numbers of bacteria in the

* "Centralbl. f. Bakt.," etc., 1901, Bd. XXX, pp. 10 and 70.
† "Zeitschrift für klin. Med.," 1902, XLIV, 5 and 6; 1903, XLVIII, 5 and 6.
‡ "Jour. Amer. Med. Assoc.," Aug. 24, 1907, p. 647.
§ "Journal of Infectious Diseases," 1909, VI, pp. 132, 571.
‖ "Jour. of Biological Chemistry," Aug., 1906, II, 1 and 2, p. 71.
** "Archiv f. Gynäk.," Bd. LXIV, Heft 3.
†† Ibid., 1896, Bd. L, Heft 3.
‡‡ "Beiträge für Geburtshülfe und Gynäkologie," Bd. III, Heft 2.

dust of the inspired atmosphere. These organisms are too numerous and too various to enumerate, and might, indeed, comprehend the entire bacterial flora. But in spite of the large numbers of organisms received, the nose retains scarcely any, its mucous membranes seeming to be provided with means of disposing of the organisms. Among those best able to withstand the destructive influences, and, therefore, most apt to be found in the deeper passages, are the pseudo-diphtheria bacillus, streptococci, pneumococci, staphylococci, Bacillus pneumoniæ (Friedländer), Bacillus subtilis and sarcina. A complete review of the subject with references to the literature has been made by Hasslauer.*

The **larynx** and **trachea** contain very few bacteria and probably have no permanent parasitic flora.

The **lungs** harbor no bacteria. A few micro-organisms doubtless reach them in the inspired air, but the defensive mechanisms soon dispose of them.

AVENUES OF INFECTION

The **skin** seems to form an effectual barrier against the entrance of bacteria into the deeper tissues. A few higher fungi—Trycophyton, Microsporon, Achorion, etc.—seem able to establish themselves in the superficial layers of the cells, invade the hair-follicles, and so reach the deeper layers, where morbid changes are produced. The minute size of the bacteria makes it possible for them to enter through lesions too small to be noticed. Garré applied a pure culture of Staphylococcus pyogenes aureus to the skin of his forearm, and found that furuncles developed in four days, though the skin was supposed to be uninjured. Bockhart moistened his skin with a suspension of the same organism, gently scratched it with his finger-nail, and suffered from a furuncle some days later.

The greater number of surgical infections result from the entrance of bacteria through lesions of the skin. It makes but little difference to what depth the lesion extends—abrasions, punctures, lacerations, incisions—the protective covering is gone and the infecting organisms find themselves in the tissues, surrounded by the tissue lymph, under conditions appropriate for growth and multiplication, provided no inhibiting or destructive mechanism be called into action.

The **digestive apparatus** is the portal through which many infections take place. The Bacillus diphtheriæ, finding its way to the pharynx, speedily establishes itself upon the surface, producing pseudomembranous inflammation there. Typhoid bacilli, dysentery amœba and bacilli, cholera spirilla and related organisms, finding their way to the intestine, where the vital conditions are appropriate, take up temporary residence there, to the injury of the host, who may suffer from the respective infections.

* "Centralbl. f. Bakt. u. Parasitenk. I. Abt. Referata," Bd. XXXVII, Nos. 1–3, p. 1, and Nos. 4–6, p. 97.

Various organisms pass from the pharynx to the tonsils and so to the lymph-nodes and deeper tissues of the neck, where their first operations may be observed.

It is supposed by some pathologists that the digestive tract is a constant menace to health in that it regularly admits bacteria, through the lacteals, and perhaps through its capillaries, to the blood, where under slightly abnormal conditions they might do harm. According to Adami,* the intestine is responsible for a condition of sub-infection depending upon the constant entrance of colon bacilli into the blood. He finds the colon bacillus in the blood, and traces it to the liver, where its final dissolution takes place in the fine dumbbell-like granules enclosed in the cells. Nicholls† confirms Adami by finding similar dumbbell or diplococcoid bodies in the epithelial denuded tissues of the mesentery of normal animals.

Nicholas and Descos‡ and Ravenel§ fed fasting dogs upon a soup containing quantities of tubercle bacilli, killed them three hours later, and examined the contents of the thoracic duct, where tubercle bacilli, some alive and some dead, were found in large numbers. van Steenberghe and Grysez‖ found that carbon particles readily passed through the intestinal mucosa, entered the lymphatics, were thrown into the venous circulation, and so carried to the lung, where anthracosis was produced.

In a subsequent paper** they believe that they have demonstrated that the tubercle bacillus like the carbon particles may also pass through the normal intestinal wall, and follow the same course to the lungs. They believe that pulmonary tuberculosis thus depends upon ingested and not inhaled micro-organisms. Montgomery†† repeated the work of van Steenberghe and Grysez at the Henry Phipps Institute, Philadelphia, but though many attempts were made by various methods, no carbon particles seemed to be transported from the alimentary to the pulmonary tissues.

But there are enough experiments recorded to make it probable that the wall of the intestine is permeable to bacteria, and that in small numbers they constantly enter the blood of healthy animals, to be disposed of by mechanisms yet to be described.

Many of the bacteria penetrating the intestine must be retained in the lymph nodes; others, as in the experiment with the tubercle bacilli, meet destruction before they reach the blood; the remainder must reach the blood alive.

The presence of colon bacilli in the greater number of the organs

* "Jour. of the American Medical Association," Dec. 16 and 23, 1899, vol. XXXIII, Nov. 25 and 26.

† "Jour. Med. Research," vol. XI, No. 2.

‡ "Jour. de Phys. et Path. gén.," 1902, IV, 910–912.

§ "Jour. Med. Research," 1904, X, p. 460.

‖ "Ann. de l'Inst. Pasteur," Dec. 25, 1905, Tome XIX, No. 12, p. 787.

** Ibid., 1910, XXIV, 316.

†† "Jour. of Med. Research," Aug., 1910, vol. XXIII, No. 1.

shortly after death has led some pathologists to assume that they readily pass through the intestinal walls during the death agony, but although experiments have been made to prove and to disprove it, the matter is still controversial. Undoubtedly in the final dissolution some change takes place in the constitution of the individual by which general invasion by bacteria is made more easy than under normal conditions.

The **respiratory apparatus** affords admission to a few micro-organisms whose activities seem more easily carried on there than elsewhere. Although it is still controversial whether the inhalation of tubercle bacilli is as frequent a mode of conveying that organism into the body as was once supposed, it cannot be denied that its inhalation will account for the far greater frequency with which tuberculosis affects the lungs than other organs of the body.

Pneumonia, caused in an immense majority of cases by the pneumococcus of Fraenkel and Weichselbaum, probably results from the entrance of the organism into the respiratory tissues directly.

The entrance of the unknown infectious agents causing measles, German measles, smallpox, and scarlatina can best be accounted for by supposing that they are inhaled into the lungs and thus enter the blood.

The **genital apparatus** is the portal of entry of micro-organisms whose early or chief operations are local. Among these are the gonococcus, which causes urethritis, vaginitis, balanitis, posthitis, endometritis, orchitis, salpingitis, vesiculitis, cystitis, oöphoritis, sometimes peritonitis, and rarely endocarditis; the bacillus of Ducrey, that causes the chancroid or soft sore; and the treponema of syphilis. In more rare cases other organisms, such as the common cocci of suppuration and the tubercle bacillus, may also be transmitted from individual to individual by sexual contact.

The **placenta** usually forms a barrier through which infectious agents find their way with difficulty. A study of this subject by Neëlow* shows that the non-pathogenic organisms do not pass from the mother through the placenta to the fetus. Some pathogenic micro-organisms, however, readily pass through, and a few diseases, such as syphilis, are well known in the congenital form. Pregnant women suffering from smallpox may be delivered of infants with marks indicative of prenatal disease. Some common infectious agents, such as the tubercle bacillus, seem to infect unborn animals with difficulty. The frequency of antenatal tuberculous infection is, however, somewhat controversial at present, Baumgarten having reached the opinion, exactly the opposite of what is commonly believed, that many children are subject to antenatal infection, though the bacilli subsequently develop and cause disease in only a few of them.

* "Centralbl. f. Bakt.," etc., Aug., 1902, I. Abt., Bd. xxxi, Orig., p. 691.

PATHOGENESIS

This subject can be understood only through a broad knowledge of the metabolic products of micro-organisms. In general it may be said that the ability of micro-organisms to do harm depends upon the injurious nature of their products. This alone, however, will not explain the phenomena of infection, for in many cases the intoxication is subsidiary in importance to the invasive power of the micro-organisms. Some bacteria having but limited toxic powers possess extraordinary powers of invasion, as Bacillus anthracis, and the intoxication becomes important only after the organisms have penetrated to all the tissues of the body. Others, with more active toxic properties, have but limited invasive powers, and a few organisms, growing with difficulty in some insignificant focus, excite actively destructive reactions in the tissues with which they come in contact. Still others, with limited invasive powers, eliminate active toxic substances, soluble in nature, that enter the circulation and act upon cells remote from the bacteria themselves, as in diphtheria and tetanus.

The *invasive power* of the organisms depends upon their ability to overcome the body defenses. This may indicate activity of the infecting organism, or weakness of the defensive mechanism. The relation of these factors is exceedingly complex, only partly understood, and will be fully discussed in the chapter upon Immunity.

For convenience toxins may be described as *intracellular* or insoluble, and *extracellular* or soluble.

The **intracellular toxins.** Until the investigations of Vaughan, Cooley and Gelston,* and later Vaughan and his associates, Detweiler,† Wheeler,‡ Leach,§ Marshall and Gelston,‖ Gelston,** J. V. Vaughan,†† Wheeler,‡‡ Leach,§§ McIntyre,‖‖ and others, it seemed remarkable that micro-organisms whose filtered cultures contained little demonstrable toxic substance are sometimes able to produce active pathogenic effects. By means of special apparatus in which the micro-organisms could be cultivated in enormous quantities, and the disintegration of the micro-organismal masses secured by subjecting them to high temperatures, to the action of mineral acids or autolysis, it was discovered that the colon bacilli, typhoid bacilli, and many supposedly harmless bacteria contain intensely active toxic substances. In all probability some of the toxic substances produced by such means are artefacts, but enough work has been done to prove that insoluble toxic substances are present in such organisms, and the toxic substances obtained by the com-

* "Journal of the American Medical Association," Feb. 23, 1901; "Trans. Assoc. Amer. Phys.," 1901; "American Medicine," May, 1901.
† "Trans Asso. Amer. Phys.," 1902. ‡ Ibid.
§ Ibid. ‖ Ibid. ** Ibid.
†† Ibid.
‡‡ "Jour. Amer. Med. Assoc.," 1904, XLII, p. 1000.
§§ Ibid., p. 1003. ‖‖ Ibid., p. 1073.

minution of culture masses made solid and brittle by exposure to liquid air, as suggested by Macfadyen and Rowland; the autolytic digestion of bacteria washed free of their culture fluids and suspended in physiological salt solution, and the dissolution of bacteria by bacteriolytic animal juices clearly prove that endotoxins exist.

It seems probable that there is considerable difference in the readiness with which these intracellular toxic substances are given up by the bacteria. From some they seem never to be set free in the bodies of animals into which the bacteria are injected; thus, Bacillus prodigiosus is usually harmless for animals, no matter what quantity is injected, yet active toxic substances can be extracted from the bodies of these organisms by appropriate chemical means. From others they are given off in small quantities either during the life of the organism or at the moment of death and dissolution, as in the case of the typhoid bacillus and streptococci, whose filtered cultures are almost harmless, though both organisms are pathogenic.

The intracellular toxins are limited in action by the distribution of the bacteria producing them. When these organisms are but slightly invasive, more or less local reaction is produced; when they are actively invasive, general reactions of varying intensity result.

The **extracellular toxins,** of which those of Bacillus tetani and Bacillus diphtheriæ can be taken as types, have been known since the early work of Brieger and Fränkel and Roux and Yersin. They seem to be excretions of the bacteria, not retained in the cells, but eliminated from them as rapidly as they are formed. Thus, in appropriate bouillon cultures of the diphtheria bacillus, the toxin is present in large quantity and is highly virulent, but if the fluid be removed from the bacteria by porcelain filtration and the remaining bacilli carefully washed, their bodies are found to be devoid of toxic powers. The poison is most concentrated where its diffusion is most restricted, thus, agar-agar cultures of the tetanus bacillus are much more toxic than bouillon cultures because the soluble principle readily diffuses through the fluid, but is held by the agar-agar.

The soluble toxin is but one of numerous metabolic products of the bacteria. Thus in culture filtrates of the tetanus bacillus there are at least two very different active substances, the *tetano-spasmin* that acts upon the nervous system with convulsive effect, and the *tetano-lysin* that is solvent for erythrocytes.

In all probability all of the culture filtrates of bacteria are highly complex because of the addition of the various metabolic products —toxins, lysins, enzymes, pigments, acids, etc.—of the bacteria, as well as because of changes produced in the medium by the abstraction of those molecular constituents upon which the bacteria have fed. This complexity makes it difficult to accurately study the toxins, which we scarcely know apart from their associated products.

The chemic nature of the toxins differs. Undoubtedly some are tox-albumins, but others are of different composition and fail to give the reactions belonging to the compounds of this group.

The variations observed in toxicogenesis under experimental conditions in the test-tube indicate that similar variations occur in the bodies of animals, and a few experiments conducted with slight variations in the composition and reaction of the media in which the bacteria grow will suffice to show that the exact effect of toxicogenic bacteria in the bodies of different animals cannot always be accurately prejudged.

The physiologic and pathogenic action of the extracellular soluble toxins differs from that of the intracellular and difficultly soluble toxins in that it is more easily diffused throughout the animal juices, and that its diffusion is independent of the invasiveness of the bacteria, so that a few organisms growing at some focus of unimportant magnitude, and causing but little local manifestation, may be able to produce a profound impression upon remote organs. This is best exemplified in the case of the Bacillus tetani, which, finding its way into the tissues under proper conditions, produces scarcely any local reaction—indeed, the lesion may be undiscoverable—yet may cause the death of the animal through the intensity of its action upon the central nervous system.

SPECIFIC ACTION OF TOXINS

The metabolic products of the greater number of injurious bacteria are characterized by irritative action upon those body cells with which they come into contact. If through the intracellular nature of the poisons and the mildly invasive character of the micro-organisms this action is restricted to the seat of original infection, a local manifestation will result. Its exact nature will, however, be modified to some extent by other qualities of the bacterial products. Thus, when in addition to their irritative action which, when mild, occasions multiplication of the cells of the connective and lymphoid tissues, and, when extreme, effects the death of the cells, the products are strongly chemotactic, suppuration will occur.

Fever and suppuration are, therefore, non-specific actions, because numerous micro-organisms share in common the qualities productive of these conditions.

If the bacteria are rapidly invasive, but still have injurious products of the intracellular variety, they are apt to share certain qualities, such as the swelling of the lymph-nodes, etc., in common, so that such lesions cannot be considered as specific. So soon as any one of the products is discovered to give some single lesion peculiar to that organism by which it is produced, or so soon as the total effect of the activity of the various products of any micro-organism produces a typical effect, differing from the total effect

of the operation of other micro-organisms, and a recognized type of disease results, it becomes possible to say that the micro-organism in question is specific.

The most striking examples of the specific action of bacterio-toxins is, however, seen in those cases where soluble extracellular metabolic products of bacterial energy are liberated into the body juices so as to be conveyed by the circulatory system to all parts of the body. Those cells most susceptible to its action are then first or most profoundly impressed by it, and definite responses brought about. Thus, the soluble toxin of tetanus causes no visible reaction in the cells with which it first comes into contact at the seat of primary infection, because these cells are either less susceptible to its influence, or are less well able to show its effects, than the cells of the nervous system to which it is secondarily carried by the blood.

SPECIFIC AFFINITY OF THE CELLS FOR THE TOXINS

The cells of the connective tissue in which the tetanus bacillus is living show little reaction, but the motor cells of the central nervous system, having a greater affinity for it, are profoundly impressed, so that convulsions of the controlled muscular system are brought about. This special excitation of the nerve cells is specific because no other bacterio-toxin is known to produce it and it is attributed to special selective affinities of the nerve cells for the poison. This affinity has its analogue among the poisons of higher plants, thus, strychnin has a similar selective affinity and is also said to be specific in action upon the motor cells.

The venoms of various serpents, especially the cobra, also have specific reactions, the cells of the respiratory centers seeming to be most profoundly affected by them.

The diphtheria bacillus, when observed in ordinary throat infections, is seen to produce a pseudomembranous angina which results in part from an irritative local action of the organism, which it shares in common with many others, and in part from some coagulating product which it shares in common with a few—pneumococcus, streptococcus, etc. Neither of these reactions is specific, but subsequent to these early manifestations comes depressant action on the nervous cells with palsy, peculiar to the products of the diphtheria bacillus, and therefore specific.

It is upon the peculiar specific reactions of the bacterio-toxins and the peculiar susceptibility of certain cells to this action that the production of distinct clinical manifestations depend.

THE INVASION OF THE BODY BY MICRO-ORGANISMS

Some bacteria whose invasiveness is insufficient to enable them successfully to maintain life in healthy tissues, occasionally get a

foothold in diseased tissues and assist in morbid changes. This is seen in what is described as *sapremia*, in which various saprophytic bacteria, possessing no invasive powers, by growing in the putrefying tissues of a gangrenous part, give rise to poisonous substances which when absorbed by the adjacent healthy tissues produce such constitutional disturbances as depression, fever, and the like.

Bacteria with limited invasive powers and intracellular toxins can at best occasion local effects. Such organisms not infrequently vary, however, and when of unusual vitality may survive entrance into the blood and lymph circulations and occasion *bacteremia*, or, as it is more frequently called, *septicemia*, a morbid condition characterized by the presence of bacteria in the circulating blood. When bacteria entering the circulation are unable to pervade the entire organisms, they may collect in the capillaries of the less resisting tissues, producing local metastatic lesions, usually purulent in character. This results in what is surgically known as *pyemia*.

The mode by which the entrance of bacteria into the circulation is effected differs in different cases. Kruse* believes that they sometimes are passively forced through the stomata of the vessels when the pressure of the inflammatory exudate is greater than that of the blood within them; that they may sometimes enter into the bodies of leukocytes that have incorporated them; that they may actually grow through the capillary walls, or that they reach the blood circulation indirectly by first following the course of the lymphatics.

Toxemia results from the absorption of the poisonous bacterial products from non-invasive bacteria, as in tetanus.

THE CARDINAL CONDITIONS OF INFECTION

Infection can take place only when the micro-organisms are sufficiently virulent, when they enter in sufficient number, when they enter by appropriate avenues, and when the host is susceptible to their action.

Virulence.—Virulence may be defined as the disease-producing power of micro-organisms. It is a variable quality, and depends upon the invasiveness of the micro-organisms, or the toxicity of their products, or both.

A few bacteria are almost constant in virulence and can be kept under artificial conditions for years with very little change. Other bacteria begin to diminish in virulence so soon as they are introduced to the artificial conditions of life in the test-tube. Still others, and perhaps the greater number, can be modified, and their virulence increased or diminished according to the experimental manipulations to which they are subjected.

Variation in virulence is not always a peculiarity of the species,

* Flügge, "Die Mikroorganismen," vol. 1, p. 271.

for the greatest differences may be observed among individuals of the same kind. Thus, the streptococcus usually attenuates rapidly when kept in artificial media, so that special precautions have to be taken to maintain it, but Holst observed a culture whose virulence was unaltered after eight years of continuous cultivation in the laboratory without any particular attention having been devoted to it. What is true of different cultures of the same organisms, is equally true of the individuals in the same culture. To determine such individual differences is quite easy among chromogenic bacteria. If these are plated in the ordinary way it will be found that some colonies are paler and some darker than others. Conn found that by repeating the plating a number of times and always selecting the palest and darkest colonies he was eventually able to produce two cultures, one brilliant yellow, the other colorless, from the same original stock of yellow cocci from milk.

Decrease of virulence under artificial conditions probably depends upon artificial selection of the organisms in transplantation from culture to culture. When planted upon artificial media, the vegetative members of the bacterial family proceed to grow actively and soon exceed in number their more pathogenic fellows. Each time the culture is transplanted, more of the vegetative and fewer of the pathogenic forms are carried over, until after the organism is accustomed to its new environment, and grows readily upon the artificial media, it is found that the pathogenic organisms have been largely or entirely eliminated and the vegetative forms alone retained.

Increase ot virulence can be achieved by artificial selection so planned as to preserve the more virulent or pathogenic organisms at the same time that the less virulent and more vegetative organisms are eliminated. In cases in which no virulence remains, the experimental manipulation of the culture is directed toward gradual immunization of the micro-organisms to the defensive mechanisms of the body of the animal for which the organism is to be made virulent. A number of methods are made use of for this purpose.

Passage Through Animals.—Except in cases where the virulence of the micro-orgainsm is invariable, it is usually observed that the transplantation of the organism from animal to animal without intermediate culture *in vitro* greatly augments its pathogenic power. Of course, this artificially selects those members of the bacterial family best qualified for development in the animal body, eliminating the others, and the virulence correspondingly increases.

The increase in virulence thus brought about is, however, not so much an increase in the general pathogenic power of the organism for all animals, as toward the particular animal or kind of animal used in the experiments. Thus, in general, the passage of bacteria through mice increases their virulence for mice, but not necessarily

for cats or horses; passage through rabbits, the virulence for rabbits, but not necessarily for dogs or pigeons, etc.

This specific character of the virulence can be explained by the "lateral-chain theory of immunity," where it will again be considered.

The Use of Collodion Sacs.—When cultures of bacteria are enclosed in collodion sacs and placed in the abdominal or other body cavities of animals, and kept in this manner through successive generations, the virulence is usually considerably increased. This is one of the favorite methods used by the French investigators. It keeps the bacteria in constant contact with the slightly modified body juices of the animal, which transfuse through the collodion, and thus impedes the development of such organisms as are not able to endure their injurious influences. Thus it becomes only another way of carrying on an artificial selection of those members of the bacterial family that can endure, and eliminating those that cannot endure the defensive agencies of those juices with which the organisms come in contact.*

The addition of animal fluids to the culture-media sometimes enables the investigator to increase, and usually enables him to maintain, the virulence of bacteria. A series of generations in gradually increasing concentrations of the body fluid should be employed, until the organism becomes thoroughly accustomed to it.

In some cases it may be sufficient to use a single standard mixture, thus: Shaw† found that he could exalt the virulence of anthrax bacilli by cultivating them upon blood-serum agar for fourteen generations, after which they were three times as active as cultures similarly transferred upon ordinary agar-agar.

The increase of virulence under such conditions probably depends upon the immunization of the bacteria to the body juices of the animals, and this whole matter will be understood after the subject "Immunity" has been considered.

Number.—The number of bacteria entering the infected animal has a very important bearing upon infection.

The entrance of a single micro-organism of any kind is scarcely ever able to cause infection because of the uncertainty of its being able to withstand the changed conditions to which it is subjected. In most cases a considerable number of organisms is necessary in order that some may survive. Park points out that when bacteria are transplanted from culture to culture, under conditions supposed to be favorable, many of them die. It seems not improbable, therefore, that when they are transplanted to an environment in which are present certain mechanisms for defending the organism against them, many more must inevitably die. The more virulent an organism is, the fewer will be the number required to infect. Marmorek,

* Directions for making and using the capsules are given in the chapter upon Animal Experimentation.

† "Brit. Med. Jour.," May 9, 1903.

in his experiments with antistreptococcic serum, used a streptoccocus whose virulence was exalted by passage through rabbits and intermediate cultivation upon agar-agar containing ascitic fluid, until one hundred thousand millionth of a cubic centimeter (*un cent milliardieme*) was fatal for a rabbit. In this quantity it is scarcely probable that more than a single coccus could have been present. Single anthrax or glanders bacilli may infect rabbits and guinea-pigs. Roger found that 820 tubercle bacilli from the culture with which he experimented were required to infect a guinea-pig, when introduced beneath the skin. Herman found that it required 4 or 5 cc. of a culture of Staphylococcus pyogenes to produce suppuration in the peritoneal cavity of an animal; 0.75 cc. to produce it beneath the skin; 0.25 cc. in the pleura; 0.05 cc. in the veins and 0.0001 cc. in the anterior chamber of the eye.

In experimenting with Bacillus proteus vulgaris, Watson Cheyne found that 5,000,000 to 6,000,000 organisms injected beneath the skin did not produce any lesion; 8,000,000 caused the formation of an abscess; 56,000,000 produced a phlegmon from which the animal died in five or six weeks and 225,000,000 were required to cause the death of the animal in twenty-four hours. In studying Staphylococcus aureus upon rabbits he found that 25,000,000 would cause an abscess, but 1,000,000,000 were necessary to cause death.

The Avenue of Infection.—The successful invasion of the body by certain bacteria can be achieved only when they enter it through appropriate avenues. Even when invasion is possible through several channels, the parasite most commonly invades through one that may, therefore, be regarded as most appropriate, and furnishes the typical picture of the infection.

Thus, gonococci usually reach the body through the urogenital mucous membranes, where they set up the various inflammatory reactions collectively known as gonorrhea—*i.e.*, urethritis, vaginitis, prostatitis, orchitis, cystitis, etc. These constitute the typical picture of the infection. The organism may also successfully invade the conjunctiva, producing blennorrhea, but there is no evidence that gonococci can successfully invade the body through the skin, the respiratory, or alimentary mucous membrane.

Typhoid and cholera infections seem to take place through the alimentary mucous membrane, and the evidence that infection takes place by inhalation is slight. It is not known to take place through the urogenital system, the conjunctiva, or the skin.

The avenue of entrance not only determines infection, but may also determine the form that it takes. Thus, tubercle bacilli rubbed into the deeper layer of the skin produce a chronic inflammatory disease, called *lupus*, that lasts for years and rarely results in generalized tuberculosis. Bacilli reaching the cervical or other lymph-nodes by entrance through the tonsils, may remain localized,

producing enlargement and softening of the nodes, or passing through them reach the circulation, in which they may be carried to the bones and joints and occasion chronic inflammation with necrosis and ultimate evacuation or exfoliation of the diseased mass, after which the patient may recover. Bacilli entering the intestine in many cases produce implantation lesions in the intestinal walls; bacilli inhaled into the lung, or conveyed to it from the intestine by the thoracic duct and veins, produce the ordinary pulmonary tuberculosis known as phthisis or consumption.

Inhaled pneumococci colonizing in the pharynx have been known to produce pseudomembranous angina; in the lungs, pneumonia; implanted upon the conjunctiva, conjunctivitis. In these cases we can look upon the type of infection as depending upon the portal through which the invading organism found its way into the tissues.

The avenue of entrance is, for obvious reasons, less important when the micro-organism is of some rapidly invasive form, whose chief operation is in the streaming blood or in the lymphatics. Anthrax in most animals is characterized by a bacteremia regardless of the point of primary infection. Bubonic plague rapidly becomes a bacteremia regardless of the entrance of the Bacillus pestis by inhalation into the lungs, or by way of the lymphatics through superficial lesions. The failure of the micro-organisms to colonize successfully when introduced through inappropriate avenues may be explained by a consideration of the local conditions to which they are subjected.

When they are introduced beneath the skin, bacteria are, in most cases, delayed in reaching the circulation, and are in the meantime subjected to the germicidal action of the lymph and exposed to the attacks of phagocytes. Many succumb to these and never penetrate more deeply into the body. Should any survive, they may be transported to the lymph-nodes and there destroyed, or, passing through these barriers without destruction, and reaching the venous channels, they have next to pass through the pulmonary capillaries, where they are apt to be caught and destroyed. Finally, should any escape all these defenses and reach the general circulation, it is to find the endothelium of the capillaries prone to collect and detain them until destruction is finally effected. The systemic circulation is also defended against such micro-organisms as might reach the veins through lesions or accidents of the abdominal viscera, by the interposition of the portal capillary network of the liver, where the bacteria are caught and many of them destroyed, or passing which, the pulmonary capillary system acts as a second barrier against them. The deeper the penetration, the more active the defense becomes, the blood itself furnishing agglutinins, bacterio-lysins, and phagocytes for the destruction of the micro-organisms and the protection of the host.

These defenses, however, are of no avail against actively invasive

organisms provided with the means of overcoming them all through *aggressins* that destroy the germicidal humors or *toxins* that kill or paralyze the cells. When these are injected directly into the streaming blood they produce their effects more rapidly than when injected beneath the skin or elsewhere, because the field of operation is immediately reached instead of through a roundabout course in which so many defenses have to be overcome. Taking anthrax bacilli, whose invasiveness has already been dwelt upon, as an example, Roger* found that when the orginisms were injected into the aorta, animals died more quickly than when they were injected into the veins and obliged to find their way through the pulmonary capillaries to the general circulation. If the injections were made into the portal vein, the animals stood a good chance of recovery, the liver possessing the power of destroying sixty-four times as many anthrax bacilli as would prove fatal if introduced through other channels.

The conditions differ, however, in different infections, for when Roger experimented with streptococci instead of anthrax bacilli, he found that if the bacilli were inoculated into the portal vein the animals died more quickly than when they were injected into the aorta, and that when the bacilli were injected into the peripheral veins the animals lived longest, the liver seeming to be far less destructive to streptococci than the lungs.

The Susceptibility of the Host.—Susceptibility is liability to infection. It is a condition in which the host is unable to defend itself against invading micro-organisms. Unusual or unnatural susceptibility is also spoken of as *predisposition* or *dyscrasia*.

Many animals and plants are naturally without any means of overcoming the invasiveness of certain parasitic micro-organisms, and are, therefore, naturally susceptible; others naturally resist their inroads, but through various temporary or permanent physiologic changes may lose the defensive power.

In general, it is true that any condition that depresses or diminishes the general physiological activity of an animal diminishes its ability to defend itself against the pathogenic action of bacteria, and so predisposes to infection. These changes are often so subtile that they escape detection, though at times they can be partly understood.

The inhalation of noxious vapors. It has long been supposed that sewer gas was responsible for the occurrence of certain infectious diseases, and when the nature of these diseases was made clear by a knowledge of their bacterial causes, the old belief still remained and many sanitarians continued to believe that defective sewage is in some way connected with their occurrence. It is difficult to prove or disprove the matter experimentally. Men who work in sewers and plumbers who breathe much sewer gas are not apparently affected by it. Alessi† found that rats, rabbits, and

* "Introduction to the Study of Medicine," p. 151.

† "Centralbl. f. Bakt.," etc., 1894, XV, p. 228.

guinea-pigs kept in cages some of which were placed over the opening of a privy, while in others the excreta of the animals were allowed to accumulate, suffered from a pronounced diminution of the resisting powers. This would seem to be inconsistent with the habits of rats, many of which live in sewers. Abbott* caused rabbits to breathe air forced through sewage and putrid meat infusions for one hundred and twenty-nine days, and found that the products of decomposition inhaled by the animals played no part in producing disease, or in inducing susceptibility to it.

Fatigue is a well-recognized clinical cause of susceptibility to disease, and experimental evidence of its correctness is not wanting. Charrin and Roger† found that white rats, which naturally resist infection with anthrax, succumbed to the infection if compelled to turn a revolving wheel until exhausted before inoculation.

Exposure to cold seriously diminishes the resisting power of the warm-blooded animals. It is an everyday experience that chilling the body predisposes to "cold" and may be the starting-point of pneumonia. Pasteur found that fowls, which resist anthrax under normal conditions, succumbed to infection if kept, for some time, in a cold bath before inoculation.

The reverse seems to be true of the cold-blooded animals, for Gibier‡ found that frogs, naturally resistant to the anthrax bacillus, would succumb to infection if kept at 37°C. after inoculation.

Diet produces some variation in the resisting powers. The tendency of scorbutics to suffer from infectious disorders of the mouth, the frequency with which epidemics of infectious disease follow famines, and the enterocolitis of marasmatic infants, illustrate the effects of insufficient food in predisposing to disease. We also find that the infectious diseases of carnivorous animals are not the same as those of herbivorous animals, and that the former are exempt from many disorders to which the latter quickly succumb. Hankin was able to show experimentally that meat-fed rats resisted anthrax infection far better than rats fed upon bread.

Intoxication of all kinds predisposes to infection. Platania§ found that such animals as frogs, pigeons, and dogs became susceptible to anthrax when under the influence of curare, chloral, and alcohol. Leo‖ found that white rats fed upon phloridzin became susceptible to anthrax. Wagner** found that pigeons become susceptible to anthrax when under the influence of chloral. Abbott†† found the resisting powers of rabbits against Streptococcus pyogenes and Bacillus coli diminished by daily intoxication with 5 to 15 c.c.

* "Trans. Assoc. Amer. Phys.," 1895.
† "Compte rendu Soc. de Biol de Paris," Jan. 24, 1890.
‡ "Compte rendu Acad. des Sciences de Paris," 1882, t. XCIX, p. 1605.
§ See Sternberg's "Immunity and Serum Therapy," p. 10; "Centralbl. f. Bakt.," etc., Bd. VII, p. 405.
‖ "Zeitschrift für Hyg.," 1889, Bd. VII, p. 505.
** "Wratsch," 1890, 39, 40.
†† "Jour. of Exp. Med.," 1896, vol. I, No. 3.

of alcohol introduced into the stomach through a tube. Salant* found that alcohol was disadvantageous in combating the infectious diseases because it diminished the glycogen content of the liver which Colla† had found an important adjunct in supporting the resisting power.

It is a common clinical observation that excessive indulgence in alcohol predisposes to certain infections, notably pneumonia, and every surgeon knows the danger of pneumonia after anesthetization with ether.

Traumatic injury and mutilation of the body are not without effect upon infection. The more extensive the damage done to the tissues, the greater the danger of infection, and the more serious the consequences of infection when it takes place.

The mutilation of the body by the removal of certain organs is of disputed importance. There is much literature upon the effect of the spleen in overcoming infectious agents, but the experimental evidence seems about equally divided as to whether an animal is more or less susceptible after the removal of this organ than it was before.

Morbid conditions in general predispose to infection. The frequency with which diabetics suffer from furuncles, carbuncles, and local gangrenous lesions of the skin; the increased susceptibility of phthisics to bronchopneumonia of other than tuberculous origin; the apparent predisposition of injured joints and pneumonic lungs to tuberculosis; the extensive streptococcus invasions accompanying scarlatina and variola; the presence of Bacillus icteroides and various other organisms in the blood and tissues of yellow fever patients, and the presence of Bacillus suipestifer in the bodies of hogs suffering with hog cholera, all show the diminution in the general resisting power of an individual already diseased.

MIXED INFECTIONS

The general prevalence of bacteria determines that few can enter and infect the body of a host without the association of other kinds. Therefore their operation in the body is subject to modifications produced in them or in the host by these associated organisms.

In experimental investigations this fact is not infrequently forgotten, and it is often remarked with surprise that the results of inoculation with pure cultures of a micro-organism may be clinically different from those observed under natural conditions.

The tetanus bacillus, which endures with difficulty the effects of uncombined oxygen, flourishes in association with saprophytic organisms by which the oxygen is absorbed. The same thing is probably true of other obligatory anaërobic organisms.

* "Jour. Amer. Med. Assoc.," 1906, XLVII, 18, Nov. 3, p. 1467.
† "Archiv. Ital. de Biologie," XXVI.

The metabolic products of one species may intensify or accelerate the action of those of an associated species, or the reverse may be true, and the products of different organisms, having different chemical composition, may neutralize one another, or combine to form some entirely new substance which is entirely different from its antecedents. Such conditions cannot fail to influence the type and course of infection.

CHAPTER IV

IMMUNITY

Immunity is ability to resist infection. It is the ability of an organism successfully to antagonize the invasive powers of parasites, or to annul the injurious properties of their products. The mechanism of immunity is complicated or otherwise according to circumstances. When the invasive action of non-toxicogenic bacteria is to be overcome, certain reactions, mostly on the part of the phagocytic cells, are called into action; when the toxic products of bacteria are to be deprived of injurious effects, the reaction seems to take place between the toxin and certain combining and neutralizing substances contained in the body juices; when bacterial invasion and intoxication are both to be antagonized, both mechanisms are engaged in the defenses, comparatively simple or exceedingly complex, according to the conditions involved. The more involved the conditions of infection become, the more complicated the defensive reactions become, until it may no longer be possible accurately to analyze them.

Some have endeavored to refer all of the phenomena of immunity to the ability of the animal to endure the bacterio-toxins, and have sought to relegate the reactions against invasion to a subsidiary place. This is undoubtedly an error, as the mechanisms are different and the prompt action of one may make the action of the other unnecessary. Metschnikoff* found that frogs injected with 0.5 cc. of cholera toxin died promptly, but that frogs injected with cultures of the cholera spirillum recovered without illness. This would suggest that the recovery of the infected frog depended upon some defensive mechanism combating the invasiveness of the bacteria and so preventing the production of the toxin to which the frog was susceptible.

Immunity must not be conceived as something inseparably associated with infection. The reactions of the body toward bacteria in the infectious diseases are identical with those toward other minute irritative bodies, and the reactions toward bacterio-toxins are identical with those toward other toxic substances, so that the only way by which a satisfactory understanding of the phenomena can be reached is by carefully comparing the reactions produced by bacteria and their products with those produced by other active bodies.

* "Immunite dans les Maladies Infectieuses," Paris, 1901, p. 150.

Immunity is called *active* when the animal protects itself through its own activities, *passive* when the protection depends upon defensive substances prepared by some other animal entering into it. Thus, if a frog be injected with anthrax bacilli, its leukocytes devour the bacteria, destroy them, and so protect the frog from infection; the immunity is active because it depends upon the activity of the frog's phagocytes. But if a guinea-pig previously given antitetanic serum be injected with tetanus toxin, and so recovers from the toxin, the resisting power, conferred by the antitoxin previously injected, does not depend upon any activity of the animal, which remains entirely passive.

Immunity is largely *relative*. Fowls are immune against tetanus, that is, they can endure, without injury, as much toxin as tetanus bacilli can produce in their bodies, and suffer no ill effects from inoculation. If, however, a large quantity of tetanotoxin produced in a test-tube be introduced into their bodies, they succumb to it. Mongooses and hedgehogs are sufficiently immune against the venoms of serpents to resist as much poison as is ordinarily injected by the serpents, but by collecting the venom from several serpents and injecting considerable quantities of it, both animals can be killed. Rats cannot be killed by infection with Bacillus diphtheriæ, and Cobbett* found that they could endure from 1500 to 1800 times as much diphtheria toxin as guinea-pigs, though more than this would kill them.

Carl Fränkel has expressed the whole matter very forcibly when he says: "A white rat is immune against anthrax in doses sufficiently large to kill a rabbit, but not necessarily against a dose sufficiently large to kill an elephant."

NATURAL IMMUNITY

Natural immunity is the natural, inherited resistance against infection or intoxication, peculiar to certain groups of animals, and common to all the individuals of those groups.

Few micro-organisms are capable of infecting all kinds of animals; indeed, it is doubtful whether any known organism possesses such universally invasive powers.

The micro-organisms of suppuration seem able to infect animals of many different kinds, sometimes producing local lesions, sometimes invading rapidly with resulting bacteremia. The tubercle bacillus is known to be pathogenic for mammals, birds, reptiles, batrachians, and fishes, though it is still uncertain whether the infecting organisms in these cases are identical or slightly differing species.

As a rule, however, the infectivity of bacteria and other micro-organisms is restricted to certain groups of animals which usually

* "Brit. Med. Jour.," April 15, 1899.

have more or less resemblance to one another; thus, anthrax is essentially a disease of warm-blooded animals, though certain exceptions are observed, and Metschnikoff has found that hippocampi (sea-horses), perch, crickets, and certain mussels are susceptible. Among the warm-blooded animals anthrax is most frequent among the herbivora, though some carnivora may also be infected.

Close relationship is not, however, a guarantee that animals will behave similarly toward infection. The rabbit, guinea-pig, and the rat are rodents, but though the rabbit and guinea-pig are susceptible to anthrax, the rat is immune. This is still better exemplified in the susceptibility of mice to glanders. The field-mouse seems to be the most susceptible of all animals to infection with Bacillus mallei; the house mouse is much less susceptible, and the white mouse is immune. Mosquitos, though closely related, are different in their immunity to the malarial parasite. The culex does not harbor the parasite at all, and of the anopheles, two very similar species seem to behave very differently. Anopheles maculipennis being the common definitive host of the parasite, while Anopheles punctipennis is not known to be susceptible to it. The same differences may exist among the members of the human species. It has been asserted that Mongolians, and especially Japanese, are immune against scarlatina, and that negroes are immune against yellow fever, but increasing information is to the contrary.

Human beings suffer from typhoid, cholera, measles, scarlatina, yellow fever, varicella, and numerous other diseases unknown among the lower animals, even those domestic animals with which they come in close contact. They also suffer from Malta fever, anthrax, rabies, glanders, bubonic plague, and tuberculosis, which are common among the lower animals. Animals, in turn, suffer from distemper, septicemia, etc., the respective micro-organisms of which are not known to infect man.

It has already been pointed out that mongooses and hedgehogs are immune against the venom of serpents from which other animals quickly die. The tobacco-worm lives solely upon tobacco-leaves, the juice of which is intensely poisonous to higher animals, and is also a good insecticide. Boxed cigars and baled tobacco are often ruined by the larvæ of a small beetle that feeds upon them, and a glance over the poisonous vegetables will show that few of them escape the attacks of insects immune against their juices.

These facts are sufficient to show that many animals are by nature immune against the invasion of microparasites of certain kinds, and that they are also at times immune against poisons. Immunity against one kind of infection or intoxication is, however, entirely independent of all other infections and intoxications. Immunity against infection usually guarantees exemption from the

toxic products of that particular micro-organism, though experiment may show the animal to be susceptible to it. Immunity against any form of bacterio-toxin usually, though not necessarily, determines that the micro-organism, though it may be able to invade the body, can do very little harm.

ACQUIRED IMMUNITY

Acquired immunity is resistance against infection or intoxication possessed by certain animals, of a naturally susceptible kind, in consequence of conditions peculiar to them as individuals. It is a peculiarity of the individual, not of his kind, and signifies a subtile change in physiology by which latent defensive powers are stimulated to action. The reactions in general correspond with those of natural immunity, and comprise mechanisms for overcoming the invasion of pathogenic organisms, for neutralizing or destroying their toxins or for both. As an acquired character and an individual peculiarity it is not transmitted to the offspring, though these sometimes also acquire immunity through the parents. Thus in studying immunity of mice against ricin, Ehrlich found that the newly born offspring of an immune mother were not immune, though they subsequently became so through her milk.

Acquired immunity differs from natural immunity in being more variable in degree and duration. The animal may be immune to-day, but lose all power of defending itself a month hence.

Natural immunity is always active, but certain forms of acquired immunity are passive.

Immunity may be acquired through *infection* or *intoxication*, and in either case may be accidental or experimental.

(A) Active Acquired Immunity.—1. Immunity Acquired through Infection.—(*a*) *Accidental Infection.*—The most familiar form of acquired immunity follows an attack of an infectious disease. Every one knows that an attack of measles, scarlatina, varicella, variola, yellow fever, typhoid fever, and other common infectious maladies, is a fairly good guarantee of future exemption from the respective disease. Immunity thus acquired is not transmissible to the offspring. Almost everybody has had measles, yet almost all children are born susceptible to it. It is not necessarily permanent, as is shown by the not infrequent cases in which second attacks of measles occur. In some cases, as after typhoid fever, the immunity is not at first observable and the patient may suffer from relapses. Later it becomes well-established and no repetition of the disease is possible for years.

Sometimes the infection, by which immunity is acquired, is not exactly similar to the disease against which it affords protection, as in the case of vaccinia, which protects against variola. It is still controversial, however, whether cow-pox is variola of the cow

or an entirely different disease. Cow-pox was, however, common in the days when smallpox was frequent, and has now become extremely rare.

(*b*) *Experimental Infection.*—1. *Inoculation:* This is an attempt to prevent the occurrence of a fatal attack of an infectious disease, by inducing a mild attack of the same disease when the individual is in good health, and at his maximum resisting power. The oldest experiments date from unknown antiquity and were practised in China and other Oriental countries for the purpose of preventing smallpox. The Chinese method of experimentally producing variolous infection was very crude and consisted in introducing crusts from cases of variola into the nose, and tying them upon the skin. The Turkish method was much more neat, in that a small quantity of the variolous pus was introduced into a scarification upon the skin of the individual to be protected. The following extract is from a letter of Lady Montague,* wife of the British Ambassador to Turkey, who brought the so-called "inoculation" method from Turkey in the early part of the eighteenth century (1718):

". . . . . Apropos of distempers, I am going to tell you a thing that I am sure will make you wish yourself here. The smallpox, so fatal, and so general amongst us, is here entirely harmless by the invention of ingrafting, which is the term they give it. There is a set of old women who make it their business to perform the operation every autumn, in the month of September, when the great heat is abated. People send to one another to know if any of their family has a mind to have the smallpox; they make parties for this purpose, and when they are met (commonly fifteen or sixteen together), the old woman comes with a nut-shell full of the matter of the best sort of smallpox, and asks what vein you please to have opened. She immediately rips open that you offer to her with a large needle (which gives you no more pain than a common scratch), and puts into the vein as much venom as can lie upon the head of her needle, and after binds up the little wound with a hollow bit of shell; and in this manner opens four or five veins. The Grecians have commonly the superstition of opening one in the middle of the forehead, in each arm, and on the breast, to mark the sign of the cross; but this has a very ill effect, all these wounds leaving little scars, and is not done by those that are not superstitious, who choose to have them in the legs, or that part of the arm that is concealed. The children of young patients play together all the rest of the day, and are in perfect health to the eighth. Then the fever begins to seize them, and they keep their beds two days, very seldom three. They have very rarely above twenty or thirty [pocks] in their faces, which never mark; and in eight days' time they are as well as before their illness. Where they are wounded, there remain running sores during the distemper, which I don't doubt is a great relief to it. Every year thousands undergo this operation; and the French embassador says pleasantly, that they take the smallpox here by way of diversion, as they take the waters in other countries. There is no example of any one that has died in it; and you may believe I am very well satisfied of the safety of this experiment, since I intend to try it on my dear little son.

"I am patriot enough to take pains enough to bring this useful invention into fashion in England; and I should not fail to write to some of our doctors very particularly about it, if I knew any one of them that I thought had virtue enough to destroy such a considerable branch of their revenue for the good of mankind. But that distemper is too beneficial to them not to expose to all their resentment the hardy wight that should undertake to put an end to it."

* See the "Letters of Lady Mary Wortley Montague;" letter to Miss Sarah Chisives dated Adrianople, April 1 (O. S.), 1717.

By both methods the very disease, variola, against which protection was desired, was induced, the only advantage of the experimental over the accidental infection being that by selecting the infective virus from a mild case of variola, by performing the operation at a time when no epidemic of the disease was raging, and by doing it at a time when the person infected was in the most perfect physical condition, the dangers of the malady might be mitigated.

There was always danger, however, that the induced disease being true variola might prove unexpectedly severe, or even fatal, and that each inoculated individual, suffering from the contagious disease, might start an epidemic.

2. *Jennerian vaccination*: In 1791 a country schoolmaster named Plett, living in the town of Starkendorf near Kiel in Germany, seems to have made the first endeavor to subject the oft-repeated observation, that persons who had acquired *cow-pox* did not subsequently become infected with *smallpox*, to experimental demonstration, by inserting cow-pox virus into three children, all of whom escaped smallpox.

The father of vaccination, and the man to whom the world owes one of its greatest debts, was Edward Jenner, who performed his first experiment on May 14, 1796, when he transferred some of the contents of a cow-pox pustule on the arm of a milkmaid named Sarah Nelmess to the arm of a boy named John Phips. After the lad had recovered from the experimental cow-pox thus produced, he subsequently introduced smallpox pus into his arm and found him fully immunized and insusceptible to the disease. This led Jenner to perform many other experiments, and record his observations in numerous scientific memoirs. The success of his work immediately attracted the attention of both scientific investigators and sanitarians, and its outcome has been the establishment of compulsory vaccination by legal enactment in nearly all civilized countries, with the result that smallpox, instead of being one of the most prevalent and most dreaded diseases, has become one of the most rare and least feared.

The immunity acquired through vaccination is active and usually of prolonged duration. It is subject to the same variations observed in other experimentally acquired immunities, these variations explaining the occasional failures which constitute the "stock in trade" of those who still remain unconvinced of the scientific basis and efficacy of the procedure.

Though a thorough analysis of the irregularities and exceptions of vaccination would be of much interest, a brief mention of the most important must suffice for the present argument.

The first controversial point is the nature of the "vaccine," or virus used in the operation. It is obtained from calves or heifers suffering from experimental cow-pox, and is a virus descended from

various spontaneous cases of cow-pox observed in places remote from one another. Experts are undecided whether cow-pox is variola modified by passage through the cow so that the transplanted micro-organisms are only capable of inducing a local instead of a general disease, or whether it is an independent affection natural to the cow.

In reality the matter is unimportant, so long as the desired effect is accomplished, and the true lineage of the virus is only a matter of scientific curiosity. As immunity is almost invariably a specific effect resulting from infection, it would seem most likely that cow-pox and smallpox were originally identical.

The advantage of "vaccination" over "inoculation" is that the induced disease is local and not dangerous except in rare cases, and that it is not contagious. The natural variations in the susceptibility of different vaccinated individuals determine that a few persons cannot be successfully vaccinated, being immune to the mildly invasive organisms of vaccinia, though perhaps susceptible to the actively invasive organisms of variola; that a few individuals shall prove abnormally susceptible to vaccinia so that the disease departs from its usual local type and generalizes, but that in nearly all cases the disease will follow the well-known type of a local lesion characterized by definite periods of incubation, vesiculation, pustulation, and cicatrization.

The occasional variations in immunity of different individuals also determine that having been vaccinated once an individual may not again become susceptible to vaccination, though he may become susceptible to the more actively invasive organisms of variola, or that he may soon become again susceptible to both diseases, or that in very rare cases no immunity against variola will result from vaccination. In most cases successful vaccination can be repeated once or twice at intervals of seven or ten years, and experience shows that the immunity against smallpox conferred by vaccination is of longer duration and usually becomes permanent after vaccination has been repeated once or twice.

Sanitarians are accustomed to speak of *efficient* and *inefficient* vaccination. These are vague terms and do not seem to be understood by the laity. Efficient vaccination is vaccination repeated as often as is necessary. It has already been shown that individual variations determine that a few individuals never become immune, hence never can be efficiently vaccinated. Other persons are efficiently vaccinated by a single operation. The term is usually interpreted to indicate that which experience has shown to be efficient in average cases.

Failures not uncommonly result from causes having nothing to do with the problems of immunity. That an operation of scarification has been performed upon a child, and that a scar has remained thereafter may mean nothing. It is not the *operation* but the *dis-*

ease that achieves the result, and if the operation be improperly done, poor—*i.e.*, old or inert—matter introduced, or if after introduction it be destroyed by the application of antiseptics, no effect can be expected. Hence all persons that have been vaccinated may not have had vaccinia, the essential condition leading to immunity. Nor does the occurrence of a local lesion act as a guarantee that vaccinia has been induced. Careful examination of the resulting lesions should always be made, that the type of the infection may be studied. It is the *disease, vaccinia*, that must occur—three days' incubation, three days' vesiculation, three days' pustulation, and subsequent cicatrization with the formation of a punctate scar.

An arm may be made very sore, may suppurate or even become gangrenous, without vaccinia having occurred or the desired benefit attained.

The accidents of vaccination were formerly numerous and sometimes disastrous because of the general inattention to the quality of the materials used, the mode of inserting them, the condition of the patient's skin, and the careless treatment of the resulting lesions. When human virus was used, that is, matter taken from a vaccinia lesion from a human being, the transmission of human diseases, such as syphilis and erysipelas, occasionally took place; now these are rare accidents indeed, because no virus is employed except that taken from carefully selected and treated calves or heifers. When no attention was paid to the quality of the bovine virus, and no governmental inspection of laboratories required, the accidental contamination of the virus occasioned a small number of accidental infections of the wound. There are a good many cases of phlegmon, gangrene and tetanus in the older literature. But these evils are becoming less and less as greater attention is given to the selection and preparation of the virus. Some accidents and some few deaths there will probably always be, just as there are occasional accidents and occasional fatal results following all kinds of trivial injuries, though care will eliminate them as the sources of accident are better understood.

3. *Pasteurian vaccination* or *bacterination:* Although the word *vaccination* is derived from the Latin *vacca*, "a cow," and was first employed in connection with Jenner's method of introducing virus modified by passage through a cow, Pasteur, in honor of Jenner, applied it to every kind of protective inoculation, and the word *bacterination* is only introduced for the purpose of indicating certain differences in the method.

In 1880 Pasteur* observed that some hens inoculated with a culture of the bacillus of chicken cholera that had been on hand for some time did not die as was expected. Later, securing a fresh and virulent culture, these and other chickens were inoculated. The former hens did not die, the new hens did. Quick to observe and

* "Compte rendu de la Soc. de Biol., 1880, 239; 315 et seq.

study phenomena of this kind, he investigated and found that when chickens were inoculated with old and non-virulent cultures they acquired immunity against virulent cultures. This led him to the recommendation of the employment of attenuated cultures as *vaccines* against the disease, and to the achievement of great success in preventing epidemics by which great numbers of the barnyard fowls of France were being destroyed.

In 1881 Pasteur,* in experimenting with Bacillus anthracis, observed that if the organism were cultivated at unusually high temperatures it lost the power of producing spores, and diminished in virulence. He also found that when the organisms had been so attenuated, they could not regain virulence without artificial manipulation. It occurred to him that such organisms, possessing feeble virulence, might be able to confer immunity upon animals into which they were inoculated, and he continued to investigate the subject until he found that by using three "vaccines" or modified cultures of increasing virulence, it was possible to render animals immune against the unmodified organisms. This method was put to practical test with great success, and has since been extensively practised in different parts of the world.

Arloing, Cornevin and Thomas,† and Kitt‡ found that exposure of the Bacillus anthracis symptomatici to a high temperature in the dry state modified its virulence and devised a practical method of protecting cattle against symptomatic anthrax by inoculating them with powdered muscle tissue containing the bacilli attenuated by drying and exposure to 85°C. This method has since been in use in many countries, and has given excellent satisfaction.

In 1889 Pasteur,§ continuing his researches upon the experimental modification of the germs of disease and their use as prophylactics, published his famous work upon rabies, and showed that, although the micro-organism of that disease had so far eluded discovery, it was contained in the central nervous system of diseased animals, where it could be modified in virulence by drying. By placing spinal cords removed from rabid rabbits in a glass jar containing calcium chlorid, he was able to diminish the virulence of the contained micro-organisms according to the duration of the exposure. The introduction of the attenuated virus was followed by the development of a certain degree of immunity. By repeated inoculation of more and more active viruses animals acquired complete immunity against street virus. These experiments formed the basis of the "Pasteur method" of treating rabies, which is nothing more than immunization with the modified germs of the disease during the long incubation period of the disease.

* "Compte rendu de la Soc. de Biol. de Paris," 1881, XCII, pp. 662–665.
† "Le Charbon Symptomatique du Bœuf," Paris, 1887.
‡ "Centralbl. f. Bakt.," etc., I, p. 684.
§ "Compte rendu de la Soc. de Biol. de Paris," 1881, CVIII, p. 1228.

Haffkine* found that the introduction of killed cultures of virulent cholera spirilla produced immunity against the living micro-organisms, and used the method with considerable success for preventing the disease. Later† he applied the same method, also with considerable success, for the prevention of bubonic plague, and A. E. Wright‡ followed pretty much the same method for the prevention of typhoid fever.

In all these cases the immunity induced by the experimental manipulations is specific in nature, and variable in intensity, according to the method of treatment adopted and the thoroughness with which it is carried out.

2. Immunity Acquired by Intoxication.—Bacterio-toxins form a miscellaneous group of active bodies of entirely different chemical composition and physiologic activity. Some are toxalbumins, some are enzymes, some are bacterie-proteins. The true nature of the greater number of these bodies is unknown, but study of their physiologic action has brought forth the important fact that their behavior toward the body cells is in no way different from the behavior of the same cells toward other chemical compounds of similar constitution, and that nearly all physiologically active bodies introduced into living organisms produce definite, though not necessarily visible, reactions.

Such reactions are now known as antigenic, and the substances by which they are induced have been called by Deutsch *antigens*.§ Since its introduction the precise meaning given the word by Deutsch has been slightly changed. An antigen is any substance which when injected into the body of a living organism is capable of producing a chemicophysiologic reaction resulting in the appearance of a neutralizing, precipitating, agglutinating, dissolving, or otherwise antagonizing substance known as an *antibody*.

The antigens are, so far as known, all colloidal substances. They may be harmful or harmless, active or inert, living or dead, organized or unorganized. The reactions are specific and the antibody has specific affinity for that antigen alone by which its formation has been excited.

All poisonous substances are not antigens, even though a certain immunity—in the sense of habituation or tolerance—may follow their repeated administration. One may become habituated or tolerant to a certain quantity of mercury or arsenic, and to certain alkaloids, such as morphin, caffein, nicotin, cocain, etc., but he does not react as to them as to antigens and no antibodies antagonistic to them are formed. To these various substances he really acquires only a slight degree of tolerance; to the effects of

* "Brit. Med. Jour.," 1891, II, p. 1278.
† "Brit. Med. Jour.," 1895, II, p. 1541.
‡ Ibid., Jan. 30, 1897, I, p. 256.
§ Deutsch und Feistmantel, "Die Impfstoffe und Sera," 1903, Leipzig, Thieme.

injurious antigens he may acquire an almost unlimited degree of immunity through the formation of the antibodies.

From remote antiquity it has been known that those who regularly consume small quantities of poisons become irresponsive to their action, and it is well known that Mithridates attempted this mode of defending himself from his enemies.

Chauveau* believed that the immunity conferred by inoculations of bacteria was due to the presence of their soluble products, but the first direct demonstration of the fact was by Salmon and Smith,† who, as early as 1886, showed that it was possible to immunize pigeons against the hog-cholera bacillus by means of repeated injection with cultures exposed to 60°C., and containing no living organisms. Charrin‡ found it possible to immunize rabbits against Bacillus pyocyaneus by injecting them with the filtered products of cultures of that organism, and Bonome§ similarly to immunize animals against Bacillus proteus, B. cholera gallinarum and the pneumococcus. Roux and Chamberland‖ and Roux** were able by the use of boiled cultures of the bacilli of malignant edema, and of quarter evil, similarly to immunize animals against these respective infections.

The subject was much further elaborated by Roux and Yersin†† in their experiments with diphtheria toxin; by Behring‡‡ in his early studies of diphtheria, and by Kitasato§§ in his experiments with tetanus.

These early experiments opened a wide field, through the investigation of which we now know that the products as well as the living or dead bacteria of most of the infectious diseases, when properly introduced into animals, can induce immunity.

(B) Passive Acquired Immunity.—Passive immunity is always acquired, never natural. It depends upon defensive factors not originating in the animal protected, but artificially or experimentally supplied to it. The fundamental principle is simple and has become the basis of serum therapeutics. If the immunized animal generates factors by which the infecting bacteria can be destroyed or the activity of their products overcome in its body, cannot these factors be removed and the benefit they confer transferred to another animal?

The first experiments in this direction seem to have been made by Babes and Lepp,‖‖ who found that the blood-serum of animals

* "Ann. de l'Inst. Pasteur," 1888, 2.
† "Centralbl. f. Bakt.," etc., 1887, II, No. 18, p. 543.
‡ "Compte rendu," de la Soc. de Biol., CV, p. 756.
§ "Zeitschrift f. Hyg.," V, p. 415.
‖ "Ann. de l'Inst. Pasteur," 1887, 12.
** Ibid., 1888, 2.
†† Ibid., 1888, II, p. 269.
‡‡ "Deutsche med. Wochenschrift," 1890, No. 50.
§§ "Zeitschrift für Hygiene," 1891, X, p. 267.
‖‖ "Annales de l'Inst. Pasteur," 1889, vol. III.

immunized to rabies showed a defensive power when injected into other animals. Ogata and Jasuhara* found that the subcutaneous injection of blood-serum from an animal immunized against anthrax enabled the injected animals successfully to resist infection. Behring and Kitasato† found that the blood-serums of animals immunized against diphtheria and tetanus, when mixed with cultures of these respective bacilli, neutralized their power to produce disease. Kitasato‡ found that if mice were inoculated with tetanus bacilli, they could be saved from the fatal infection by the intra-abdominal injection of some blood-serum from a mouse immunized against tetanus, even after symptoms of the disease had appeared. Ehrlich§ showed that the blood-serums of animals immunized against abrin and ricin could save other animals from the fatal effects of these respective toxalbumins; Phisalix and Bertrand,‖ and, later, Calmette** found the blood-serum of animals, immunized against the venoms of serpents, similarly possessed the power of neutralizing the poisonous effects of the venoms. Kossel†† found that the blood-serum of animals, immunized against the poisonous blood-serum of eels, contained a body which destroyed or neutralized the effects of the eels' serum.

Thus, it is shown that in each case in which defensive reactions are stimulated in experiment animals, the reactions are accompanied by the appearance in the blood-serum of those animals of factors that can be utilized to defend other animals in whose bodies no similar reactions have taken place.

Passive immunity may also be brought about in a few cases by the injection into the intoxicated animal of substances, other than immunity products, that have a specific affinity for the poison. Thus Wassermann and Takaki‡‡ found that when the crushed spinal cord of a rabbit was mixed *in vitro* with tetanus toxin, the poison was quickly absorbed by the nerve-cells, so that the mixture became inert and could be injected into animals without harm. Wassermann also found that the same effects could be produced in the bodies of animals, and that when the crushed spinal cord was injected into an animal a few hours previously, or a few hours after a fatal dose of tetanus toxin, enough of the combining elements remained in the blood to fix the toxin before it anchored itself to the central nervous system of the intoxicated animal. Myers§§ found that the ground-up tissue of the adrenal bodies was able to fix and thus annul the poisonous effects of cobra venom *in vitro*.

* "Centralbl. f. Bakt.," etc., 1890, IX, p. 25.
† "Deutsche med. Woch.," 1890, No. 49.
‡ "Zeitschrift für Hygiene," 1892, XII, p. 256.
§ "Deutsche med. Wochenschrift," 1891, Nos. 32 and 44.
‖ "Compte rendu Acad. des Sciences de Paris," CXVIII, p. 556.
** "Ann. de l'Inst. Pasteur," 1894, VIII, p. 275.
†† "Berliner klin. Woch.," 1898, p. 152.
‡‡ "Berliner klin. Wochenschrift," Jan. 3, 1898.
§§ "Lancet," July 2, 1898.

In all these cases the neutralizing effects are either accomplished or initiated by factors prepared experimentally, and forced upon the animal in whose body their activities are manifested.

EXPERIMENTAL INVESTIGATION OF THE PROBLEMS OF IMMUNITY

Very important contributions were made by Ehrlich,* in his work upon the vegetable toxalbumins, ricin, abrin, and robin, that were found to be antigens capable of producing anti-ricin, anti-abrin and anti-robin respectively, each antibody being capable of neutralizing the effect of its specific antigen. Kossel† investigated the reactions produced by toxic eels' blood and found that immunity could be established against their hemolytic action, and that specific antibodies were formed. Phisalix and Bertrand‡ showed that immunity could also be produced in guinea-pigs against the action of viper venom, and that a specific antibody, "*antivenene*" was the source of the immunity.

The investigation of other active bodies was soon begun. In 1893 Hildebrand§ studied emulsin and found that it produced a definite reaction with the formation, in animals injected, of an anti-emulsin. v. Düngern‖ studied proteolytic enzymes of various bacteria, and showed that when gelatin-dissolving enzymes were repeatedly injected into animals, definite reactions took place, and in the serum a body appeared that inhibited the action of the ferment in a test-tube. Gheorghiewski** immunized animals to cultures of Bacillus pyocyaneus, and found that the reaction provoked caused the appearance in the serum of some body that prevented the formation of the blue pigment so characteristic of the organism. Morgenroth†† applied the same principle to rennet, finding that it produced definite reactions, with the formation of an antibody inhibiting the coagulation of milk. Bordet and Gengou‡‡ found that the fibrin ferment of the blood of one animal was active in the body of another animal, producing an inhibiting substance by which the coagulation of the blood of the first animal could be delayed.

The studies of Kraus§§ showed a new fact, that when filtered cultures of the cholera spirillum were introduced into animals, the serum of these animals, added to the filtered culture in a test-tube, caused the appearance of a delicate flocculent precipitate, *specific precipitate*.

* "Deutsche med. Woch.," 1891, Nos. 32 and 44.
† "Berliner klin Wochenschrift," 1898.
‡ Atti d XI Congr. med. internaz. Roma, 1894, II, 200–202.
§ "Virchow's Archives," Bd. CXXXI.
‖ "Münchener med. Woch.," Aug. 15, 1898.
** "Ann. de l'Inst. Pasteur," 1899.
†† "Centralbl. f. Bakt.," etc., 1899, XXVI, p. 349.
‡‡ "Ann. de l'Inst. Pasteur," 1903, XVII, p. 822.
§§ "Wien. klin. Woch.," 1897.

Wassermann and Schütze* found that when cow's milk was repeatedly injected into rabbits, their serum acquired the property of occasioning a precipitate when added to cows' milk, but not when added to goats' or any other milk. If, however, the rabbit had been repeatedly injected with goats' milk or human milk, its serum would precipitate with those milks respectively, and not with cow's milk. The reaction was thus shown to be specific.

Myers† found that the repeated intraperitoneal injection of egg-albumen into rabbits caused their serum to give a dense precipitate when added to solutions of egg-albumen.

Tchistowitch‡ found that eels' serum injected into animals produced a reaction in which immunity to its poisonous action was associated with the ability of their serum to produce a precipitate when added to the eels' serum.

Closely connected with these various reactions are certain others variously spoken of as cytotoxic, cytolytic, hemolytic, bacteriolytic, etc. The first observation bearing upon these was made by R. Pfeiffer,§ who found that when guinea-pigs received frequent intraperitoneal injections of cholera spirilla and became thoroughly immunized, their serum behaved very peculiarly toward the bacteria in the peritoneal cavity of freshly infected animals, in that it caused them to become aggregated into granular masses and subsequently to disappear. This became known as "Pfeiffer's phenomenon." The serum of the immunized animal was devoid of action by itself, the serum of the infected animal was inactive, but the combination of the two brought about dissolution of the micro-organisms. Later it was shown by Metschnikoff‖ that the living animal was not a factor in the process, but that what was seen in the peritoneal cavity could be reproduced in a test-tube, though not quite as well.

Bordet** made frequent injections of defibrinated rabbits' blood into guinea-pigs, and obtained a serum that had a solvent action upon the rabbit's corpuscles *in vitro*, and showed that the induced hemolysis resembled in all points the bacteriolysis.

Ehrlich†† and Morgenroth studied the hemolytic action of the serum of goats that had been frequently injected with the defibrinated blood of sheep and goats, and were able to point out the mechanism of the corpuscle solution or *hemolysis*. It was found to depend upon two associated factors, one of which, the *lysin* or solvent, was present in normal blood, and was called "addiment" or "*complement*," and another present only in the serum of the reactive animals, called the "*immune body*" or "intermediate body." The former was labile and easily destroyed by heat, the latter

* "Deutsche med. Woch.," 1900.
† "Lancet," 1900, II.
‡ "Ann. de l'Inst. Pasteur," vol. XIII, 406.
§ "Deutsche med. Wochenschrift," 1896, No. 7.
‖ "Ann. de l'Inst. Pasteur," 1895.
** Ibid., 1898, XII.
†† "Berliner klin. Wochenschrift," 1899.

stabile and not affected by heat up to the point of coagulation. The experiments were confirmed by von Düngern and many others. It is to be observed in passing that this reaction differs from the direct solution of the corpuscles *in vitro* by cobralysin, which was studied by Myers,* and tetanolysin, studied by Madsen,† in that it is intermediate, and only brought about by the coöperation of two factors, while the action of the lysins of venom, the tetanus bacillus, the streptococcus, Bacillus pyocyaneus, and other micro-organisms, is direct and immediate.

Myers found, however, that the hemolytic substance of venom, and Madsen that the hemolytic products of Bacillus tetani, also produce reactions in animals, and that when successful immunization against them was accomplished, the serums of the experiment animals became antidotal or inhibiting to the action of the respective lysins.

Von Düngern‡ found that by injecting dissociated epithelial cells from the trachea of oxen into the peritoneal cavity of guinea-pigs, it was possible to produce *epitheliolysins;* Lindemann,§ that emulsions of kidney substance injected into animals caused them to form nephrolysins or *nephrotoxins;* Landsteiner‖ and Metschnikoff** in the same manner successfully prepared *spermatoxin* by injecting the spermatozoa of one animal into the peritoneal cavity of another. Metalnikoff†† found that if he introduced the spermatozoa of a guinea-pig into the peritoneum of another, the spermatoxic serum produced was solvent for the spermatozoa of both. Both Metschnikoff and Metalnikoff also found that the spermatoxin when introduced into animals was active in producing anti-spermatoxin by which the destructive action of the serum upon spermatozoa could be inhibited.

Metschnikoff‡‡ and Funck§§ found that animals treated with emulsions of the spleen, and mesenteric lymph-nodes of one kind of animal, produced sera whose action was agglutinative and solvent for leukocytes and lymph-cells. Delezene‖‖ found that dissociated liver cells injected into animals similarly caused the formation of a specific cytotoxic serum.

All of these reactions are indirect and intermediate, and take place under appropriate conditions both in the bodies of animals and in the test-tube.

Thus the number of antigenic reactions that can be brought about in the bodies of animals seems to be limitless, and, strange

* "Trans. Path. Soc. of London," LI.
† "Zeitschr. f. Hyg.," 1899, XXXIII, p. 239.
‡ "Münchener med. Wochenschrift," 1899.
§ "Ann. de l'Inst. Pasteur," 1900.
‖ "Centralbl. f. Bakt.," etc., 1899, XXV.
** "Ann. de l'Inst. Pasteur," 1899.
†† Ibid., 1900.
‡‡ Ibid., 1899.
§§ "Centralbl. f. Bakt.," etc., 1900, XXVII.
‖‖ "Compte rendu de l'Acad. des Sciences," 1900, CXXX, pp. 938, 1488.

as it may seem, the antibodies produced in the body of one animal may act as antigens when introduced into another. Thus, Ehrlich and Morgenroth in their studies of hemolysis found that serums rich in immune bodies produced reactions yielding anti-immune bodies, which inhibited the activities of the respective immune bodies by whose stimulation they were produced.

The reactions which when repeated may lead to immunity and to the formation of antibodies seem to be followed by constitutional disturbances much more profound than would be supposed from the apparent freedom from symptoms manifested by the animal. As early as 1839 Magendie observed that if a rabbit was given an injection of albumin, and then, some days later, a second injection, it was made very ill and might die. About 1900 Mattson in private conversation called the author's attention to the fact that when guinea-pigs used for testing antitoxic serums were subsequently injected with another dose of serum, they commonly died. Not being understood, the matter was not thought worthy of publication. Otto* speaks of this fatal action of serums as the "Theobald-Smith phenomenon," the fact having first been pointed out to him by Smith.

The first to realize the importance of the condition seem to have been Portier and Richet,† who studied the effect of extracts of the poisonous tentacles of actiniens upon dogs which were found to die more quickly and from smaller doses given at a second injection than at the first. To this increase of sensitivity to the poison brought about by the initial dose they gave the name *anaphylaxis* (αν negative, φυλαξις protection, destroying protection or breaking down the defenses).

The therapeutic employment of diphtheria antitoxic serum was scarcely popularized before the medical profession was shocked by the sudden death of the healthy child of a noted German professor after a prophylactic injection, and in 1896 Gottstein‡ was able to collect eight deaths following the use of the serum, four of them being persons not ill with diphtheria. von Pirquet and Schick§ also pointed out that in a certain proportion of cases the injection of horse-serum in man is followed by urticarial eruptions, joint-pains, fever, swelling of the lymph-nodes, edema and albuminuria, these symptoms usually appearing after an incubation period of eight to thirteen days, and constituting what they call the "serum disease," or *allergia*. Sometimes these reactions are immediate; sometimes death appears imminent, and, as has been observed, death sometimes occurs.

The investigation of the subject was taken up in 1905 by Rosenau

* von Lenthold, "Gedenkschrift," Bd. 1, pp. 9, 16, 18.
† "Compte rendu de la Soc. de Biol. de Paris," 1902.
‡ "Therap. Monatschrift," 1896.
§ "Die Serumkrankheit," Leipzig and Wien, 1905.

and Anderson,* who pursued it with great interest and industry, by Gay,† Gay and Southard,‡ and others.

Experimental study shows that when an animal is injected with an alien protein of almost any kind, a reaction takes place that usually is not completed under six days. If a second injection is given before the reaction is perfected, the mechanism of immunity is set in action, and the animal proceeds to defend itself through the various means described. If the second administration be deferred, however, until the first reaction is completed, it seems to find the animal in a state of disturbed biologic equilibrium, the nature of which is not understood, but which is characterized by a profound disturbance that may terminate in death. The reaction is quite specific; the sensitization, once effected, may continue throughout the remainder of the life of the animal and be transmitted from the mother to her offspring through her blood. The reaction can be brought about by feeding the protein or by injecting it. It has an important bearing upon infection and immunity, the chief example being seen in the tuberculin reaction.

The symptomatology of anaphylaxis is interesting and characteristic. When it is desirable to study it, a guinea-pig is first given a sensitizing dose of horse-serum. This may be very small. Rosenau and Anderson found one guinea-pig to be sensitized by *one-millionth of a cubic centimeter*. In most of their work they used less than $\frac{1}{250}$ cc. It is necessary to wait until the effects of this first injection are completely over before giving the poisoning dose. This period of incubation lasts about twelve days. After the lapse of this time, the second dose, usually about $\frac{1}{10}$ cc., is given. Both doses are given by injection into the peritoneal cavity.

The symptoms come on almost immediately after the second dose. The animal is profoundly depressed, extremely uneasy, pants for breath, and suffers from intense itching of the face. It soon falls, continues to gasp for breath, and dies within an hour. The disturbances in the body of the animal are sufficient to account for the symptoms. Extensive lesions exist, the first to be described by Rosenau§ affecting the mucous membrane of the stomach, which appeared ecchymotic and ulcerated. Gay and Southard‖ found hemorrhages in most of the organs, and believe anaphylaxis to depend upon the presence, in the blood of the sensitized animal, of a substance to which they have given the name *anaphylactin*. Besredka and Steinhardt** found that by the repeated injection of

* "Journal of Medical Research," 1906, XV, p. 207; "Bull. No. 29 of the Hygienic Laboratory," Washington, D. C., 1906; "Bull. No. 36," 1907, Ibid.; "Jour. Med. Research," 1907, XVI, No. 3, p. 381; "Jour. Infectious Diseases," 1907, IV, No. 1, p. 1, "Jour. Infectious Diseases," 1907, vol. IV, p. 552.

† "Jour. Med. Research," May, 1907, XVI, No. 2, p. 143.

‡ Ibid., June, 1908, XVIII, No. 3, p. 385.

§ "Bull. No. 32 of the Hygienic Laboratory," Washington, D. C., October, 1906.

‖ "Jour. Med. Research," July, 1908, XIX, No. 1, pp. 1, 5, 17.

** "Ann. de l'Inst. Pasteur," February 25, 1907, XXI, No. 2, pp. 117–127.

horse-serum into guinea-pigs, the intervals being too short to permit anaphylaxis, *antianaphylactin* could be prepared. It seems difficult, however, to imagine how such a substance could remain in the blood throughout the entire subsequent life of the animal.

Vaughan has endeavored to explain anaphylaxis by assuming that when the strange protein in the blood reaches the cells it is slowly broken down by enzymic action, but that the cells, having once acquired the property of destroying it, seize eagerly upon the protein the next time it is offered, disintegrate it rapidly, and so disseminate throughout the body the degradation products, some of which may be toxic and account for the reaction.

Anaphylaxis is not a disturbance of the cells of the body, as some have thought, but is at least in part a disturbance of the composition of the blood, as can be shown by the occurrence of what is known as *passive anaphylaxis*. If the blood-serum of a sensitized animal be withdrawn and injected into a normal animal of the same kind, it carries the sensitization with it. The new animal, however, does not become sensitized at once, but only after some days, hence it is equally true that the disturbance is not solely in the blood, else why should not the sensitization be immediately present upon the injection of the serum?

Anaphylaxis may, furthermore, be local. Thus, when certain substances like tuberculin are dropped in the eye there is no effect, but when a second application is made, after some weeks, the eye may be reddened.

Anaphylaxis may play a rôle in infection. In cases where an attack of an infectious disease leaves no immunity, the body may be left hypersensitive to subsequent attacks.

EXPLANATION OF IMMUNITY

Before the facts now at our disposal had been gathered together, and before the phenomena of immunity against infection had been compared with those of intoxication, Pasteur* and Klebs† endeavored to explain acquired immunity by supposing that micro-organisms living in the infected animal used up some substance essential to their existence, and so died out, leaving the soil unfit for further occupation. This was known as the "exhaustion theory." Wernich‡ and Chauveau§ thought it more probable that the micro-organisms after having lived in the body left behind them some substance inimical to their further existence. This was known as the "retention theory." These hypotheses are of historic interest only, and deserve no more than passing mention, as they both fail to explain natural immunity or immunity against intoxication.

* "Compte rendu de la Soc. de Biol. de Paris," XCI.
† "Arch. f. experimentelle Path. u. Pharmak.," XIII.
‡ "Virchow's Archives," Bd. LXXVIII.
§ "Compte rendu de la Soc. de Biol. de Paris," XC and XCI.

Karl Roser* observed that the leukocytes of the bodies of higher animals sometimes enclosed bacteria in their cytoplasm. Koch, Sternberg, and others, confirmed the observation, but no attention was paid to it until Metschnikoff† correlated it with other known facts and original observations, and came to the conclusion that the enclosed bacteria had been eaten by the leukocytes in which they were killed and digested, and that the behavior of the cells toward the bacteria afforded an explanation of the mechanism by which recovery from the infectious diseases takes place. The original conception upon which this "*theory of phagocytosis*" was founded, refers recovery in many, if not all of the infectious diseases, to the successful destruction of the invading bacteria by the body cells, especially the leukocytes. These devouring cells Metschnikoff called *phagocytes*, and of them he recognized two classes, the *microphages*, which are white blood-corpuscles, and the *macrophages*, which are larger cells derived from the endothelial and other tissues.

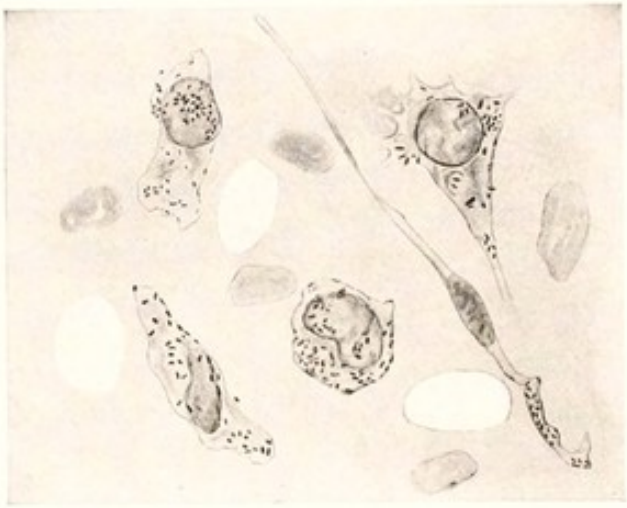


Fig. 17.—Phagocytosis; the omentum immediately after injection of typhoid bacilli into a rabbit. Meshwork showing a macrophage, intermediate forms and a trailer, all containing intact bacilli (Buxton and Torry).

Metschnikoff, his associates, and his pupils soon collected evidence sufficient to show that phagocytosis, if not the chief factor in defending the body from infectious organisms, is at least an important one. Many of the most interesting facts are described in Metschnikoff's books, "Etudes sur l'Inflammation" and "Immunite dans les Maladies Infectieuses," which every interested student of the subject should read.

These studies show that in nearly all cases in which animals are naturally immune against infection, the leukocytes are active in their phagocytic behavior toward them; that in acquired immunity, the leukocytes previously inactive, become active toward them;

* "Beiträge zur Biologie niederster Organismen," Inaugural Dissertation, Marburg, 1881.

† "Virchow's Archives," Bd. xcvi, p. 177; "Ann. de l'Inst. Pasteur," 1887, t. 1, p. 321.

that the enclosure of bacteria within the cells sometimes results in the death of the cells, sometimes in the death of the bacteria; that phagocytosis is much more active in diseases in which the bacteria have limited toxicogenic powers, and in which they probably exert a positively chemotactic influence upon the cells, than in cases in which the bacteria are strongly toxicogenic and probably exert an injurious and negatively chemotactic influence upon them, and that when the toxicogenic power of the bacteria is great, many of the phagocytes are killed and dissolved—*phagolysis*. Study of the primitive forms of animal life shows that amebæ constantly feed upon smaller organisms, some almost exclusively upon bacteria, which they are able to kill and digest through an intracellular enzyme demonstrated by Mouton,* and called *amebadiastase*, and regarded as a form of trypsin. The intracellular digestion of cœlenterate animals is accomplished by means of *actinodiastase*, an enzyme discovered by Fredericq, and studied by Mesnil. It seems to be related to papine and digests albuminoids. The digestion of erythrocytes and tissue fragments is accomplished through an enzyme of the macrophages, which Metschnikoff calls *macrocytase*, that of bacteria through an enzyme of the microphages, which he calls *microcytase*. In phagolysis these respective ferments are liberated into the plasma, imparting to it a bactericidal and bacteriolytic action similar to that normally peculiar to the cytoplasm of the cells. The dissemination of the enzymes in phagolysis, with resulting bacteriolytic power of the blood plasma and serum, is a later modification of the original conception of Metschnikoff, that the invading parasites were eaten up by the phagocytes, and was made necessary by the investigation of the bactericidal property of the body juices. The experiments of Wright and Douglas† indicate that the action of the phagocytes upon the bacteria is not immediate, but only subsequent to a preparative action upon the organisms by substances contained in serum, to which they have given the name "*Opsonins*" (Lat. *opsono*, "I prepare a meal for").

Long before Metschnikoff began his studies of the phagocytes Traube and Gscheidel‡ observed that the blood-plasma possessed the power of destroying the vitality of bacteria. Grohman§ next observed that not only the intravascular, but also the extravascular blood possessed this property. Further studies of the subject were made by von Fodor.‖ The systematic investigation of the bactericidal activity of blood-serum *in vitro* was next taken up by Flügge,** and more particularly by Nuttall,†† who found that dif-

* "Compte rendu de l'Acad. des Sciences de Paris," 1901, CXXXIII, p. 244.

† "Proc. Royal Society of London," 1904, LXXXII, p. 357.

‡ "Jahresberichte der schles. Ges. f. vaterl. Kultur," 1874.

§ "Untersuchungen aus dem physiol. Institut zu Dorpat," Dorpat, 1884; Krüger.

‖ "Centralbl. f. Bakt.," etc., 1890, VII, p. 753.

** "Zeitschrift für Hygiene," Bd. IV, S. 208.

†† Ibid., Bd. IV, 353.

ferent blood-serums possessed the power of killing bacteria in large numbers, but that the bactericidal power of the serum soon disappeared, after which the serum became a good culture-medium for the very bacteria it had formerly destroyed. Metschnikoff objected to the observations, declaring that all the phenomena were ultimately referable to the leukocytes, so Nuttall investigated pericardial fluid and the aqueous humor of the eye, which were also found to possess bactericidal powers.

The matter was next taken up by Buchner and his associates,* who showed that the blood-plasma and blood-serum possessed exactly the same bactericidal effects as the total blood. Buchner and Nuttall both showed that the exposure of the bactericidal fluids to a temperature of 56°C. for a few hours entirely destroyed their activity, though low temperatures were without effect upon them. Buchner found that the exposure of the serum to sunlight and oxygen also destroyed the bactericidal power. Neutralization of alkaline serum did not destroy its activity, but when the serum was dialyzed and the NaCl removed from it, the germicidal power was lost, to return again when it was restored. Buchner called the bactericidal principle *alexin*.

Many interesting facts were collected bearing upon the bactericidal substance or alexin. Thus Moro† showed that it was proportionally more active in sucking infants than in adults, and Ehrlich and Brieger‡ found that it passed from mother to offspring in the milk.

At first Buchner regarded alexin as an albumin, but later§ he came to look upon it as a proteolytic enzyme, this view no doubt resulting from an endeavor to explain the relation of alexin to immunity against intoxication, in which it was necessary to show that alexin not only killed bacteria, but also destroyed toxins.

Hankin‖ endeavored to show that there were differences between the substances destroying the bacteria and those acting upon their toxic products. To the whole group he applied the term *defensive proteins*. Those present in natural immunity he called *sozins*, those found in acquired immunity *phylaxins*. Sozins with bactericidal activity he further described as *mycosozins*, those with toxin-destroying activities as *toxosozins*. Phylaxins with bactericidal action were called *mycophylaxins;* those with toxin-destroying properties *toxophylaxins*.

Metschnikoff found it unnecessary to modify his ideas, but persisted in referring all the phenomena to the phagocytes or to enzymes derived from them.

At this point it will be evident to the reader that the phagocytic

* "Centralbl. f. Bakt.," etc., 1889, Bd. v, 817; vi, 1; "Archiv für Hygiene," 1891, x, S. 727; "Centralbl. f. Bakt.," etc., 1890, vii, 76.

† "Jahresb. f. Kinderheilkunde," v, 396.

‡ "Zeitschrift für Hyg.," 1893, xiii, 336.

§ "Münch. med. Woch.," 1899.

‖ "Centralbl. f. Bakt.," etc., xii, Nos. 22, 23; xiv, No. 25.

theory and the humoral theory contain indubitable evidence that both the body cells and humors are important factors in defending the body against invading organisms, and that in each we see mechanisms operative in certain cases. But we have seen that both Metschnikoff and Buchner are obliged to strain a point in order to meet the requirements of increasing knowledge of the subject of immunity.

Thus, when we come to analyze Buchner's theory of alexins, we find that if natural immunity depends upon the ability of the alexins to destroy bacteria, that which takes place *in vitro* should correspond with that which takes place *in vivo*, and that the invasion of the animal's body by bacteria should be accompanied by diminution of the bactericidal substance in its blood, which should be used up before the bacteria can be successful in their invasion. Experimental evidence is, however, at hand to show that this is not always true.

Behring and Nissen* found that there was a definite relation between the bactericidal power of the blood *in vitro* and the resisting powers of a large number of animals studied, but Lubarsch† showed the remarkable exceptions of the rabbit, which is highly susceptible to anthrax, though its blood is highly bactericidal to the anthrax bacillus, and the dog, which is scarcely susceptible to anthrax, though its blood is scarcely bactericidal to the bacillus.

Flügge‡ found the bactericidal power of the blood greatly lessened in thirty-six hours after anthrax infection, and Nissen that a definite number of bacteria could be killed by a bactericidal serum, after which the alexin became inactive. The diminution of the bactericidal power was shown to occur both in the animal and in the test-tube. He also showed that the reactions of the bactericidal serums were specific, and that when a culture of one kind of bacteria was injected into an animal, the immediate effect was to diminish the activity of the serum for that species, though not necessarily for other species. The diminution of bactericidal energy was shown by him to depend upon the presence of the bacteria, as the injection of filtrates of bacterial cultures did not affect the bactericidal properties of the serum. This was a very important observation.

There is a correspondence between the behavior of the phagocytes and the body juices. When the activity of the phagocytes toward the bacteria is increased, the bactericidal activity of the serum is usually intensified. But immunity is only partly explained by alexins and bacteriolysis, for it embraces the ability of the organism to endure the effects of toxins some of which are in no way connected with bacteria.

Tolerance to certain toxins is, of course, natural to many animals,

* "Zeitschrift für Hygiene," 1890, VIII, 412.
† "Centralbl. f. Bakt.," etc., 1889, VI, 481.
‡ "Zeitschrift für Hygiene," IV, 208.

and tolerance to usually destructive toxins natural to a few. This toxin-neutralizing or annulling factor cannot be identical with the bacteria-destroying mechanism. Cobbett,* Roux and Martin,† and Bolton‡ have shown that horses that cannot be supposed ever to have come into contact with diphtheria bacilli, vary considerably in their resistance to diphtheria toxin, and that the serum of the resisting horses contains something that destroys or neutralizes the toxin *in vitro*, as well as exerts a protective influence upon animals into which it is injected. This substance exerts no inimical action upon the diphtheria bacilli, beyond what a normal serum would do, therefore cannot be alexin, but must be *antitoxin*. Abel§ found that the blood of healthy men occasionally contained some substance capable of neutralizing diphtheria toxin; Stern found one normal serum capable of protecting against typhoid infection and Metschnikoff one that protected against cholera infection. Fischel and Wunschheim‖ found newly born babies immune against diphtheria, presumably because of the presence of a small quantity of demonstrable protective substance in the blood. These are, however, peculiar and exceptional cases.

The most suggestive and fascinating theory of immunity is that of Ehrlich, and is known as the "Seitenkettentheorie" or the "*Lateral-Chain Theory.*"**

He began his studies by an investigation into the nature of toxins and their mode of action. The discovery that there was no constant relation between the intoxicating and antitoxin combining powers of diphtheria toxic bouillon led him to the conclusion that the toxin molecules possessed two different affinities, which he described as *haptophorous* or combining, and *toxophorous* or poisoning. The former were constant, the latter variable. The deterioration in the strength of the toxic filtrates of bouillon cultures of diphtheria bacilli was shown to depend upon the transformation of the

* "Lancet," Aug. 5, 1899, II, p. 532.
† "Ann. de l'Inst. Pasteur," 1894, VIII, p. 615.
‡ "Jour. of Experimental Medicine," July, 1896, I, No. 5.
§ "Centralbl. f. Bakt.," etc., 1895, XVII, p. 36.
‖ "Zeitschr. für Heilkunde," 1895, XVI, p. 429–482.

** The writings of Ehrlich and his associates are so numerous and scattered, and often so fragmentary, that instead of referring to the literature according to the method adopted in other parts of this work, the reader who desires to consult the original articles can best do so by making use of the following: Ehrlich, "Die Werthbemessung des Diphtherie Heilserums," Klinisches Jahrbuch, 1897; Ehrlich, "Die Konstitution des Diphtheriegiftes," Deutsche med. Woch., 1898; "Gesammelte Arbeiten zur Immunitätsforschung," August Hirschwald, Berlin, 1904—this work contains the collected papers of Ehrlich and his associates; Aschoff, "Ehrlich's Seitenkettentheorie und ihre Anwendung auf die Künstlichen Immunusirungs-prozesse," Jena, 1902, and the chapter upon "Wirkung und Entstehung der Aktiven Stoffe im Serum noch der Seitenkettentheorie," by Ehrlich and Morgenroth in Kolle and Wassermann's "Handbuch der Pathogene Mikroorganismen," Jena, 1904, Gustav Fischer. Readers unacquainted with the German language may find the essential facts in Ehrlich's Croonian Lecture, Proceedings of the Royal Society of London, 1900, LXVI, p. 424, and in Welch's "Huxley Lecture," Medical News, 1902, LXXXI, 2, p. 721.

toxin into *toxoids* which were not poisonous, and was shown to be quite independent of the antitoxin combining affinity of the filtrate which remained unaltered. The inevitable interpretation seemed to be the existence in the bouillon of the haptophorous and toxophorous groups described. Similar toxophorous and haptophorous groups were shown to exist in other toxins—tetanolysin by Madsen, venoms by Myers, and milk-curdling ferments by Morgenroth. The neutralizing action of the antibodies produced in the blood of animals immunized to these various substances depends upon the immediate and direct combination or union of haptophorous groups in the antibodies with corresponding haptophorous groups of the respective toxins or active bodies.

The physiological activities of toxins differ from those of alkaloids and other poisons in three fundamentals: first, in their ability to produce antibodies in the bodies of animals into which they are injected; second, the manifestation of poisonous action only after a definite incubation period, and third an extremely labile composition, by which the toxin becomes quickly transformed to toxoids.

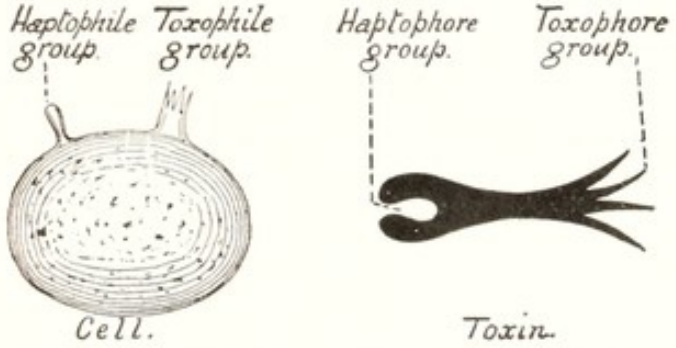


Fig. 18.—Diagram to represent the combining groups of the cell and of the toxin respectively (after Ehrlich) (Hewlett).

Study of the physiological action of toxins upon the cells resulted in showing that certain definite specific affinities existed, and that the union of the toxin with the cell antedated the production of symptoms. In some cases it was even found possible to disconnect the anchored toxin by bringing to the cells haptophorous groups for which the haptophorous elements of the toxin molecule were known to have an active affinity. Dönitz determined the quantity of tetanus antitoxin which, injected into the circulating blood immediately after the toxin, absolutely neutralized it and rendered all of the circulating toxin innocuous. If the same quantity of antitoxin was given seven or eight minutes after the injection of the toxin, death occurred from tetanus, exactly as if no antitoxin had been given. Evidently the toxin had anchored itself to the nerve-cells too quickly for the antitoxin to reach and combine with it. Heymans found that if an animal was injected with tetanus toxin and its entire blood withdrawn immediately afterward and replaced by

transfusion, it died of typical tetanus because in the brief interval between the toxin injection and the transfusion, the toxin molecules became anchored to the cell.

The ability of the cells thus to anchor the toxin is supposed by Ehrlich to depend upon the existence of haptophorous combining affinities, which he describes as *receptors*. He views the mode of toxin reception as depending upon a mechanism either identical with or analogous to that by which cellular nutrition is maintained, and points out that in the case of methylene-blue and other colored substances, which afford an opportunity to make ocular observations upon the absorption of the pigment by the cells, only certain cells absorb the colors.

Cell nutrition is therefore probably carried on through the agency of receptors by which appropriate nutrient haptophorous groups are apprehended and utilized.

The following somewhat lengthy quotation from his "Croonian Lecture upon the Lateral Chain Theory of Immunity," delivered before the Royal Society of London, March 22, 1900, explains the theory in Ehrlich's own words:

"We now come to the important question of the significance of the toxophile groups in organs. That these are in function especially designed to seize on toxins cannot be for one moment entertained. It would not be reasonable to suppose that there were present in the organism many hundreds of atomic groups destined to unite with toxins, when the latter appeared, but in function really playing no part in the processes of normal life, and only arbitrarily brought into relation with them by the will of the investigator. It would, indeed, be highly superfluous, for example, for all our native animals to possess in their tissues atomic groups deliberately adapted to unite with abrin, ricin, and crotin, substances coming from far-distant tropics."

"One may, therefore, rightly assume that these toxophile protoplasmic groups in reality serve normal functions in the animal organism, and that they only incidentally and by pure chance possess the capacity to anchor themselves to this or that toxin."

"The first thought suggested by this assumption was that the atom group referred to must be concerned in tissue change; and it may be well here to sketch roughly the laws of cell metabolism. Here we must, in the first place, draw a clear line of distinction between those substances which are able to enter into the composition of the protoplasm, and so are really assimilated, and those which have no such capacity. To the first class belong a portion of the food-stuffs, *par excellence;* to the second almost all our pharmacological agents, alkaloids, antipyretics, antiseptics, etc."

"How is it possible to determine whether any given substance will be assimilated in the body or not? There can be no doubt that assimilation is in a special sense a synthetic process—that is to say, the molecule of the food-stuff concerned enters into combination with the protoplasm by a process of condensation involving loss of a portion of its water. To take the example of sugar, in the union with protoplasm, not sugar itself as such, but a portion of it, comes into play, the sugar losing in the union some of its characteristic reactions. The sugar behaves here as it does, *e.g.*, in the glucosids, from which it can only be obtained through the agency of actual chemical cleavage. The glucosid shows no traces of sugar when extracted in indifferent solvents. In a quite analogous manner the sugar entering into the composition of albuminous bodies (glycoproteids) cannot be obtained by any method of extraction, at least not until chemical composition has previously taken place. It is, therefore, generally easy by means of extraction experiments to decide whether any given combination in which the cells take part is, or is not, a synthetic one. If alkaloids, aromatic amines, antipyretics, or anilin dyes be introduced into the animal body, it is an easy matter,

by means of water, alcohol, or acetone, according to the nature of the body, to remove all these substances quickly and easily from the tissues."

"This is most simply and convincingly demonstrated in the case of the anilin dyes. The nervous system stained with methylene-blue or the granules of the cells stained with neutral red at once yield up the dye in the presence of alcohol. We are, therefore, obliged to conclude that none of the foreign bodies just mentioned enter synthetically into the cell complex, but are merely contained in the cells in their free state." . . . . "Hence with regard to the pharmacologically active bodies in general, it is not allowable to assume that they possess definite atom groups, which enter into combination with corresponding groups of the protoplasm. This corresponds, as I may remark beforehand, with the incapacity of all these substances to produce antitoxins in the animal body. We must, therefore, conclude that only certain substances, food-stuffs, *par excellence*, are endowed with properties admitting of their being, in the previously defined sense, chemically bound by the cells of the organism. We are obliged to adopt the view that the protoplasm is equipped with certain atomic groups, whose function especially consists in fixing to themselves certain food-stuffs of importance to the cell-life." We may assume that the protoplasm consists of a special executive center, in connection with which are nutritive side-chains, which possess a certain degree of independence and which may differ from one another according to the requirements of the different cells. And as these side-chains have the office of attaching to themselves certain food-stuffs, we must also assume an atom-grouping in these food-stuffs themselves, every group uniting with a corresponding combining group of a side-chain.

Fig. 19.—Shows how the haptophores having united, the toxophores find a secondary adaptation to the cell, and so can poison it (after Ehrlich) (Hewlett).

The relationship of the corresponding groups, *i.e.*, those of the food-stuff and those of the cell, must be specific. They must be adapted to one another, as, *e.g.*, male and female screw (Pasteur), or as lock and key (E. Fischer). From this point of view, we must contemplate the relation of the toxin in the cell."

"We have already shown that the toxins possess for the antitoxins an attaching haptophore group, which accords entirely in its nature with the conditions we have ascribed to the relation existing between the food-stuffs and the cell side-chains. And the relation between toxin and cell ceases to be shrouded in mystery if we adopt the view that the haptophore groups of the toxins are molecular groups fitted to unite not only with the antitoxins, but also with the side-chains of the cells, and that it is by their agency that the toxin becomes anchored to the cells."

"We do not, however, require to suppose that the side-chains, which fit the haptophore group of the toxins, that is, the side-chains which are toxophile, represent something having no function in the normal cell economy. On the contrary, there is sufficient evidence that the toxophile side-chains are the same as those which have to do with the taking up of the food-stuffs by the protoplasm. The toxins are, in opposition to other poisons, of extremely complex structure, standing in their origin and chemical constitution in very close relationship to the proteids and their nearest derivatives. It is, therefore, not surprising that they possess a haptophore group corresponding with that of a food-stuff. Alongside of the binding haptophore group, which conditions their union to the protoplasm, the toxins are possessed of a second group, which in regard to the cell is not only useless but actually injurious. And we remember that in the case of the diphtheria toxin there was reason to believe that there existed along-

side of the haptophore group another and absolutely independent toxophore group." . . . . "As has been said, the possession of a toxophile group by the cell is the necessary preliminary and cause of the poisonous action of the toxin." . . . . "If the cells of these organs [organs essential to life] lack side-chains fitted to unite with them, the toxophore group cannot become fixed to the cell, which therefore suffers no injury, *i.e.*, the organism is naturally immune. One of the most important forms of natural immunity is based upon the circumstance that in certain animals the organs essential to life are lacking in those haptophore groups which seize upon definite toxins. If, for example, the ptomaine occurring in sausages, which for man, monkeys, and rabbits is toxic in excessively minute doses, is for the dog harmless in quite large quantities, this is because the binding haptophore groups being wanting, the ptomaine cannot, in the dog, enter into direct relation with organs essential to life." . . . . "The haptophore group exercises its activity *immediately* after injection into the organism, while in all toxins—with the perhaps solitary exception of snake-venom—the toxophore group comes into activity after the lapse of a longer or shorter incubation period which may, *e.g.*, in the case of diphtheria toxin, extend to several weeks."

"The theory above developed allows of an easy and natural explanation of the origin of antitoxins. In keeping with what has already been said, the first stage in the toxin action must be regarded as the union of the toxin by means of its haptophore group to certain 'side-chains' of the cell protoplasm. This union is, as animal experiments with a great number of toxins show, a firm and enduring one. The side-chain involved, so long as the union lasts, cannot

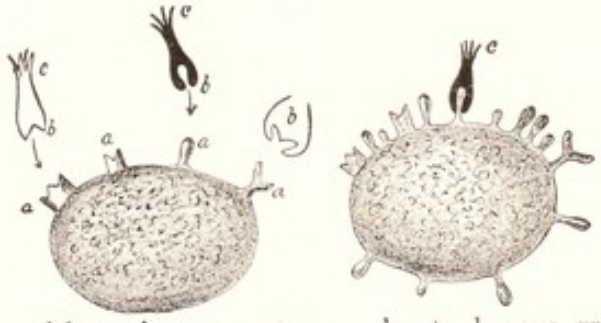
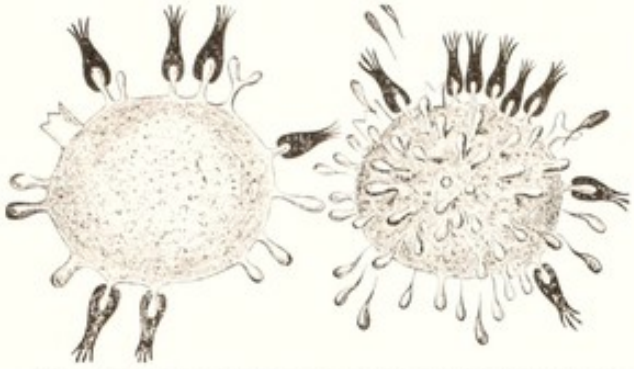


Fig. 20.—Cells with various receptors or haptophorous groups of the first order (*a*), adapted to combination with the haptophorous groups (*b*) of various chemical compounds brought to them. It will be noted that there is no mechanism by which the toxophorous elements of the molecules (*c*) can be brought to the cell.

exercise its normal nutritive physiological function—the taking up of food-stuffs. It is, as it were, shut out from participating, in the physiological sense, in the life of the cell. We are, therefore, now concerned with a defect which, according to the principles so ably worked out by Professor Carl Weigert, is repaired by regeneration. These principles, in fact, constitute the leading conception of my theory. If after union has taken place new quantities of toxin are administered at suitable intervals and in suitable quantities, the side-chains, which have been reproduced by the regenerative process, are taken up anew into union with the toxin, and so again the process of regeneration gives rise to the formation of fresh side-chains. In the course of the progress of typical systematic immunization, as this is practised in the case of diphtheria and tetanus toxin especially, the cells become, so to say, educated or trained to reproduce the necessary side-chains in ever-increasing quantity. As Weigert has confirmed by many examples, this, however, does not take place by the simple replacement of the defect; the compensation proceeds far beyond the necessary limit; indeed, overcompensation is the rule. Thus the lasting and ever-increasing regeneration must finally reach a stage at which such an excess of side-chains is produced that, to use a trivial expression, the side-chains are present in too great a quantity for the cell to carry and are, after the manner of a secretion, handed over as needless ballast to the blood. Regarded in accordance with this conception, the *antitoxins represent nothing more than side-chains reproduced in excess during regeneration and therefore pushed off from the protoplasm and so coming to exist in the free state.*"

"In the first place, our theory affords an explanation of the specific nature of the antitoxins, that tetanus antitoxin is only caused to be produced by tetanus toxin, and diphtheria antitoxin through diphtheria toxin. This very specific nature of the affinity between toxin and cell is the necessary preliminary and cause of the toxicity itself. Further, our theory makes it easy to understand the long-lasting character of the immunity produced by one or several administrations of toxin, and also the fact that the organism reacts to relatively small



Figs. 21 and 22.—Show the regeneration of the cell-haptophores or receptors to compensate for the loss of those thrown out of service.

quantities of toxin by the production of very much greater quantities of antitoxin. By the act of immunization, certain cells of the organism become converted into cells secreting antitoxin at the same rate as this is excreted. New quantities of antitoxin are constantly produced, and so throughout a long period the antitoxin content of the serum remains nearly constant. The secretory nature of the formation of antitoxins has been very strikingly illustrated by the beautiful experiments of Salmonson and Madsen, who have shown that pilo-

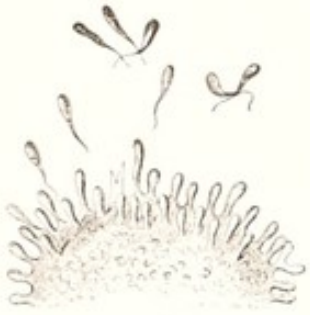


Fig. 23.—Shows the number of haptophores regenerated by the cell becoming excessive; they are thrown off into the tissue juice.

Fig. 24.—Explains what antitoxins are and how they are formed. The liberated receptors in the tissue juice and in the blood, possess identical combining affinities with those upon the cell, and meeting the adapted haptophorous elements in the blood, combine with them, thus keeping them from the cells.

carpine, which augments the secretion of most glands, also occasions in immunized animals a rapid increase in the antitoxin content of the serum."

"The production of antitoxins must, in keeping with our theory, be regarded as a function of the haptophore group of the toxin, and it is easy therefore to understand why, out of the great number of alkaloids, none are in a position to cause the production of antitoxins. Conversely, indeed, I recognize in this incapacity of the alkaloids, in opposition to the toxins, to produce antitoxins a further and salient proof of the truth of the deduction I have previously based on chemical grounds, that the alkaloids possess no haptophore group which

anchors them to the cells of organs. To formulate a general statement, the capacity of a body to cause the production of antitoxin stands in inseparable connection with the presence of a haptophore atomic group. In the formation of antitoxin the toxophore group of the toxin molecule is, on the contrary, of absolutely no moment. But the toxoid modification of the toxins, in which the haptophore group of the toxin is retained, while the toxophore group has ceased to be active, possesses the property of producing antitoxins. Indeed, in some cases of extremely susceptible animals, immunity can only be attained by means of the toxoids, and not by the too strongly acting toxins." . . . . "The symptoms of illness due to the action of the toxophore group, therefore, play no part in the production of antitoxin." The effect of enzymes upon the organism with the production of antibodies, and the "specific precipitins" caused by the injection of milk, albumin, and peptones into animals may be looked upon as "having their origin in the most widely diverse organs, and representing nothing more than nutritive side-chains, which in the course of the normal nutritive processes have been developed in excess and pushed off into the blood."

"Much more complex than in the cases hitherto discussed are the conditions when, instead of the relatively simple metabolic products of microbes, the living micro-organisms themselves come to be considered, as in immunization against cholera, typhoid, anthrax, swine-fever, and many other infectious diseases. Thus there come into existence, alongside of the antitoxins produced as a result of the action of the toxins, manifold other reaction products. This is because the bacterium is a highly complicated living cell of which the solution in the organism yields a great number of bodies of different nature, in consequence of which a multitude of 'antikörper' are called into existence. Thus we see, as a result of the injection of bacterial cultures, that there arise alongside of the specific bacteriolysins, which dissolve the bacteria, other products, as, for example, the 'coagulins' (Kraus, Bordet), *i.e.*, substances which are able to cause the precipitation of certain albuminous bodies contained in the culture fluid injected; also the much-discussed agglutinins (Durham, Gruber, Pfeiffer), the antiferments (von Düngern), and no doubt many other bodies which have not yet been recognized. It is by no means unlikely that each of these reaction products finds its origin in special cells of the body; on the other hand, it is quite likely that the formation of any single one of these bodies is not of itself sufficient to confer immunity. Thus, in the case of the introduction of bacteria into the body we have to do with a many-sided production of different forms of 'antikörper,' each of which is directed only against one definite quality or metabolic product of the bacterial cell. Accordingly, in recent times, the practice of using for the production of immunization definite toxic bodies isolated from the bacterial cells has been more and more given up, and for this purpose it is now regarded as important to employ the bacterial cells as intact as possible." . . . "The most interesting and important substances arising during such an immunizing process are without doubt the bacteriolysins." . . . . "Belfanti and Carbone first discovered the remarkable fact that horses which had been treated with the blood-corpuscles of rabbits contain in their serum constituents which are poisonous for the rabbit, and for the rabbit only." . . . "Bordet showed shortly thereafter that in the case quoted there was present in the serum a specific hemolysin which dissolved the corpuscles of the rabbit. He also proved that these hemolysins—as had already been shown by Buchner and Daremberg in the case of similarly acting bodies which are present in normal blood—lost their solvent property on being maintained during half an hour at a temperature of 55°C. Bordet added, further, a new fact, that the blood-solvent property of those sera which had been deprived of solvent power by heat, the solvent action could be restored if certain normal sera were added to them. By this important observation an exact analogy was established with the facts of bacteriolysis as elicited by the work of Pfeiffer, Metschnikoff, and Bordet."

. . . "In collaboration with Dr. Morgenroth, I have sought in regard to this question, for which hemolysis offered prospects favorable to experimentation, to make clear the mechanism concerned in the action of these two compounds—the stable, which may be designated 'immune body,' and the unstable, which may be designated 'complement'—which acting together effect the solution of the red blood-corpuscles. For this purpose, in the first place, solutions containing either only the 'immune body' or only the 'complement' were brought in contact with suitable blood-corpuscles, and after separation of the fluid and the corpuscles

by centrifugalization, we investigated whether these substances had been taken up by the red corpuscles or remained behind in the fluid. The proof of its location in the one position or in the other was readily forthcoming, since to restore the hemolysin to its former activity, it was only necessary to add to the 'immune body' a fresh supply of 'complement,' or to the 'complement' a fresh supply of 'immune body' in order that the presence of the hemolysin in its integrity might be shown by the occurrence of solution of the red cells. The experiments proved that, after centrifugalizing, the 'immune body' is quantitatively bound to the red blood-corpuscles, and that the 'complement,' on the contrary, remains entirely behind in the fluid. The presence of the two components in contact with blood-corpuscles only occasions the solution of these at higher temperatures, and not at 0°C. And an active hemolytic serum (with 'immune body' and 'complement' both present) having been placed in contact with red blood-corpuscles and maintained for a while at 0°C., it was found after centrifugalizing that, under these circumstances also the 'immune body' had united with the red blood-corpuscles, but that the 'complement' remained in the serum. This experiment showed that both components must, at a temperature of 0°C., have existed alongside of one another in a free condition." . . . .

"But when analogous experiments were undertaken at a higher temperature it was found that both components were retained in the sediment.

"These facts can only be explained by making certain assumptions regarding the constitution of the two components, *i.e.*, of the 'immune body' and the 'complement.' In the first place, two haptophore groups must be ascribed to the 'immune body,' one having affinity for a corresponding haptophore group of the red blood-corpuscles and with which at a lower temperature it quickly unites, and another haptophore group of a lesser chemical affinity, which at a higher temperature becomes united with the 'complement' present in the serum. Therefore at the higher temperature the red blood-corpuscles will draw to themselves those molecules of the 'immune body' which in the fluid have previously become united to the 'complement.' In this case the 'immune body' represents in a measure the connecting chain which binds the complement to the red blood-corpuscles and so brings them under its deleterious influence. Since under the influence of the 'complement'—at least, in the case of the bacteria—appearances are to be observed (for example, in the Pfeiffer phenomenon) which must be regarded as analogous to digestion, we shall not seriously err if we ascribe to this 'complement' a ferment-like character." . . . . "Having obtained a precise conception of the method of action of the lysins of the serum—of the hemolysins, and thereby also of the bacteriolysins—it becomes possible for us to attempt to solve the mystery of the origin of these bodies. I have in the beginning of this lecture fully developed the 'side-chain theory,' according to which the antitoxins are merely certain of the protoplasm 'side-chain' which have been produced in excess and pushed off into the blood.

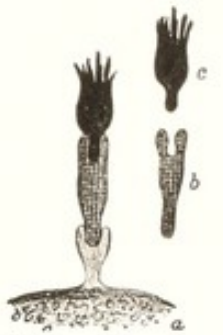


Fig. 25.—Combination of cell (*a*), amboceptor (*b*), and complement (*c*). The amboceptor may unite with the cell, but cannot affect it alone. The complement cannot unite with the cell except through the amboceptor, having no adaptation to the cell directly.

"The toxins as secretion products of the cells are in all likelihood still relatively uncomplicated bodies; at least by comparison with the primary and complex albumins of which the living cell is composed.

"If we now recognize that the different lysins arise only through absorption of highly complex cell material—such as red blood-corpuscles or bacteria—then the explanation, in accordance with what I have said, is that there are present in the organism 'side-chains' of a special nature, so constituted that they are endowed not only with an atomic group by virtue of the affinities of which they are enabled to pick up material, but also with a second atomic group, which, being ferment-loving in its nature, brings about the digestion of the material taken up. Should the pushing off of these 'side-chains' be forced, as it were, by immunization, then the 'side-chains' thus set free must possess both groups, and will, therefore, in their characteristics entirely correspond with what we have placed beyond doubt as regards the 'immune body' of the hemolysin."

An analysis of this theory shows complete natural immunity to depend upon the absence of haptophore groups (receptors) by which the toxins can be united to the cells. Extreme sensitivity or susceptibility probably depends upon the adapted haptophores being present or at least most numerous upon the cells of highly vital organs; comparative insensitivity or insusceptibility upon the fact that the greater number of haptophore groups are attached to comparatively unimportant cells whose combining affinities have to be satisfied before combination with more vital cells can be accomplished. In some cases natural immunity is increased by the presence of free haptophore groups (antitoxin) in the blood.

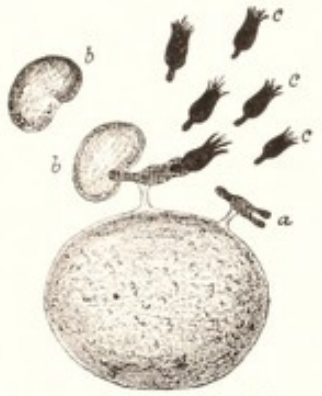


Fig. 26.—Cell with receptors of the second order (*a*) by which the cells fix useful molecules, of albumins, etc., on one hand (*b*), and zymogen molecules (*c*) on the other hand, and make use of the one substance through the action of the other.

Acquired immunity against toxins depends upon the regeneration of the cellular haptophores or receptors which, being liberated into the body juices, fix the haptophores of the toxin molecules before they are able to reach the cells themselves. Antitoxins and other anti-bodies, including the lysins, consist of liberated cellular haptophores or receptors, the former having a single combining affinity, the latter a double combining affinity, by which they unite, on the one hand, with the cell to be dissolved, on the other with the complement by which it is to be dissolved. Antibodies having this double combining affinity have been called "*amboceptors*" by Ehrlich. They are variously known in different writings as "immune bodies," amboceptors, *substance sensibilisatrice*, desmon, and *fixateur*. The "*complement*" or "addiment" of Ehrlich is also called alexin and cytase. Ehrlich conceives every amboceptor and every complement to be specific, but Bordet and others, while admitting that the amboceptor is specific, hold that there is but one complement or cytase.

It has already been said that Metschnikoff's primitive conception of the body being defended against infection through the phagocytic incorporation and digestion of the microparasites, has had to be modified to conform to the increasing information upon the immunity reactions. He has persistently clung to the idea that the phagocytes are the essential factors, but has changed the conception of "phagocytosis" to make it applicable to the new requirements. He now teaches that when invasive micro-organisms enter the body, chemotactic influences determine that they shall be met by phagocytes. If the invading micro-organisms are too powerful and the phagocytes are killed, phagolysis or dissolution of the phagocytes liberates their enzymes into the blood. These liberated enzymes still act deleteriously upon the invaders, tending to ag-

glutinate—aggregate them in clumps—and sensitize them to the future action of other phagocytes by which they may be taken up. Through extensive phagolysis, and the liberation of large quantities of the enzyme contents of the phagocytes into the blood, the plasma and serum acquire a "fixing" or "sensitizing" quality from the *macrocytase* of the macrophages, which is the "*fixateur*" or "*substance sensibilisatrice*," and a bacteria-dissolving quality forms another enzyme, *microcytase*, from the microphages. Thus, we find that Metschnikoff is prepared to account for the "amboceptor" or "immune body" of Ehrlich, which is the *macrocytase*, and the "complement," which is the "*microcytase*." In cases where the bacteria exert a negatively chemotactic influence upon the leukocytes, no immunity exists.

The antitoxins are similarly accounted for by Metschnikoff: the cellular digestive enzymes exert their action not only upon the microparasites, but also upon their products, fixing or otherwise altering them until they can be finally destroyed.

It will thus be seen that the two chief theories of immunity, though they appear discordant when explained independently of one another, can be fairly well harmonized. Ehrlich believes the immune bodies to be the products of those cells of the body with whose haptophile combining groups the haptophore groups of the antigen engaged, and does not attribute the function to any particular group of cells; Metschnikoff attributes all the activities to the phagocytes, and especially the leukocytes. Ehrlich looks upon the phenomena as chemical and pictures them as taking places independently of the cells; Metschnikoff looks upon them as vital and brought about by the agency of living cells. Both theories are ultimately chemical.

The fundamental ideas embodied in the "lateral-chain theory" of immunity may, by reversing the hypothesis and considering the bacterial instead of the body cells to be upon the defensive, be made to explain other phenomena of immunity. Walker* seems to have been the pioneer in this field, and his researches show that it is possible to immunize bacteria against "immune serums" by cultivating them in media containing increasing proportions of the immune serums. The bacteria thus cultivated were of increased virulence. The idea was further amplified by Welch in his Huxley Lecture.† The microorganismal cells must be regarded as endowed with receptors of their own, fitted for combination with adapted haptophorous elements in the juices reaching them, and therefore capable of reacting toward such substances exactly as do the cells of the host. As the host reacts toward the active products of the bacteria, so the bacteria react toward the defensive products of the host, and as the cells of the former are stimulated to the production of immune bodies that shall facilitate bacteriolysis, so the latter are stimulated to antagonize their action by producing neutralizing bodies. These neutralizing bodies by which the defenses of the host are broken down are among those described by Bail‡ as "*aggressins*."

Thus, as the cells of the host invaded are constantly reacting to the active

* "Jour. of Path. and Bact.," March, 1902, VIII, No. 1, p. 34.

† "British Medical Journal," Oct. 11, 1902, p. 1105; "Medical News," Oct. 18, 1902.

‡ "Wiener klin. Woch.," 1905, Nos. 9, 14, 16, 17; "Berl. klin. Woch.," 1905, No. 15; "Zeitschr. f. Hyg.," 1905, Bd. 1, No. 3.

bodies produced by the invading parasites, so the latter are reacting toward the defensive products of the former. If the reactive processes of the host predominate, immunity and the destruction of the parasites result; if those of the bacteria predominate, increased virulence, facilitated invasion, and death of the host may result. This hypothesis also serves to make clear why micro-organisms entering the body not infrequently show a marked tendency to colonize in certain organs and tissues in preference to others.

Supposing accident to determine the tissue in which the primary infection has taken place, a longer or shorter residence in that tissue, with the resulting more or less marked acquired immunity against the defensive activities of that tissue, endow the organism with a higher degree of virulence for it than for other tissues, so that if at some future time the organism entering the circulation of a new host were able to colonize in any tissue of the body, its activities could be more easily and more successfully manifested in that to which it had already become accustomed, and to which it had acquired a peculiar adaptability. This adaptability has been made the subject of interesting experimental demonstration by Forssner* in his work upon the intravenous injection of streptococci.

SPECIAL PHENOMENA OF INFECTION AND IMMUNITY

Certain phenomena which present themselves in the course of infection and immunity, to which reference has already been casually made, must now be considered in detail.

SPECIFIC PRECIPITATION

Specific precipitation is the coagulation or precipitation of an antigen by its specific antibody. In 1897 Kraus† while studying the "specific reactions produced by homologous serums with germ-free filtrates of bouillon cultures, of cholera, typhoid and plague bacteria," observed that immune serum brought into contact with the respective culture filtrate occasioned a precipitate specific in nature, to which he gave the name "specific precipitate."

Bordet‡ and Tchistowitch§ showed that the phenomenon was of wide occurrence and had a broad significance, for they discovered that when the serum of one animal was injected into another animal of different kind, some reaction took place in the injected animal, which caused a precipitate to form whenever the serums of the two animals were being subsequently brought together in a test-tube. The same was found true of milk. When an animal was injected with the milk of a different kind of animal, its serum acquired the property of causing a precipitate to form when its serum and filtered milk were mixed together in a test-tube. The substance or factor inducing the precipitation was called "*precipitin*" or "*coagulin*." Myers,‖ Jacoby,** Nolf,†† and others showed that the faculty of provoking specific precipitins was common to many albuminous bodies—albumen, globulin, albumose, peptone, ricin, etc. Kraus in his original communication dwelt upon the *specific nature of the precipitation*, and was corroborated by Fish,‡‡ Wasser-

* "Nordiskt Medicinskt Archiv," 1902, Bd. XXXV, p. 1.
† "Wiener klin. Woch.," 1897, No. 32.
‡ "Ann. de l'Inst. Pasteur," 1899, p. 173.
§ "Ann. de l'Inst. Pasteur," 1899, p. 406.
‖ "Centralbl. f. Bakt.," etc., 1900, Bd. XXXIII, and "The Lancet," 1900, II, p. 98.
** "Archiv für exper. Path. u. Pharmak.," 1900.
†† "Ann. de l'Inst. Pasteur," 1900, p. 297.
‡‡ "Courier of Medicine," St. Louis, Feb., 1900.

mann,* Morgenroth, and others, by whom it has been shown that the reaction is sufficiently accurate to make possible the differentiation of human and goat's milk. The most important practical application of the specific character of the precipitins, however, came through Uhlenhuth† and Wassermann,‡ who made use of it for the differentiation of bloods for forensic purposes.

Uhlenhuth gave rabbits intraperitoneal injections of 10 cc. of defibrinated blood at intervals of from six to eight days and found the blood-serum strongly precipitant after the fifth. He used such serum for testing the reaction with the bloods of oxen, horses, donkeys, pigs, sheep, dogs, cats, deer, hares, guinea-pigs, rats, mice, rabbits, chickens, geese, turkeys, pigeons, and men.

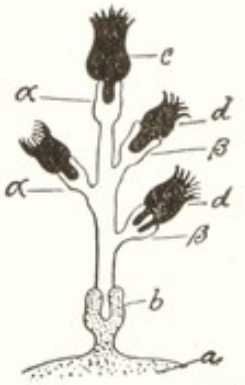


Fig. 27.—Polyceptor (Ehrlich and Marshall) such as can be conceived to occur in hemolysis and bacteriolysis where various complements are engaged. *a*, Receptor of bacterial cell; *b*, cytophil group of the amboceptor; *c*, dominating complement; *d*, subordinate complement; α, β, complementophil groups of the amboceptor, α for the dominating, β for the subordinate complements.

The method of making the test is important, as carelessness of detail will interfere with the accuracy of the result. The blood to be tested is diluted about 1 : 100, or until it has a feeble red color, with tap water, and then freed from corpuscular stroma by filtration or decantation. Two cubic centimeters of it are placed in a small test-tube, and further diluted with an equal quantity of physiological salt solution (if more water be added a precipitate of globulin might take place and spoil the experiment). To such a prepared blood solution, from six to eight drops of the immune serum are added. If the diluted blood come from the same kind of animal as that whose blood was used to immunize the animal furnishing the test serum, immediate clouding takes place, and a flocculent precipitate forms. The precipitate never occurs with any other blood.

Wassermann and Schutze§ prepared a test serum by injecting rabbits with human blood. They tested its precipitating powers upon twenty-three other kinds of blood and found no precipitate except with the blood of a baboon, but the reaction in that case was not nearly so marked as with human blood.

The most interesting and one of the most important biological applications of this phenomenon is by Nuttall, whose work, "Blood Immunity and Blood Relationship" (Cambridge, 1904), should be read by all who wish to study the subject for its scientific interest as a means of determining the blood relationship of animals, or its

* "Verhandl. d. Kong. f. innere Med.," 1900, 501, Wiesbaden.

† "Deutsche med. Woch.," 1900 and 1901.

‡ "Samml. klin. Vortr. von Volkman," Leipzig, Verlag von Breitkopf and Härtel, 1902.

§ "Deutsche med. Wochenschrift," 1900, No. 30.

practical medicolegal importance in recognizing blood-stains. Nuttall comes to the following conclusions:

"(1) The investigations we have made confirm and extend the observations of others with regard to the formation of specific precipitins in the blood-serum of animals treated with various sera. (2) These precipitins are specific, although they may produce a slight reaction with the sera of allied animals. (3) The substance in serum which brings about the formation of a precipitin, as also the precipitin itself, are remarkably stable bodies. (4) The new test can be successfully applied to a blood which has been mixed with those of several other animals. (5) We have in this test the most delicate means hitherto discovered of detecting and testing bloods, and consequently we may hope that it will be put to forensic use."

Further perfection in the technic of the precipitation experiments can be found in a paper by Nuttall and Inchley.*

The precipitinogen is capable of acting as an antigen and the injection into animals of serum containing it results in the formation of anti-precipitins.

AGGLUTINATION

Agglutination is a phenomenon of infection and immunity in which the serum or other body juice of the infected animal so acts upon the infecting micro-organism as to destroy its power of movement, and cause it to sediment in clusters in the liquid in which it is suspended. This phenomenon was first observed by Charrin and Roger† in the course of experiments with Bacillus pyocyaneus. They found that when bacillus pyocyaneus was introduced into a test-tube containing the diluted serum of an animal infected with or immunized against it, the bacilli ceased their active movements, became aggregated in clusters and settled to the bottom of the tube, leaving the supernatant fluid clear. Observations confirming and enlarging upon the subject were made by Metschnikoff,‡ Issaeff§ and others. Gruber and Durham‖ made an elaborate and now classic study of the subject, first employing the term "agglutination" to the phenomenon, and "agglutinins" to the substances in the serum by which it might be brought about. They found that when cholera or typhoid bacilli are mixed with their respective immune serums, the organisms lose motility and become aggregated in clusters, masses or "clumps." They further showed the reaction to be specific within certain limitations, *i.e.*, typhoid immune serum agglutinated typhoid-like bacilli but no others, etc., and they saw in the phenomenon a practical means for the differentiation of different, closely related bacteria, an application that has, indeed, become a useful one.

It remained for Widal** to show that it had a much more important

* "Journal of Hygiene," 1904, IV, p. 201.
† "Compte rendu de la Soc. de Biol.," 1899, p. 667.
‡ "Ann. de l'Inst. Pasteur," 1891, V.
§ Ibid., 1893, VII.
‖ "Münchener med. Woch.," 1896, No. 9.
** "Société Médicale des Hopitaux," June 26, 1896.

application, in that the micro-organism being known, the effect produced by a serum upon it would be an indication of the infection of the animal from which the serum was secured. The first practical application was made in connection with the diagnosis of typhoid fever, and the brilliant success attending it has led to the test being known as the "Widal reaction."

The agglutinins are stable substances that resist drying and can be kept dry and active for years. Widal and Sicard found that they pass with difficulty through a porcelain filter and do not dialyze. They are precipitated in part by 15 per cent. of sodium chloride that throws down fibrinogen and further precipitated with magnesium sulphate, which throws down globulins. They therefore thought them to be intimately related to the globulins and to fibrinogen. A temperature of 60°C. diminishes their activity, but they are not destroyed below 80°C. Sunlight has no effect upon them.

Metschnikoff looks upon agglutination as preliminary to phagocytosis and to bacteriolysis, and thinks it the effect of enzymes in the serum preparing and clustering the bacteria to be taken up by the phagocytes. Ehrlich* finds in the agglutinins nothing more than receptors of what he denominates the II order, each of which possesses a zymophore and an agglutinophore group.

Malvoz† found that the addition of chemical substances, such as safranin, vesuvin, and corrosive sublimate, to cultures of the typhoid bacilli would cause their agglutination. Typhoid bacilli retained on the Chamberland filter and washed for a long time, could no longer be agglutinated, and were found to have lost their flagella and to be without motion. This led Dineur,‡ who made additional experiments, to conclude that agglutination depended upon the flagella. Malvoz§ found that bacteria were sometimes agglutinated by their own metabolic products. He prepared a fresh culture of the first vaccine of the anthrax bacillus by thoroughly distributing it through ½ cc. of distilled water, and then added a loopful of a six-day-old culture. After standing for a few hours typical agglutinations were observed under the microscope.

H. C. Ernst and Robey‖ found that flagella have nothing to do with agglutination, which subsequent experiment has shown to be correct, as non-flagellated bacteria can be agglutinated by their respective serums quite as well as the flagellated forms.

Bail,** Joos††, Eisenberg and Voll‡‡ have shown that all of the agglutinins possess haptophore and agglutinophore groups, either of which may be destroyed without the other. Thus typhoid

* See Nothnagel's "Specielle Pathologie und Therapie," 1901, VIII.
† "Ann. de l'Inst. Pasteur," 1897, No. 6.
‡ "Bull. de l'Acad. de Med. de Belgique," 1898, IV, p. 705.
§ "Ann. de l'Inst. Pasteur," Aug. 25, 1899.
‖ "Trans. Cong. Amer. Phys. and Surg.," 1900, p. 26.
** "Archiv f. Hyg.," 1902, XLII, Heft 4.
†† "Zeitschr. f. Hyg.," 1901, XXXVI, p. 422.
‡‡ Ibid., 1902, XL, p. 155.

agglutinative serum when exposed to a temperature of 65°C. loses the agglutinophores, and no longer clumps the bacteria, though it retains the haptophores, and when brought into contact with the bacteria combines with them, producing no agglutination, but preventing the action of unheated agglutinogenic serum.

Buxton and Vaughan* found that bacteria differ both in their agglutinogenic powers and their agglutinability, both of which must be taken into account in studying the subject.

Theobald Smith† has shown that there are two kinds of agglutinins, one of which acts upon the bacteria directly, the other through the flagella. The occurrence of these two bodies explains some of the incompatible results of previous experiments.

The reaction is one of the most delicate known to us for the identification of bacteria. It is so specific that, in the case of many organisms, it is even possible to tell from what original source they may have come, and always to tell to what variety they belong. It is, moreover, a comparatively simple method that can be used by physicians with little technical skill. The various serums necessary can be obtained from the large public and commercial laboratories where animals immunized against various cultures can always be kept on hand and periodically bled. The serums, sealed in small tubes, can be kept an almost unlimited length of time and shipped to any distance ready for use when opened and diluted.

There is no uniform technic by which to apply the test. Scarcely any two laboratories employ the same method, but the results are uniform and the method to be employed, provided it is free from error, is that found most convenient to the individual operator.

The agglutination test now subserves two important functions: 1, *the diagnosis of any infectious disease, provided the infecting organism be at hand;* 2, *the recognition of any micro-organism, provided specific serum be at hand.*

Technic of Agglutination Tests

If possible, a culture of the micro-organism, grown upon agar-agar, is to be selected for the purpose. A good-sized platinum loopful of the culture is taken up and distributed as uniformly as possible throughout a few cubic centimeters of distilled water. This is best done by placing the water in a test-tube and then rubbing the culture upon the glass just above the level of the fluid, until it is thoroughly emulsified, permitting it to enter the water little by little and, finally, washing it all down into the fluid. This gives a distinctly cloudy fluid, too concentrated to use. Of this one adds enough to each of a series of watch-glasses or test-tubes, each containing an equal volume of distilled water (say 2 cc.), to make the fluid opalescent by reflected light though transparent by transmitted light. The same quantity should be added to each, so that they form a uniform series. The patient's blood or serum is next diluted and added so that the watch-glasses or tubes receive a 1:10, 1:20, 1:30, 1:40, 1:50, 1:60, 1:80, 1:100, 1:150, 1:200, 1:300, or a laboratory serum of high agglutinative value, 1:1000, 1:2000, 1:5000, 1:10,000, 1:50,000, and 1:100,000.

If watch-glasses are used, they are stood upon a black surface, covered,

* "Jour. Med. Research," July, 1904.
† Ibid., 1904, vol. x, p. 89.

and examined in fifteen, thirty, and sixty minutes by simply looking at the dark surface through the fluid. If agglutination occur, the original opalescence gives place to a slightly curdy appearance, as the uniformly suspended bacteria aggregate in clumps.

If test-tubes are employed, they are best observed by tilting them and looking through a thin layer of the contained fluid at a dark surface or at the sky. In either case the flocculent collections of agglutinated bacteria can be seen.

The test can also be made and observed under the microscope by the hanging-drop method, but in working with such small quantities much of the accuracy of the technic is apt to be lost.

Some knowledge is required in order to form correct deductions from the experiments. Thus, with typhoid bloods, the agglutination of the typhoid bacillus usually occurs within an hour in dilutions of 1:50, but the agglutinability of the culture employed should be known before the experiment is undertaken.

Similarly, when the method is employed for the differentiation of bacteria the agglutinative value of the serum should be known to begin with.

The agglutinins are capable of acting as antigens and when injected into animals effect reactions followed by the formation of antibodies inhibiting their own activity.

ANTITOXINS

Antitoxins are immunity products by which the injurious actions of toxins are annulled. In the synopsis of immunity experiments already given, the history of the discovery and development of the antibodies has been outlined, together with references to the original contributions in which they were made public.

In the section upon the "Explanation of Immunity" we have seen that the best mode of accounting for the occurrence of antitoxins is afforded by Ehrlich in the lateral-chain theory. He regards them as cell haptophiles—receptors—that are formed in excess of the requirements, by cells frequently stimulated by the presence of bacterial products possessing adapted haptophores. The receptors are under normal conditions engaged in maintaining the proper nutrition of the cell; under abnormal conditions (as when preempted by the inert or injurious haptophores of the bacterial products) are obliged to increase in number to compensate for the damage done the cell. Antibody formation can be induced only by antigens or bodies that bear a resemblance to the normal nutrient substances absorbed by the cells in that they are provided with haptophore groups corresponding with the haptophile groups of the cells and so adapted for union with them. Mineral and alkaloidal substances have no such adaptations, but bacterial products, the toxalbumins of various higher plants, venoms, enzymes, and other protein combinations have. The possession of the haptophile groups determines whether or not the cell can stimulate antibody formation, and the ability to produce antibodies shows the existence of the haptophore groups.

The attachment of the haptophore groups to the cells is usually shown by morbid action of the cells in cases where there are associated toxophore and toxophile groups, as in the case of the bacterio-

toxins, but may not be discovered if there are none. The combination of the toxin-haptophores with the cell-haptophiles can be demonstrated in the test-tube by crushing the cerebral substance of a rabbit, and adding tetanus toxin. The toxin becomes fixed by combination with the cell haptophiles or receptors, loses its further combining powers and fails to affect animals into which it is subsequently injected. The increased formation of receptors in consequence of repeated stimulation has been shown by the effect of abrin upon the conjunctiva. If dropped into one eye until the conjunctiva is thoroughly immune against its action, the cells of this eye develop a greatly increased capacity for absorbing—*i.e.*, fixing—the abrin as compared with those of the other eye. Thus if the two conjunctival membranes be dissected out and a certain quantity of abrin triturated with each, the haptophiles of the cells of the immunized membrane fix the poison so that it is no longer able deleteriously to affect animals, while no such effect takes place with the other membrane.

The ability to stimulate the formation of antibodies is entirely independent of any toxic action and is entirely the work of the haptophiles. This is best shown in the fact that diphtheria toxin that has been heated or otherwise manipulated until its toxic action is lost, still retains the power of combining with antitoxin, or of producing antibodies.

The cells furnishing the haptophile groups or receptors whose presence in the blood gives it its antitoxic quality vary in number or quality in different animals. Thus, in the warm-blooded animals the rapidity with which tetanus toxin is anchored to the cells of the central nervous system seems to indicate that those cells, if not the only cells in the body passing the adapted receptors by which it is anchored, are the chief cells by which it is absorbed. In the alligator, however, other cells seem to fix the toxin before it reaches or connects with those of the nervous system, so that the alligator, though immune against the action of the toxin, is able to make antitoxin as well as susceptible animals.

Each introduction of appropriate antibody forming substance is followed by an outpouring of the antibody far in excess of what would neutralize it, so that after a systematic treatment has been carried out for some time, the neutralizing value of the blood may be *a thousand times* what would be necessary to neutralize the total quantity of active substance introduced into the animal.

Each antibody is specific in action, as must be evident from its mode of formation. Should it be found, however, that several active bodies possessed haptophore groups of identical structure, the antibody formed by any of them might be found to possess common neutralizing powers for all.

The animal whose blood contains antibodies enjoys immunity from the active body by which they were formed only so long as

they are present. In some cases, however, animals that have been long subjected to the immunization treatment, and whose blood contains large quantities of free antitoxin, unexpectedly become abnormally sensitive (hypersensitivity) to the toxin, and may die after receiving a very small dose. This may be attributed to a difference in the combining activity of the receptors attached to the cells, and those separated and free in the serum. If the former developed a greater affinity for the toxin than the latter, it would unite with them by preference and intoxication ensue. If the treatment by which the antitoxins are produced is interrupted, they immediately begin to lessen in quantity, and eventually disappear. Their occurrence in the blood determines that they shall be found in all the body juices, though in varying quantity.

Their chemical composition, which experiment shows to be of protein nature, determines that when practical use is to be made of them, they must not be administered by the stomach, as digestion is usually followed by their destruction. In infants, the protein digestion being feeble, antitoxins pass from the mother's milk to the blood of the sucking offspring without digestion, but the administration of antitoxins by this method at later periods of life is followed by effects too uncertain to be depended upon. For practical therapeutic purposes, therefore, the administration must always be made hypodermically or intravenously.

Diphtheria Antitoxin.—This was first utilized for practical therapeutic purposes by Behring.* As usually prepared by the administration of the toxin, it is essentially an antitoxin and has no destructive action upon the diphtheria bacilli. In therapeutics it is employed to neutralize or "fix" the toxin circulating in the blood, not to destroy the bacilli, or to effect the regeneration of the tissues injuriously acted upon by the toxin. Martin is of the opinion that such purely antitoxic serums are inferior to those containing other immunity products, such as bacteriolysins, and recommends that the whole culture instead of the filtered culture be used in the immunization of the animal. If this is done, the bacteriolytic effect is added to the antitoxic effects of the serum.

The serum may be used to prevent or to cure diphtheria.

The antitoxin is commercially manufactured at present by immunizing horses against increasing quantities of diphtheria toxin until the proper degree of immunity has been attained, then withdrawing the antitoxic blood. The details are as follows:

I. The Preparation of the Toxin.—The toxic metabolic products of the Bacillus diphtheriæ are for the most part freely soluble, and are therefore best prepared in cultures grown in fluid media. The medium best adapted to the purpose is that recommended by Theobald Smith.†

To make it, the usual meat infusion receives the addition of a culture of

* "Deutsche med. Wochenschrift," 1890, Nos. 49 and 50; "Zeitschrift für Hygiene," etc., 1892, XII, p. 1; "Die Blutserumtherapie," Berlin, 1902.

† "Journal of Experimental Medicine," May and July, 1899, p. 373.

Bacillus coli, and is stood in a warm place overnight. The colon bacilli ferment and remove the muscle and other sugars. The infusion is then made into bouillon, titrated so that the reaction equals + 1.1 when tested with phenolphthalein. It then receives an addition of 0.2 per cent. of dextrose, and is sterilized in the autoclave. To secure the best toxic product, the bacilli at hand must be carefully studied and that naturally possessing the strongest toxicogenic power employed for the cultures. The greatest toxicity seems to develop between the fifth and seventh days. If the culture is permitted to remain in the incubating oven beyond this period, the toxin gradually is transformed to toxoid and its activity declines. The fatal dose for a 250–300 gram guinea-pig should be about 0.001 cc. given hypodermically.

II. The Immunization of the Animals.—All commercial manufacturers of diphtheria antitoxic serums now use horses, as recommended by Roux, instead of the sheep, dogs, and goats with which the earlier investigators worked. The horse is readily immunized, gives an abundant supply of blood which clots readily and yields a beautiful clear amber serum.

The horse selected should be in perfect health, and should be tested with mallein and tuberculin to avoid obscure glanders and tuberculosis.

A small dose of the toxic bouillon—say 0.1 cc.—should be given in the beginning, as one occasionally finds exceptionally susceptible animals that will succumb to larger doses. If a marked local and general reaction follows, it may be better to try another animal. If no reaction is brought about, the immunization is carried on as rapidly as possible. The toxin is injected hypodermatically into the tissues of the neck, the skin being thoroughly cleaned and disinfected before each injection. The doses are cautiously increased and may often be doubled each day. If any unfavorable symptoms arise, treatment must be interrupted for a day or two. The animal yields good antitoxic serum when it can endure several doses of 500 cc. of the strong toxin mentioned above.

III. Bleeding.—When the withdrawal of a small quantity of blood by a hypodermic needle introduced into the jugular vein shows that the serum contains a maximum antitoxic strength (300 to 1000 units per cubic centimeter), the horse is ready to bleed. Some horses can be bled without resistance, but most of them require to be fastened in appropriate stocks. The blood is taken from the jugular vein, which is superficial, of large size, and easily accessible. The skin is carefully shaved over an area about 9 square inches in extent, thoroughly disinfected. A small incision is made over the center of the vein, which is made prominent by pressure at the base of the neck, and the point of a small sterile trocar being inserted in the incision through the skin, it is directed obliquely upward into the vein. The blood is allowed to flow through a sterile tube attached to the cannula into sterile bottles prepared to receive it. A large horse may furnish 7 to 9 liters; small horses, 5 to 7 liters.

IV. Preparation of the Serum.—The blood is stood away in a cool place until the clot retracts after coagulation and the clear serum separates. The serum is then withdrawn under strict aseptic precautions. It is variously prepared for the market. Some manufacturers bottle it without any added preservative; some add a crystal of thymol; some Pasteurize it; some add carbolic acid; some add trikresol.

The plain serum would be ideal, but the danger of subsequent contamination through careless treatment makes it rather better to have an antiseptic added. Trikresol is probably the most satisfactory of these, though it throws down a precipitate that necessitates the filtration of the product, and leaves the serum slightly opalescent.

V. Determining the Potency of the Serum.—The potency of the serum is expressed as so many "*immunizing units.*" Only one method of testing is in use at the present time, though to understand it, it seems wise to mention the original method from which it was derived.

(A) *Behring's Method.*—Behring's unit was an arbitrary standard chosen in consequence of certain conditions existing at the time it was devised. It is difficult to understand apart from the circumstances governing its creation, but may be defined as "*Ten times the least quantity of antitoxin serum that will protect a standard (300 gram) guinea-pig against ten times the least certainly fatal dose of toxic bouillon.*"

The method of determining it is not difficult to those skilled in laboratory technic, and is as follows:

1. Determine accurately the least certainly fatal dose of a sterile diphtheria toxic bouillon for a standard guinea-pig.

2. Determine accurately the least quantity of the serum that will protect the guinea-pig against *ten times* the above determined least fatal dose of toxin.

3. Express the required dose of antitoxic serum as a fraction of a cubic centimeter and multiply by 10; the result is one unit.

Example: It is found that 0.01 cc. of a toxic bouillon kills at least 9 out of 10 guinea-pigs, and is therefore the least certainly fatal dose. Guinea-pigs receive ten times this dose of the toxic bouillon plus varying quantities of the serum to be tested, measured by dilution—say $\frac{1}{2000}$ cc., $\frac{1}{2500}$ cc., $\frac{1}{3000}$ cc. The first two live. The fraction $\frac{1}{2500}$ is now multiplied by 10; $\frac{1}{2500} \times 10 = \frac{1}{250}$ = 1 unit. So we find that each cubic centimeter of the serum contains 250 units.

This method would be satisfactory were it not for certain variations in the toxic bouillon by which the strength is worked out. Ehrlich,* in an elaborate investigation of these changes, has clearly proved that an ever-changing toxin cannot be a satisfactory standard, because it does not possess uniform combining affinity for the antitoxin. He shows by a labored scheme that the toxicity of the bouillon is no index to its antitoxin-combining power, which, of course, must be the foundation of the test. The toxin, under natural conditions, is changed with varying rapidity into toxoids, of which he demonstrates three groups—prototoxoids, syntoxoids, and epitoxoids. The epitoxoids have a greater antitoxin-combining power than the toxin itself, yet have no toxic action upon the guinea-pigs, hence cause confusion in the results.

To secure a satisfactory measure of the antitoxic strength of a serum, it is therefore more important to first determine the antitoxin-combining power of the toxin or toxic bouillon to be used than to determine its guinea-pig fatality, and this is what Ehrlich endeavors to do.

(B) *Ehrlich's Method.*—In this method the unit is the same as in Behring's method, but its determination is arrived at by a very important modification of the method, by which the standard of measurement is a special antitoxin of known strength, by which the antitoxin-combining power of the test toxic bouillon is first determined. Ehrlich began by determining the antitoxic value of a serum as accurately as possible by the old method, and then used that serum as the standard for all further determinations. The serum was dried in a vacuum, and two grams of the dry powder were placed in each of a large number of small vacuum tubes, connecting with a small bulb of phosphoric anhydride. In this way the standard powder was protected from oxygen, water, and other injurious agents by which variations in its strength could be initiated. Periodically one of these tubes was opened and the contained powder dissolved in 200 cc. of a mixture of 10 per cent. aqueous solution of sodium chloride and glycerin. The subsequent calculations are all based upon the strength of the antitoxin powder. In Ehrlich's first test serum 1 gram of the dry powder represented 1700 units. Of the solution mentioned, 1 cc. represented 17 units; $\frac{1}{17}$ cc., one unit.

Having by dilution—1 cc. of the first dilution in 17 of water—secured the standard unit of antitoxin in a convenient bulk for the subsequent manipulations, it is mixed with varying quantities of the toxic bouillon to be used for testing the new serums, until the least quantity is determined that will cause the death of a 250 gram guinea-pig in exactly four days, when carefully injected beneath the skin of the animal's abdomen. This quantity of toxin is the *test dose*. If the toxic bouillon was "normal" in constitution, it should represent 100 of the least certainly fatal doses that formed the basis of the old method of testing, but as toxic bouillons contain varying quantities of toxoids it may equal anywhere from fifty to one hundred and fifty times that dose.

The test dose of toxic bouillon, having been determined, remains invariable throughout the test as before, the serum to be tested for comparison with the standard being modified. The calculation is, however, different because the guinea-pig is receiving, not ten times, but more nearly one hundred times the least fatal dose, and the quantity of the antitoxic serum that preserves life beyond the fourth day is itself the unit.

Example: The sample of serum issued as the standard contains 17 units per cubic centimeter. Serum 1 cc. + water 16 cc. = 1 cc. is the unit. 1 cc. of the dilution containing one antitoxic unit is mixed with 0.01, 0.025, 0.05, 0.075, 0.1 cc. of the toxic bouillon. All the animals receiving less than 0.1 cc. live.

* "Klinisches Jahrbuch," 1897.

A new series is started, and the guinea-pigs all weighing exactly 250 grams, receive 1 unit of the antitoxin plus toxic bouillon 0.08, 0.09, 0.095, 0.097, 0.1, 0.11, 0.12, etc. It is found that all receiving more than 0.097 die in four days, but that the animal receiving that dose, though very ill, lives longer. The test dose may then be assumed to be 0.1, or it may be calculated more closely if desired.

To test the serum itself, guinea-pigs weighing exactly 250 grams are now all given toxic bouillon 0.1 cc. plus varying quantities of the serum—$\frac{1}{200}$, $\frac{1}{300}$, $\frac{1}{400}$, etc. All live except those receiving less than $\frac{1}{400}$, which die about or on the fourth day. The serum can then be assumed to have 400 units per cubic centimeter unless it be desired to test more closely.

Standard test serums for making tests of antitoxic serums by the Ehrlich method were first shipped at small expense from the Kaiserliches Institut für Serum-Therapie at Höchst-on-the-Main. At present the Hygienic Laboratory of the United States Public Health Service has legal control of the manufacture of therapeutic serums and kindred products in the United States, issuing licenses to those engaged in legitimate manufacture, and furnishing a standard test serum, similar to that of Ehrlich, to those entitled to receive it.

A full description of "The Immunity Unit for Standardizing Diphtheria Antitoxin," by M. J. Rosenau, Director of the Hygienic Laboratory, can be found in Bulletin No. 21 of the U. S. Public Health and Marine Hospital Service, Washington, 1905.

As the quantity to be injected at each dose diminishes according to the number of units per cubic centimeter the serum contains, it is of the highest importance that therapeutic serums be as strong as possible. Various methods of concentration have been suggested. Bujwid* and H. C. Ernst† found that when an antitoxic serum is frozen and then thawed, it separates into two layers, the upper stratum watery, the lower yellowish, the antitoxic value of the yellowish layer being about three times that of the original serum, the upper layer consisting chiefly of water.

The most satisfactory method of securing a useful concentration is by the employment of the *globulin precipitation* as recommended by Gibson,‡ which is briefly as follows: The diluted citrated plasma is precipitated with an equal volume of saturated ammonium sulphate solution and the antitoxic proteins separated by extracting the precipitate with saturated sodium chloride solution. The soluble antitoxic proteins are then reprecipitated from the saturated sodium chloride solution with acetic acid. This filtered precipitate is then partially dried between filter-papers and dialyzed in running water. This yields a final product which when dried *in vacuo* is readily soluble in salt solution and is free from many of the offensive substances in the horse serum. Steinhardt and Bauzhaf§ found that the thera-

* "Centralbl. f. Bakt. u. Parasitenk.," Sept., 1897, Bd. XXII, Nos. 10 and 11, p. 287.

† "Jour. Boston Soc. of Med. Sci.," May, 1898, vol. II, No. 8, p. 137.

‡ "Jour. Biol. Chem.," I, p. 161; III, p. 253.

§ "Jour. Infectious Diseases," March, 1908, vol. II, pp. 202 and 264.

peutic value of the plasma was not appreciably impaired through the process of eliminating the albumins and other non-antitoxic proteins by the salting out methods employed, and the final dialyzation of the concentrated product, thus disproving the objection of Cruveilhier* on this point.

Tetanus antitoxin was first prepared by Behring and Kitasato.† It can be employed for the prevention or cure of tetanus. For the former purpose, hypodermic injections of the serum may be given in cases with suspicious wounds, or the wounds may be dusted with a powder made by pulverizing the dried serum. For treatment the serum must be administered in frequently repeated large doses by hypodermic or intravenous injection. The results are less brilliant than those attained with diphtheria antitoxin because of the avidity with which the cells of the central nervous system take up the tetanus toxin, and the firmness of the union formed. An analysis of a great number of cases has, however, shown that the recoveries following the free administration of the serum exceed those effected by other methods of treatment by about 40 per cent.

By the gradual introduction of tetanus toxin Behring and Kitasato‡ have been able to produce a powerful antitoxic substance in the blood of animals.

The method of obtaining *tetanus antitoxic serum* is like that employed for securing diphtheria antitoxic serum (*q.v.*).

Madsen§ found that for each of the specific poisons, tetanolysin and tetanospasmin, a specific antitoxin is produced, the one annulling the convulsive, the other the hemolytic, properties of the toxin. The usual therapeutic serums contain both of these.

Different standards for measuring the strength of the tetanus toxin and different definitions of the unit of measurement are given in different countries, so that great confusion and dissatisfaction were experienced until a special committee of the Society of American Bacteriologists met in New York, Dec. 27 and 28, 1906, and in collaboration with the United States Public Health and Marine Hospital Service, Hygienic Laboratory, formulated a standard unit which has become the legal unit of measurement for the United States. It is thus defined:

"The immunity unit for measuring the strength of tetanus antitoxin shall be ten times the least quantity of antitetanic serum necessary to save the life of a 350-gram guinea-pig for ninety-six hours against the official test dose of a standard toxin furnished by the Hygienic Laboratory of the Public Health and Marine Hospital Service." The unit is thus officially defined, Oct. 25, 1907, in Treasury Circular No. 61.

Testing tetanus antitoxic serums immediately became a matter

* "Ann. de l'Inst. Pasteur," 1904, XVIII, p. 249.
† "Deutsche med. Wochenschrift," 1890, No. 49.
‡ Ibid.
§ "Zeitschrift für Hygiene," 1899, XXXIII, p. 239.

of great simplicity. The governmental laboratory furnishes the "test toxin" whose strength is guaranteed, and what follows is a simple matter of dilution, admixture with the serum to be tested, and the injection of animals that are carefully observed for a few days.

The entire subject, historical, theoretical, and practical, is treated in Bulletin No. 43, 1908, of the Hygienic Laboratory upon "The Standardization of Tetanus Antitoxin," by Rosenau and Anderson.

Antivenene or Anti-venomous Serum.—This was discovered by Phisalix and Bertrand* and made practical for therapeutic purposes by Calmette.† Calmette found that cobra venom contained two principles, one of which, labile in nature and readily destroyed by heat, was destructive in action upon the tissues with which it came into direct contact; the other, stable in nature, was death-dealing through its action upon the respiratory centers. By heating the venoms and thus destroying the irritative principle, he was able to immunize animals against the other, which he looked upon as the important element of the venom. The immunized animals furnished an anti-serum, which entirely annulled the effect of the toxin (modified venom) used in treating them. This serum was found to protect rabbits and other animals against both modified and unmodified cobra venom, and was used successfully in the treatment of a number of human beings who had been bitten by cobras. Calmette, however, erroneously concluded that because in most venoms studied he was able to find a larger or smaller proportion of the respiratory poison, it constituted the essential element of the venom to be antagonized. Arguing from this standpoint, he recommended his antivenene in all cases of snake-bite, regardless of the variety of serpent. C. J. Martin‡ and others showed that Calmette was wrong, and that his antivenene was useless in the treatment of the bites of the Australian serpents, and the experiments of the author have shown it to be useless in the treatment of the bites of the American snakes. In the venoms of our snakes—the rattlesnake, copper-head, and moccasin—the poison is essentially locally destructive in action, the fatal influence upon the respiratory centers being of secondary importance. Flexner and Noguchi,§ Noguchi‖ and Madsen and Noguchi,** however, applied Ehrlich's principle to the investigation, destroyed the toxophorous group of the venom molecules, and succeeded in producing an anti-serum useful in antagonizing the active principle—*hemorrhagin*—of the Crotalus venom.

* "Compt. rendu de l'Acad. des Sciences de Paris," Feb. 5, 1894, CXVIII, p. 356.

† "Compt. rendu de la Soc. de Biol. de Paris," Feb. 10, 1894, 10 Series, I, p. 120.

‡ "Intercolonial Medical Journal of Australia," 1897, II, p. 537.

§ "Journal of Experimental Medicine," 1901–1905, VI, p. 277.

‖ Ibid., 1906, VIII, p. 614.

** Ibid., 1907, IX, p. 18.

Antivenene is useful in the treatment of cobra invenomation, as Calmette has shown by cases treated in his own laboratory. The serums of Noguchi and others are equally useful in their respective invenomations, but the opportunity for successfully employing antivenenes is very small. Few persons are bitten where the remedy is at hand, and the effects of venom of all kinds are so rapid that immediate treatment is required. In India and a few other reptile infected countries, as well as in zoological gardens where venomous serpents are kept, and in laboratories where the snakes are kept for experimental purposes, it is well to be provided with a supply of the serum, but it has no wide sphere of usefulness.

CYTOTOXINS

Cytotoxins are immunity products that exert a specific destructive action upon cellular antigens. They are essentially cell-dissolving products of immunity. The solution of the cells, of whatever kind, takes place through the complement, native to the blood, fixed to the cells by the specific amboceptor. The complement is presumably always the same and is present in all normal blood; the amboceptor is an "immune body" susceptible of artificial production or increase, and specifically differs according to the particular cell through whose antigenic activity it was produced.

Hemolysis.—The phenomena of hemolysis or the solution of erythrocytes, caused by heterologous serums were first studied by Creite* and Landois,† who studied hemoglobinuria following transfusion. Subsequent observations were made upon corpuscular agglutination and solution by venoms by Mitchell and Stewart‡ and by Flexner and Noguchi§, and upon the effects upon corpuscles of warm-blooded animals, of the poisonous serum of certain eels by Mosso,‖ Camus and Gley,** and Kossel.†† The serious consideration of the subject was, however, deferred until Belfanti and Carbone‡‡ showed that if horses were injected with red corpuscles of rabbits, the serum thereafter obtained from the horses would be toxic for rabbits; Bordet§§ had shown that the serum of guinea-pigs injected several times with 3 to 5 cc. of the defibrinated blood of rabbits acquired the property of rapidly dissolving the red corpuscles of the rabbit in a test-tube, and Ehrlich and Morgenroth‖‖ had shown the mechanism of the

* "Zeitschrift f. ration. Med.," 1869, Bd. XXXVI—quoted by Nuttall in his "Blood Immunity and Relationships."
† "Zur Lehre von der Bluttransfusion," Leipzig, 1875.
‡ "Transactions of the College of Physicians of Philadelphia," 1897, p. 105.
§ "Journal of Exp. Med.," 1901–1905, VI, p. 277.
‖ "Archiv. f. Exp. Path. and Pharmak.," XXV, pp. 111 and 135.
** "Compt. rendu de la Soc. de Biol. de Paris," 1898, p. 129.
†† "Berliner klin. Wochenschrift.," 1898.
‡‡ "Jour. de la R. Acad. d. Med. de Torino," 1898, No. 8.
§§ "Ann. de l'Inst. Pasteur," 1898, XII, 688.
‖‖ "Berliner klin. Wochenschrift," 1899.

hemolytic action. From this time on the literature of hemolysis rapidly grew and the subject assumed a more and more important place in the domain of chemico-physiological research.

The *technic of hemolysis* is comparatively simple, and it is intended in this chapter to do no more than offer the student a simple method of performing experiments which he can modify to suit his own purposes.

For the study of hemolysis and hemo-agglutination it is necessary to prepare a 5 per cent. suspension of the blood-corpuscles in an isotonic salt (NaCl) solution. To do this the blood of the animal is permitted to flow into a sterile tube and is immediately stirred with a small stick or a platinum wire until completely defibrinated. Some salt solution (0.85–0,9 per cent.) is then added and the mixture shaken. It is then placed in a sterile centrifuge tube and rotated until the corpuscles are packed in a mass at the bottom. The supernatant fluid is poured off, replaced by an equal volume of salt solution, and shaken until the corpuscles are again thoroughly distributed. It is then again centrifugated and the fluid again poured off, after which 95 parts (by volume as compared with the corpuscular mass) of the salt solution are added and the fluid thoroughly shaken to distribute the corpuscles. This slightly greenish-red fluid is the 5 per cent. solution of corpuscles. It is, of course, not permanent, and easily spoils if bacteria enter. It also gradually deteriorates through changes in the corpuscles, so that it is not usually useful after the third day, even when kept on ice.

The hemolytic substance to be investigated must be isotonic with the corpuscles and therefore must be dissolved in, or diluted with, the same salt solution as that used for making the corpuscular suspension. Neglect to observe this requirement may lead to error by diminishing the tonicity of the solution and inducing spontaneous or hypotonic disintegration of the corpuscles.

To secure a specifically hemolytic serum one injects an animal—say a rabbit or guinea-pig—with increasing doses of the washed blood corpuscles of the animal for whose corpuscles the serum is to be made hemolytic, the doses being given intraperitoneally about six times, at intervals of a week. The animal is then bled, the blood permitted to coagulate, the serum separated and filtered, if necessary.

The contact of the corpuscles and the hemolytic substance is best conducted in small test-tubes holding about 2 cc. of the mixed fluids. It is usually best to work with a constant volume of the blood-corpuscle suspension and varying quantities or concentrations of the hemolytic substances. Two observations are to be made, one after thirty minutes' sojourn in the thermostat at 37°C., the other after twenty-four hours in the ice-box, both observations being made on the same series of tubes. Hemolysis is shown by the appearance of a beautiful clear red color of the formerly cloudy greenish suspension. One must notice the difference between partial and complete hemolysis, different additions of the hemolytic substance being required for these results.

Cytolysis.—The phenomena of hemolysis corresponds to those by which many other cells, vegetable and animal, are destroyed and dissolved through the activity of immunity products. Delezene* first produced a leukolytic or leukocyte-destroying serum by injecting animals with the leukocytes of a heterologous species; Metalnikoff,† by injecting the spermatozoa of one animal into another of another species, produced a spermatoxic or spermalytic serum; von Düngern,‡ a serum capable of dissolving the ciliated epithelium scraped from the trachea of an ox by injecting the dissociated epithelial cells into an animal, Delezene§ found that

* "Compt. rendu de l'Acad. de Sciences de Paris," 1900.

† "Ann. de l'Inst. Pasteur," 1899.

‡ "Münchener med. Wochenschrift," 1899.

§ "Compt. rendu de l'Acad. de Sciences de Paris," 1900, CXXX, pp. 938 and 1488.

by injecting an animal with the dissociated liver cells of a heterologous animal, a hepatolytic serum could be produced.

The technic of these investigators is not difficult. It is, however, first necessary to prepare a homogeneous tissue pulp for injection into the animal that is to furnish immune serum. For this purpose it is necessary to grind the tissues, when solid, in some kind of mill, one of the best forms of apparatus being that of Latapie.* After the pulp is made, it is diluted to a convenient extent with physiological salt solution and then injected into the experiment animal in the same manner as is the blood for making the hemolytic serum. After animal has received a number of injections made at intervals of a few days and is thought to be "immunized" it is bled and the serum separated. The remaining steps in the experiment do not differ essentially from those of hemolytic experiments. The tissue suspension, having about the same concentration as the 5 per cent. NaCl suspensions of the corpuscles, is used as the constant quantity and the immune serum used as the variable quantity. The tissue suspension or antigen, the immune serum or amboceptor, and the complement in normal guinea-pig serum are brought into contact in small test-tubes, kept for twenty-four hours in the refrigerator, and the amount of solution gauged by the naked eye supplemented by microscopical examination of the tissue elements.

Fig. 28.—Latapie's instrument for preparing tissue pulp.

Bacteriolysis.—The first observations upon bacteriolysis were made in 1874 by Traube and Gscheidel,† who found that freshly drawn blood was destructive to bacteria. The matter was pursued by numerous subsequent investigators and was explained by Buchner as depending upon *alexines*. Pfeiffer‡ described the peculiar reaction known as "Pfeiffer's phenomenon." Ehrlich and Morgenroth§ and Bordet‖ described the mechanism of cytolysis, explaining the "Pfeiffer phenomenon" and paving the way for future experiments.

Direct destruction of bacteria by blood-serum and body juices is rare, and occurs only when the serum contains appropriate

* "Ann. de l'Inst. Pasteur," 1902, XVI, p. 947.
† "Jahresb. der schles. Ges. f. vaterl. Kultur," 1874.
‡ "Deutsche med. Wochenschrift," 1896, No. 7.
§ "Berliner klin. Wochenschrift," 1899.
‖ "Ann. de l'Inst. Pasteur," 1898, XII.

quantities of both factors involved—*i.e.*, amboceptor and complement. For the usual bacteriolytic investigations it is, therefore, necessary to consider three factors: 1, The bacteria to be destroyed; 2, the serum furnishing the complement; and 3, the serum furnishing the immune body.

Technic.—1. The bacteria to be destroyed should be prepared in the form of a homogeneous suspension in physiological salt solution, similar to that employed for making the agglutination tests (*q. v.*). It is best to use the surface growths from agar-agar, well rubbed upon the side of a test-tube containing the fluid, which is permitted to contact with the mass from time to time by inclining the tube so that the fluid is able to carry away the bacteria as they are distributed.

If quantitative estimations are to be made, the number of bacteria in the suspension must be known or at least a standard quantity must be employed, as the destructive process is a chemical one, in which the destructive agents are themselves used up.

2. The serum furnishing the complement is a normal serum—that is, the serum from a healthy animal that has undergone no manipulation. The guinea-pig is the animal preferred.

3. The serum containing the amboceptor or the immune body is obtained from an animal that has been given a high degree of immunization against the bacterium to be destroyed or dissolved. The complement contained in this serum should be destroyed by heating for a short time to 55°C.

These three having been prepared, an appropriate quantity of the bacterial suspension is placed in a small test-tube, and an appropriate quantity of the diluted normal serum added. To this mixture of two constants, varying quantities of the immune serum are added and the tube stood away for twenty-four hours on ice. In almost every case it will be found that the immune serum contains a great quantity of agglutinating substance, so that the bacteria all fall to the bottom in a short time. This is independent of bacteriolysis. The bacterial destruction is gauged by the disappearance of the bacteria or by their failure to grow when transplanted to appropriate culture media.

By making the bacterial suspension and complementary serum constant quantities (taking care that not too many bacteria be present), one is able to estimate the value of the immune serum. By using the bacterial suspension and a heated immune serum (containing no complement) as constants and varying the addition of complementary serum, one can estimate the respective values of several complementary serums. By using both serums as constant factors and varying the number of bacteria, one can determine the exact bacteriolytic value of the mixture. By taking out and planting drops from time to time the rapidity of bacteriolysis can be determined, and by plating out the drops and counting the colonies one may arrive at percentages of destruction and express the bacteriolytic process in the form of a curve.

THE DEVIATION OF THE COMPLEMENT, OR THE "NEISSER-WECHSBERG PHENOMENON"

A peculiar phenomenon has been observed and studied by Neisser and Wechsberg.* When an animal whose blood-serum is normally possessed of a high degree of germicidal power is immunized by repeated injections of a bacterial antigen, its serum when examined by the usual methods fails to show the usual increase in the specific bactericidal action toward that particular organism, though it retains its general bacteria-destroying power. If, however, the serum be greatly diluted, its action is changed, so that it loses its general bacteria-destroying power and develops marked increase in the specific destructive action upon the particular bacteria used

* "Münch. med. Wochenschrift," April 30, 1901, XLVIII, No. 13, p. 697.

in the experiment. Neisser and Wechsberg attribute the peculiar reaction to the fact that there being more amboceptors than complements in the serum, some of the former satisfy their combining affinities by attaching themselves to the bacteria, some by attaching themselves to the complement, instead of forming combinations of all three. If under these circumstances the serum containing

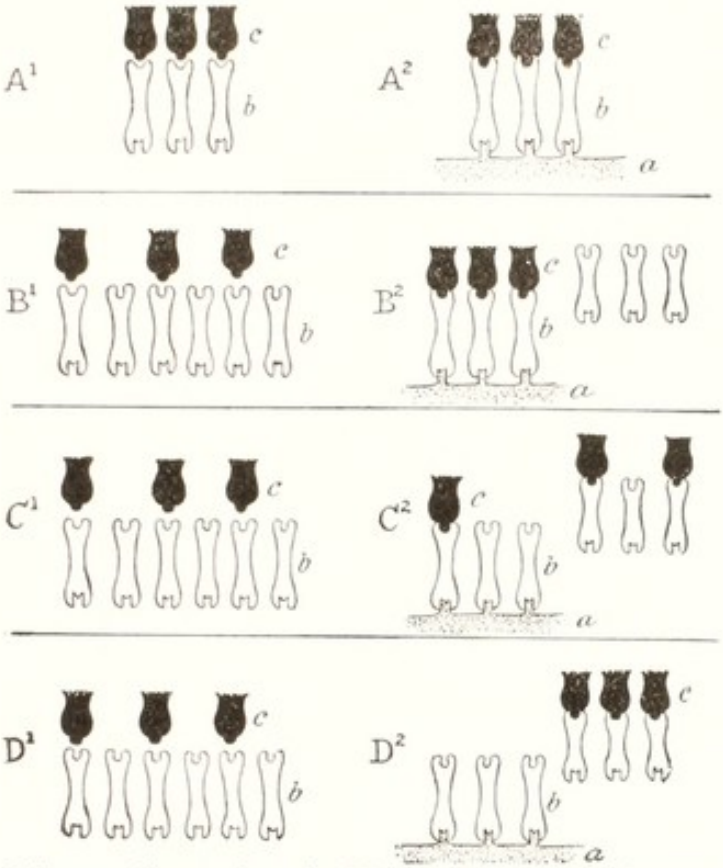


Fig. 29.—Diagram illustrating the Neisser-Wechsberg phenomenon of "deviation of complement." In A^1 the three black units (*c*) represent the quantity of complement necessary for the dissolution of a bacterium, and the three white units (*b*) the intermediate bodies or amboceptors through which they may act. A^2 shows these properly proportioned units properly combined and anchored to the bacterial cell which will be destroyed. If an excess of amboceptor units be present, as is suggested in B^1, the resulting combinations and the consequent results may vary according to the differing combining affinities. Thus, B^2 shows an unchanged affinity, *i.e.*, only those amboceptors unite with bacterial cells that are charged with complement. C^2 shows equal affinity of the amboceptors for complement and for the bacterial cell, so that charged or uncharged units attach themselves to the cell, diminishing the complementary action. D^2 shows the possible result when the affinity of the amboceptor for the bacterial cell is diminished after charging with complement, so that though the complement and amboceptor combine, there can be no destruction of the bacterium. Thus, excess of the amboceptor units may "deviate the complement" and prevent its action.

the amboceptors is diluted until their number becomes approximately equal to the number of complements introduced, any deviation resulting from inequality of the combining affinities becomes improbable. Bordet and Gay,* however, have performed experiments tending to show that these elements do not really unite, thus seem-

* "Ann. de l'Inst. Pasteur," June 25, 1906, XX, No. 6, pp. 267–498.

ing to controvert the theory of Neisser and Wechsberg, and Bolton* has shown that normal serum may kill relatively more bacteria when diluted than when undiluted.

THERAPEUTIC USES OF BACTERIOLYTIC SERUMS

It was at first hoped that some of these serums and especially the bacteriolytic serums would have a wide therapeutic application in cases in which non-toxicogenic bacteria were invading the body, but experiment and experience have shown that the laws governing their action greatly limit their application, and that their effects, when not beneficial, are bound to be harmful. The difficulty lies in the fact that when we manufacture such serums we prepare only the immune body, there being no increase of the complement.

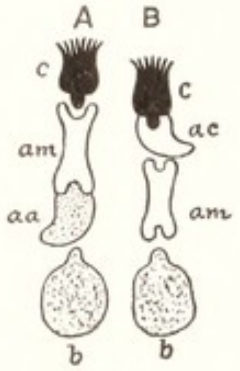


Fig. 30.—Schematic representation of the interfering action of anti-amboceptors, and anti-complements. A, Anti-amboceptor action: *c*, Complement; *am*, amboceptor; *aa*, anti-amboceptor preventing the amboceptor from connecting with the cell. B: *c*, Complement; *ac*, anti-complement preventing the complement from connecting with the amboceptor, *am*.

To introduce this by itself does the patient no good, because in most cases the existing infection has brought about the formation of as much or more "immune body" than can be utilized by the complement. To give injections of active bodies that cannot be utilized is shown by Comus and Gley† and Kossel‡ to be followed by the formation of antibodies—in this case "anti-immune bodies"—by which their effect is neutralized. Should anti-immune bodies be formed by this meddlesome medication, the state of the infected animal would be worse than before, because it would now be preparing that which by neutralizing the combining affinities of its own immune bodies, would prevent them from combining with the elements to be destroyed and so activating the complements.

No satisfactory method of experimentally increasing the complement has been devised. If, as Metschnikoff supposes, the complement is microcytase derived from disintegrated leukocytes, aseptic suppurations with active phagolysis should result in marked increase of the complement. As a matter of fact, this does take place, but the increase is so slight that the serum is not practically valuable.

Therapeutic serums whose practical application is based upon their cytolytic activity must, of necessity, contain both the essential factors involved in cytolysis, and should contain them in such proportions that, regardless of other elements in the blood, they can exercise their combining and dissolving functions.

* "The Bacteriolytic Power of the Blood-serum of Hogs," Bull. No. 95 of the Bureau of Animal Industry, U. S. Dept. of Agriculture.

† "Compte rendu de l'Acad. de Sciences de Paris," Jan. 1, 1898, 126.

‡ "Berl. klin. Woch.," 1898, S. 152.

We are unable experimentally to accomplish these prerequisites, therefore are not in the position to accurately apply bacteriolytic serums in practice.

COMPLEMENT FIXATION

In 1901 Bordet, while investigating the nature of the complementary substance, made a discovery that has now become of great importance, that is, the "Bordet-Gengou phenomenon," or, as it is now known, the "fixation of the complement." His method of procedure was as follows: Blood-corpuscles were sensitized with appropriate amboceptors and then treated with freshly-drawn normal serum. Hemolysis resulted. If now he added to the mixture some sensitized blood-corpuscles of a different species, they did not hemolyze. Clearly, the complement had been used up in the first hemolysis.

He next found that if, instead of employing blood-corpuscles for the first test, he used sensitized bacteria—*i.e.*, bacteria treated with an immune serum containing the amboceptors appropriate for effecting their solution—the complement would similarly be used up, "fixed," so that when he subsequently added sensitized red blood-corpuscles there was no hemolysis.

This reaction was naturally quantitative, the result as described depending upon the fact that no more complement (normal serum) was used in the original hemolysis or bacteriolysis than was necessary and so none left "unfixed" to effect the lysis or solution of the second factor introduced.

Bordet interpreted his results as indicating that there was only one complementary or solvent substance, and though Ehrlich subsequently published what he looked upon as proofs to the contrary, the opinion of Bordet prevails.

In addition, however, Bordet's experiments have been of practical use. As affording a means of quantitative experimentation they have enabled investigators to measure the quantity of complement in normal bloods and in immunized bloods, and so led to the discovery that for each kind of animal and for each individual animal the complement is subject to very little variation. In the course of some three years they were followed by the investigations of Neisser and Sachs upon antigens, and made to subserve the useful purpose of recognizing and differentiating antigenic substances. Thus, when a certain antibody and its complement are combined they can only attach themselves to the particular specific antigen by which the antibody has been developed. But, what is still more important, they have led to the invention of methods by which the presence of specific amboceptors may be determined where they are suspected, and so have made possible means of arriving at a correct diagnosis in certain obscure cases of disease in man.

The most important of these measures is the Wassermann reac-

tion for the diagnosis of syphilis (*q.v.*). By careful perusal of the chapter upon the method of performing the Wassermann reaction the student will learn the general details of the technic of complement fixation, and can modify them to correspond to the requirements of other cases in which complement fixation is to be studied.

DEFENSIVE FERMENTS

Defensive ferments are enzymic substances that make their appearance in the body juices in a short time after any unusual protein substance is intentionally or accidentally thrown into the blood. They were discovered by Abderhalden* who found that when substances capable of digestive transformation in the animal economy, by any means obtain access to the blood, ferments capable of effecting such transformations also quickly appear in the blood in increased quantity, effect the transformation and then quickly disappear. The appearance and disappearance of the enzymes is supposed to depend upon "mobilization" of defensive ferments, of which the body presumably has reserve supplies. The most common source of supply is supposed to be the leukocytes.

The Abderhalden Reaction.—The subject was first investigated with reference to the presence of a proteolytic ferment in the blood of pregnant woman, whose office was the defense of the mother against the syncytial and chorionic cells of the offspring which with their products may occasionally get into the circulation.

If such a ferment were present in the blood, it ought to be demonstrably capable of effecting transformations in the sub-stratum by whose presence it has been called forth. To determine it, therefore, it should only be necessary to apply the blood serum to the substratum for a brief time, and then determine by sufficiently delicate tests that some transformation has been effected. For the latter Abderhalden has made use of two separate tests:

The first of these is rarely employed, the second is now regularly employed.

I. The Optical Test.—This depends upon the fact that in the transformation of protein substances, aminoacids may be formed, some of which are optically active. The contact of the enzymic serum and the appropriate sub-stratum is permitted to take place, then after the appropriate length of time, the polariscope is employed to determine whether rotation differences obtain because of the presence of transformation products.

II. The Dialysis Test.—This test not requiring apparatus or skill of unusual or special kind, has met with greater favor and is now in daily use. Its first employment was for the demonstration of the presence, in the blood, of an enzyme that would transform placental tissue. As no such enzyme appeared in the blood except placental

*"Schützfermente des tierische Organismus," Berlin, 1912; Berlin, 1913.

tissue was in the body, it became *a test for the determination of the existence of pregnancy*. The method required but little in the way of special apparatus or reagents. The chief requirements being small "dialyzing shells" or thimbles, which are made by Schleichter and Schull, and are commercially known as No. 579*a*. They are procurable through importing agents dealing in laboratory apparatus. These shells must be tested before using, and it is best to test a large number at the same time. Each must be impervious to albumen, but readily permeable to peptones, aminoacids and other cleavage products of protein digestion.

The shells or "thimbles" are tested thus by Kolmer:*—

They are first soaked in sterile distilled water for half an hour or more, until they are softened. Each then receives about 2.5 cc. of a 5 per cent. solution of egg-albumen in distilled water, thoroughly mixed and free from flakes or shreds. In filling the shell, care should be exercised that none of the albumen solution by any chance falls upon the outside. The shell is then picked up with forceps and transferred to a short tube containing about 20 cc. of sterile distilled water. This tube should be so wide that the column of water is not so deep as the shell is high, and not so broad that the shell is in danger of oversetting. As bacteria may not have been successfully excluded and by multiplying may cause proteolytic cleavage of the albumen, it is well to cover the fluid in the thimble and that in the tube outside of it, with a thin layer of toluol. The outer tube is plugged or corked, and the whole is stood in the incubating oven where it is kept at 37°C. for sixteen to eighteen hours. At the end of this time, 10 cc. of the water in the outer tube is removed by a pipette, and tested by the biuret reaction to determine whether any albumen has penetrated the thimble. For this purpose the fluid, in a test-tube, receives 2.5 cc. of a 33 per cent. solution of sodium hydroxid and is shaken gently. One cubic centimeter of a 0.2 per cent. cupric sulphate solution is permitted to trickle down the side of the tube and overlie the contents. If a delicate violet is produced at the line of junction of the two liquids, albumen has escaped from the thimble into the water outside. Under such circumstances the thimble is, of course, useless and should be thrown away. If there is any uncertainty about the reaction, the tube can be stood away for eight hours or so longer (twenty-four hours in all) and the remaining water subjected to the ninhydrin test (see below).

The good shells or thimbles are next to be tested for permeability to peptones. Before this they should be carefully washed in running water and boiled for thirty seconds.

A 1 per cent. solution of Hochst "silk peptone" is made in distilled water, and of it 2.5 cc. is pipetted into each thimble to be tested, taking care, as before, that none of the solution by accident drops on the outside of the shell. The shell is now placed in the 20 cc. of sterile distilled water in the wide tube such as was used before, covered with toluol and stood in the incubator at 37°C. After twenty-four hours, a pipette is thrust through the toluol and 10 cc. of the water taken up. The finger being held over the top of the pipette, the tube is wiped outside with care, so as to get off any toluol, and the fluid then delivered into a test-tube. Here it receives 0.2 cc. of a 1 per cent. solution of ninhydrin, and is boiled for exactly one minute. If the peptone has dialyzed, a deep blue color develops after standing for a short time. The thimble that permits no transfusion of peptone is worthless and should be thrown away.

The good thimbles are now again thoroughly washed in running water for a minute, or so, and are then transferred to a vessel of sterile distilled water containing chloroform to saturation and covered with toluol.

In making the Abderhalden test it is imperative that the glassware used should be chemically clean, that the reagents be pure, that the preparations be kept sterile and that the thimbles and substrata should be handled with forceps, not with the fingers.

*"Infection, Immunity and Specific Therapy." Phila., 1915; p. 253.

To make the test for pregnancy known as the "Abderhalden reaction," the foundation of all the other tests of the protective or defensive ferments, it is necessary to prepare a substratum upon which the enzyme in the blood may act.

To do this one obtains a healthy placenta, removes the blood clots, cord and membranes, and washes it in running water. When it is clean on the outside, it is cut into small pieces—1 cm. cubes—which are placed upon a towel or on a wire sieve and washed in running water. The purpose of the washing is to remove every trace of blood serum and of blood pigment. From time to time the bits of tissue are moved about and squeezed by the fingers, and occasionally they are crushed together in a towel. The process is completed when the tissue has become perfectly white in color. It now receives 100 times its weight of distilled water (1 gram-1 cc.), to which are added five drops of glacial acetic acid per 1000 cc., and is boiled for ten minutes. The fluid is then thrown away, the tissue fragments are caught in a sieve or cloth, more distilled water added, this time without the acetic acid, and it is boiled again. This is repeated for six times. After the sixth boiling, some of the water is transferred to a tube and tested for proteins with ninhydrin. If the faintest blue color develops upon boiling, the process of washing the tissue by boiling it with clean water, must be repeated again and again until the ninhydrin produces no discoloration after boiling for a minute, and standing for one-half hour. The tissue is then caught on a cloth, finally looked over for any objectionable components, and transferred to a jar of sterile distilled water saturated with chloroform and covered with toluol.

The blood of the patient is obtained with a Keidel tube or with a sterile syringe from which latter it is at once transferred to a sterile test-tube. When the blood has firmly coagulated, the expressed serum is removed by a sterile pipette to a sterile centrifuge tube and any cells it may still contain are thrown out by centrifugation.

The technic of the test is more simple than the preparation and preliminary tests it entailed. The glassware being chemically clean and sterile, the thimbles all tested and sterile, and the substratum (placental tissue) ready one proceeds as follows:

A fragment of the placental tissue is removed from the container with sterile forceps and blotted with sterile filter or blotting paper to absorb the toluol and chloroform. It is then placed upon a sterile filter paper and weighed; about 0.5 gram should be placed in each of two thimbles. 1.5 cc. of the serum to be tested is cautiously pipetted into one thimble; 1.5 cc. of sterile distilled water into the other. Each is then transferred with forceps to a large tube containing 20 cc. of sterile distilled water, and the surface of each fluid is covered with toluol. The tubes are now stood in the thermostat at 37°C. for twenty-four hours, at the end of which time a sample of the fluid in each outer tube is tested by boiling for one minute with ninhydrin (0.2 cc. of a 1 per cent. solution, to 10 cc. of the fluid). The reaction is not read for thirty minutes after boiling. If the conditions are all favorable, *i.e.*, the serum used be from a pregnant woman, the tissue used as substratum be placenta, the enzyme in the serum acts upon the substratum and transforms its albumins to peptones and amino-acids; if the transfusion is perfect in both thimbles, and neither thimble leaks (this has, of course, been previously tested and security can be counted upon now) the fluid surrounding the thimble containing the serum should give a bright blue color or positive reaction, and that surrounding the thimble containing the water no color or a negative reaction.

By the test we are then able to determine, the substratum being known, whether the serum contains an enzyme capable of acting upon or transforming it; or the enzymic character of the serum being known, it may be possible to tell something about the substratum.

The general consensus of opinion is in favor of this reaction as being a useful adjunct in making the diagnosis of pregnancy. But its applicability may not be limited to the diagnosis of pregnancy for Freund and Abderhalden,* Frank and Heiman† and many others have used it as an adjunct in the diagnosis of cancer, and various other investigators have shown that modifications of the method makes it applicable for purposes of diagnosis or investigation of other conditions in which defensive enzymes may be present in the blood. For each of these investigations the specific substratum must be prepared, and in making each test, the application of the enzyme-containing serum to the sterile and appropriate substratum must be made in the tested thimbles with the precautions given above.

The method is not exclusively adapted for investigation of proteolytic enzymes in the serum, but to diastatic and lipolytic ferments as well and Abderhalden has shown that it has uses in these fields. How much importance attaches to the enzymes thus mobilized in the blood in the conditions comprehended in the studies of immunity is as yet uncertain. That there is some bearing of the one upon the other cannot be doubted. The Abderhalden reactions seem to be less specific than the immunity reactions and appear more as reactions *en gros*, while the immunity reactions previously studied were reactions *en detail*, but it may well be that this apparent difference depends upon the newness of the former reactions and the crudity of the methods employed as contrasted with the more elaborate study of the latter and the more delicate methods used.

* Münch. med. Wochenschrift, 1913, XIV, 763.
† Berl. klin. Wochenschrift, 1913, L, No. 14.

CHAPTER V

METHODS OF OBSERVING MICRO-ORGANISMS

It is of the utmost importance to examine micro-organisms alive, and as nearly as possible in their normal environment, then to supplement this examination by the study of dead and stained specimens.

The study of the living organism has the advantage of showing its true shape, size, grouping, motility, reproduction, and natural history. It has the disadvantage of being somewhat difficult because of its small size and transparency.

So long as bacteria were observed only in the natural condition, however, it was impossible to find them in the tissues of diseased animals, and it was not until Weigert suggested the use of the anilin dyes for coloring them that their demonstration was made easy and their relationship to pathologic conditions established.

The beauty and clearness of stained specimens, and the ease with which they can be observed, have led to some serious errors on the part of students, who often fail to realize the unnatural condition of the stained bacteria they observe. It only needs a moment's consideration to show how disturbed must be the structure of an organism after it has been dried, fixed, boiled, or steamed, passed through several chemic reagents, dehydrated and impregnated with stains, etc., to suggest how totally unnatural its appearance may become.

It is, therefore, necessary to examine every organism, under study, in the living condition, and to control all the appearances of the stained specimen by comparison.

I. THE STUDY OF LIVING BACTERIA

The simplest method of observing live bacteria is to take a drop of liquid containing them, place it upon a slide, put on a cover, and examine.

While this method is simple, it cannot be recommended, as evaporation at the edges causes currents of liquid to flow to and fro beneath the cover, carrying the bacteria with them and making it almost impossible to determine whether the organisms under examination are motile or not. Should it be desirable that such a specimen be kept for a time, so much evaporation takes place that in the course of an hour or two it has changed too much to be of further use.

The best way to examine living micro-organisms is in what is called the *hanging drop*. A hollow-ground slide is used, and with the aid of a small camel's-hair pencil a ring of vaselin is drawn on the slide about, not in, the concavity. A drop of the material to be examined is placed in the center of a large clean cover-glass and then placed upon the slide so that the drop hangs in, but does not touch, the glass. The micro-organisms are thus hermetically sealed in an air chamber, and appear under almost the same conditions as in the culture. Such a specimen may be kept and examined from day to day, the bacteria continuing to live until the oxygen or nutriment is exhausted. By means of a special apparatus in which the microscope is placed, the growing bacteria may be watched at any temperature, and exact observations made.

The hanging drop should always be examined at the edge, as the center is too thick.

In such a specimen it is possible to determine the shape, size,

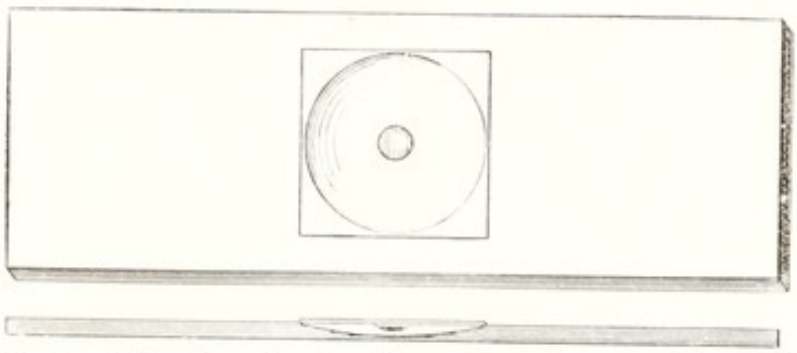


Fig. 31.—The "hanging drop" seen from above and in profile.

grouping, division, sporulation, and motility of the organism under observation.

Care should be exercised to use a rather small drop, especially for the detection of motility, as a large one vibrates and masks the motility of the sluggish forms.

When the bacteria to be observed are in solid or semi-solid culture, a small quantity of the culture should be mixed in a drop of sterile bouillon or other fluid.

For observing the growth of bacteria where it is desirable to prevent movement, Hill* has invented an ingenious device which he calls the "*hanging block*." His directions for preparing it are as follows:

"Pour melted nutrient agar into a Petri dish to the depth of about one-eighth or one-quarter inch. Cool this agar, and cut from it a block about one-quarter inch to one-third inch square and of the thickness of the agar layer in the dish. This block has a smooth upper and under surface. Place it, under side down, on a slide and protect it from dust. Prepare an emulsion, in sterile water, of the organism to be examined if it has been grown on a solid medium, or use a broth culture; spread the emulsion or broth upon the upper surface of the block as

* "Journal of Medical Research," March, 1902, vol. VII, No. 2; new series, vol. II.

if making an ordinary cover-slip preparation. Place the slide and block in a 37°C. incubator for five to ten minutes to dry slightly. Then lay a clean sterile cover-slip on the inoculated surface of the block in close contact with it, usually avoiding air-bubbles. Remove the slide from the lower surface of the block and invert the cover-slip so that the agar block is uppermost. With a platinum loop run a drop or two of melted agar along each side of the agar block, to fill the angles between the sides of the block and the cover-slip. This seal hardens at once, preventing slipping of the block. Place the preparation in the incubator again for five or ten minutes to dry the agar-agar seal. Invert this preparation over a moist chamber and seal the cover-slip in place with white wax or paraffin. Vaselin softens too readily at 37°C., allowing shifting of the cover-slip. The preparation may then be examined at leisure."

With this means of examining the growing cultures, Hill has acquired interesting knowledge of the fission and budding of Bacillus diphtheriæ.

If the specimens to be examined must be kept for some time at an elevated temperature, some such apparatus as that of Nuttall will be found useful.

II. STAINING BACTERIA

In the early days of bacteriology efforts were made to facilitate the observation of bacteria by the use of nuclear dyes. Both carmin and hematoxylin tinge the nuclei of the bacteria a little, but so unsatisfactorily that since Weigert introduced the anilin dyes for the purpose, all other stains have been abandoned. The affinity between the bacteria and the anilin dyes is peculiar, and in certain cases can be used for the differentiation of species.

The best anilin dyes made at the present time, and those which have become the standard for all bacteriologic work, are made in Germany by Dr. Grübler, and in ordering stains the name of this manufacturer should be specified.

Readers interested in the biochemistry of the subject will do well to refer to the excellent papers by Arnold Grimme,* upon "The Important Methods of Staining Bacteria, etc.," and Marx,† upon "The Metachromatic and Babes-Ernst Granules."

In this work special methods for staining such bacteria as have peculiar reactions will be given together with the description of the particular organisms, general methods only being discussed in this chapter.

Preparations for General Examination.—For bacteriologic purposes thin covers (No. 1) are required, because thicker glasses may interfere with the focussing of the oil-immersion lenses. The cover-glasses must be *perfectly clean*. It is therefore best to clean a large quantity in advance of use by immersing them first in a strong mineral acid, then washing them in water, then in alcohol, then in ether, and finally keeping them in ether until they are to be used. Except that it sometimes cracks, bends, or fuses the edge of the glass, a

* "Centralbl. f. Bakt.," etc., 1902, Bd. XXXII, Nos. 2, 3, 4, and 5.

† Ibid., 1902, XXXII, Nos. 10 and 11, p. 108.

more convenient method is to wipe the glasses as clean as possible with a soft cotton cloth, seize them with *fine-pointed* forceps, and pass them repeatedly through a small Bunsen flame until it becomes greenish-yellow. The hot glass must then be slowly elevated above the flame, so as to allow it to anneal. This manœuver removes the organic matter by combustion. It is not expedient to use covers twice for bacteriologic work, though if well cleansed by immersion in acid and washing, they may subsequently be employed for ordinary microscopic objects.

The fragility of the covers and their likelihood to be broken or dropped at the critical moment, make most workers prefer to *stain directly upon the slide*. The slide should be thoroughly cleaned, and if the material to be examined is spread near one end, the other may serve as a convenient handle. The slide is also to be preferred if a number of examinations are to be made simultaneously or for comparison, as it is large enough to contain a number of "smears."

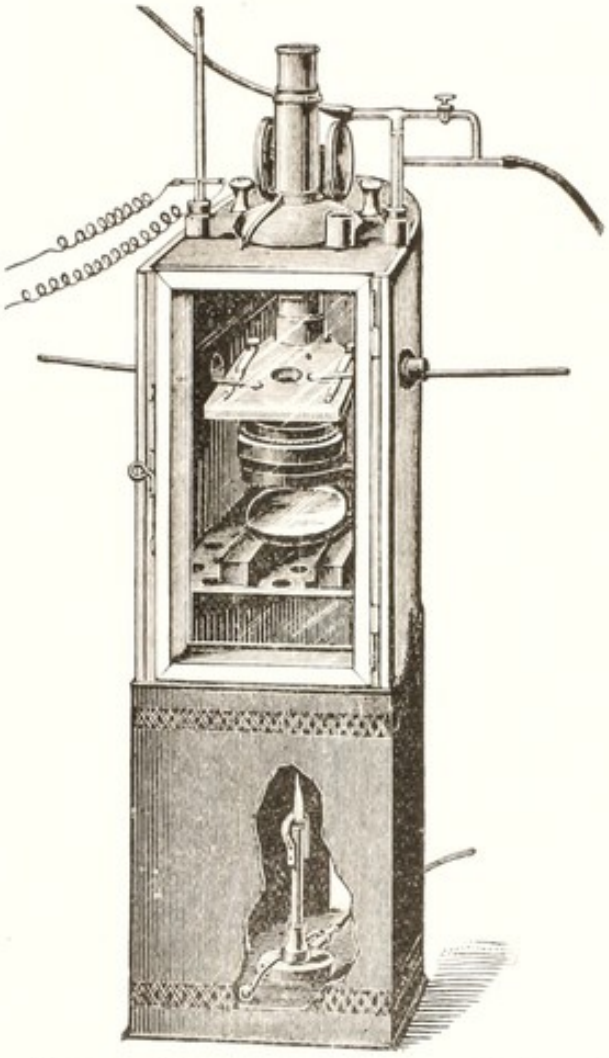


Fig. 32.—Apparatus for keeping objects under microscopic examination at constant temperatures (Nuttall).

Simple Method of Staining.—The material to be examined must be spread in the thinnest possible layer upon the surface of the perfectly clean cover-glass or slide and dried. The most convenient method of spreading is to place a minute drop on the glass with a platinum loop, and then spread it evenly over the glass with the flat wire. Should it be stained at once it would all wash off, so it must next be fixed to the glass by being passed *three times through a flame*, experience having shown that when drawn through the flame three times the desired effect is usually accomplished. The Germans recommend that a Bunsen burner or a large alcohol lamp be used, that the arm describe a circle a foot in diameter, each revolution occupying a second of time, and the glass being made to pass through

the flame from apex to base three times. This is supposed to be exactly the requisite amount of heating. The rule is a good one for the inexperienced.

Inequality in the size of various flames may make it desirable to have a more accurate rule. Novy* suggests that as soon as it is found that the glass is so hot that it can no longer be held against the finger it is sufficiently heated for fixing.

After fixing, the preparation is ready for the stain. Every laboratory should be provided with "*stock solutions*," which are saturated solutions of the ordinary dyes. For preparing them Wood† gives the following parts per 100 as being sufficiently accurate:

Alcoholic solutions (96 per cent. alcohol)		Aqueous solutions (distilled water)	
Fuchsin	3.0 grams.		
Gentian violet	4.8 "	Gentian violet	1.5 grams.
Methylene-blue	7.0 "	Methylene-blue	6.7 "
(70 per cent. alcohol)			
Scharlach R	3.2 "		
Soudan III	0.2 "		
(50 per cent. alcohol)			
Thionin	0.6 "	Thionin	1.2 "

Of these it is well to have fuchsin, gentian violet, and methylene-blue always made up. The stock solutions will not stain, but form the basis of the staining solutions. For ordinary staining an *aqueous solution* is employed. A small bottle is nearly filled with distilled water, and the stock solution added, drop by drop, until the color becomes just sufficiently intense to prevent the ready recognition of objects through it. For exact work it is probably best to give these stains a standard composition, using 5 cc. of the saturated alcoholic solution to 95 cc. of water. Such a watery solution possesses the power of readily penetrating the dried cytoplasm of the bacterium.

Cover-glasses are apt to slip from the fingers and spill the stain, so when using them it is well to be provided with special forceps which hold the glass in a firm grip and allow of all manipulations without danger of soiling the fingers or clothes. The ordinary sharp-pointed forceps are unfit for the purpose, as capillary attraction draws the stain between the blades and makes certain the soiling of the fingers. In using the special forceps the glass should not be caught at the edge, but a short distance from it, as shown in the cut. This altogether prevents capillary attraction between the blades. When the material is spread upon the slide no forceps are needed, and the method correspondingly simplified. Sufficient stain is allowed to run from a pipet upon the smear to flood it, but not overflow, and is allowed to remain for a moment or two, after which it is thoroughly washed off with water. The smear upon a slide is then dried and examined at once, a drop of oil of cedar being placed

* "Laboratory Work in Bacteriology," 1899.

† "Chemical and Microscopical Diagnosis," N. Y., 1905, D. Appleton & Co., p. 683.

directly upon the smear, and no cover-glass used. If the staining has been done upon a cover-glass, it can be mounted upon a slide with a drop of water between, and then examined, though this is less satisfactory than examination after drying it and mounting it in Canada balsam.

Sometimes the material to be examined is solid or too thick to spread upon the glass conveniently. Under such circumstances a drop of distilled water or bouillon can be added and a minute portion of the material mixed in it and spread upon the glass.

When the bacteria are contained in urine or other non-albuminous fluid, so that the heat used for fixing has nothing to coagulate and fix the organisms to the glass, a drop of Meyer's glycerin-albumen can be added with advantage, though the precaution must be taken to see that this mixture contains no bacteria to cause confusion with those in the material to be studied.

The entire process is, in brief: (1) Spread the material upon the glass; (2) dry—do not heat; (3) pass three times through the flame; (4) stain—one minute; (5) wash thoroughly in water; (6) dry; (7) mount in Canada balsam.

Fig. 33.—Stewart's cover-glass forceps.

To Observe Bacteria in Sections of Tissue.—Hardening.—It not infrequently happens that the bacteria to be examined are scattered among or inclosed in the cells of tissues. The demonstration then becomes a matter of difficulty, and the method employed must be modified according to the particular kind of organism. The success of the method will depend upon the good preservation of the tissue to be studied. As bacteria disintegrate rapidly in dead tissue, the specimen for examination should be secured as fresh as possible, cut into small fragments, and immersed in absolute alcohol from six to twenty-four hours, to kill and fix the cells and bacteria. The blocks are then removed from the absolute alcohol and kept in 80 to 90 per cent. alcohol, which does not shrink the tissue. Solutions of bichlorid of mercury* may also be used and are particularly useful when the bacteria are to be studied in relation to the cells of the tissues.

* Zenker's fluid:

Bichromate of potassium	2.5 grams
Sulphate of sodium	1.0 "
Bichlorid of mercury	5.0 "
Water	100.0 "

At the time of using add 5 grams of glacial acetic acid. Permit the specimens to remain in the solution for a few hours only, then wash for twenty-four hours in running water and transfer to 80 per cent. alcohol.

Tissues preserved in 95 per cent. alcohol, Müller's fluid, 4 per cent. formaldehyd, and other ordinary solutions rarely show the bacteria well.

Embedding.—The ordinary *methods of embedding* suffice. The simpler of these are as follows:

I. Celloidin (Schering).—The solutions of celloidin are made in equal parts of absolute alcohol and ether and should have the thickness of oil or molasses. From the hardening reagent (if other than absolute alcohol) pass the blocks of tissue through:

Ninety-five per cent. alcohol, twelve to twenty-four hours;
Absolute alcohol, six to twelve hours;
Thin celloidin (consistence of oil), twelve to twenty-four hours;
Thick celloidin (consistence of molasses), six to twelve hours.

Place upon a block of vulcanite or hard wood, allow the ether to evaporate until the block can be overturned without dislodging the specimen; then place in 80 per cent. alcohol until ready to cut. The knife must be kept flooded with alcohol while cutting.

Celloidin is soluble in absolute alcohol, ether, and oil of cloves, so that the staining of the sections must be accomplished without the use of these reagents if possible.

Celloidin sections can be fastened to the slide, if desired, by firmly pressing filter paper upon them and rubbing hard, then allowing a little vapor of ether to run upon them.

II. Paraffin.—Pure paraffin having a melting-point of about 52°C. is used. The hardened blocks of tissue are passed through:

Ninety-five per cent. alcohol, twelve to twenty-four hours;
Absolute alcohol, six to twelve hours;
Chloroform, benzole, or xylol, four hours;
A saturated solution of paraffin in one of the above reagents, four to eight hours.

The block is then placed in melted paraffin in an oven or paraffin water-bath, at 50°–55°C., until the volatile reagent is all evaporated, and the tissue impregnated with paraffin (four to twelve hours), and finally embedded in freshly melted paraffin in any convenient mold. In cutting, the knife must be perfectly dry.

The cut paraffin sections can be placed upon the surface of slightly warmed water to flatten out the wrinkles, and then floated upon a clean slide upon which a film of Meyer's glycerin-albumen (equal parts of glycerin and white of egg thoroughly beaten up and filtered, and preserved with a crystal of thymol) has been spread. After drying, the slides are placed in the paraffin oven for an hour at 60°C., so that the albumen coagulates and fixes the sections to the glass.

When sections so spread and fixed upon the slide are to be stained, the paraffin must first be dissolved in chloroform, benzole, xylol, oil of turpentine, etc., which in turn must be removed with 95 per cent. alcohol. The further staining, by whatever method desired, is accomplished by dropping the reagents upon the slide.

III. Glycerin-gelatin.—As the penetration of the tissue by celloidin is attended with deterioration in the staining qualities of the tubercle bacillus, it has been recommended by Kolle* that the tissue be saturated with a mixture of glycerin, 1 part; gelatin, 2 parts; and water, 3 parts; cemented to a cork or block of wood, hardened in absolute alcohol, and cut as usual for celloidin with a knife wet with alcohol.

Staining.—Simple Method.—For ordinary work the following simple method can be recommended: After the sections are cut and cemented to the slide, the paraffin and celloidin should be removed by appropriate solvents. The sections are immersed in the ordinary aqueous solution of the anilin stain and allowed to remain about five minutes, next washed in water for several minutes, then decolorized in 0.5 to 1 per cent. acetic acid solution. The acid removes the stain from the tissues, but ultimately from the bacteria as well, so that one must watch carefully, and so soon as the color has almost disappeared from the sections, they must be removed and transferred to absolute alcohol. At this point the process may be interrupted to allow the tissue elements to be countercolored with alum-carmin or any stain not requiring acid for differentiation, after which the sections are dehydrated in absolute alcohol, cleared in xylol, and mounted in Canada balsam.

Fig. 34.—Coplin's staining jar.

The greater number of applications can be made by simply dropping the reagents upon the slide while held in the fingers. Where exposure to the reagents is to be prolonged, the Coplin jar or some more capacious device must be employed.

Pfeiffer's Method.—The sections are stained for one-half hour in diluted Ziehl's carbol-fuchsin (*q.v.*), then transferred to absolute alcohol made feebly acid with acetic acid. The sections must be carefully watched, and so soon as the original, almost black-red color gives place to a red-violet color they are removed to xylol, to be cleared preparatory to mounting in balsam.

Löffler's Method.—Certain bacteria that do not permit ready penetration by the dye require some more intense stain. One of the best of these is Löffler's alkaline methylene-blue:

Saturated alcoholic solution of methylene-blue	30
1 : 10,000 aqueous solution of caustic potash	100

*Flügge's "Die Mikroorganismen," vol. 1, page 534.

The cut sections of tissue are stained for a few minutes and then differentiated in a 1 per cent. solution of hydrochloric acid for a few seconds, after which they are dehydrated in alcohol, cleared in xylol, and mounted in balsam.

Some bacteria, such as the typhoid fever bacillus, decolorize readily so that the use of acid should be avoided, washing in water or alcohol being sufficient.

Gram's Method of Staining Bacteria in Tissue.—Gram was the fortunate discoverer of a method of impregnating bacteria with an insoluble color. It will be seen at a glance that this is a marked improvement on the methods given above, as the stained tissue can be washed thoroughly in either water or alcohol until its cells are colorless, without fear that the bacteria will be decolorized. The details of the method are as follows: The section is stained from five to ten minutes in a solution of a basic anilin dye, pure anilin (anilin oil) and water. This solution, first devised by Ehrlich, is known as Ehrlich's solution. The ordinary method of preparing it is to mix the following:

Pure anilin	4
Saturated alcoholic solution of gentian violet	11
Water	100

Instead of gentian violet, methyl violet, Victoria blue, or any pararosanilin dye will answer. The rosanilin dyes, such as fuchsin, methylene-blue, vesuvin, etc., will not react with iodin, and so cannot be used for the purpose. The anilin-oil solutions do not keep well; in fact, seldom longer than six to eight weeks, sometimes not more than two or three; therefore it is best to prepare but a small quantity by pouring about 1 cc. of pure anilin into a test-tube, filling the tube about one-half with distilled water, shaking well, then filtering as much as is desired into a small dish. To this the saturated alcoholic solution of the dye is added until the surface becomes distinctly metallic in appearance.

Friedländer recommends that the section remain from fifteen to thirty minutes in warm stain, and in many cases the prolonged process gives better results.

From the stain the section is given a rather hasty washing in water, and then immersed from two to three minutes in Gram's solution (a dilute Lugol's solution):

Iodin crystals	1
Potassium iodid	2
Water	300

The specimen while in the Gram solution turns a dark blackish-brown color, but when removed and carefully washed in 95 per cent. alcohol again becomes blue. The washing in 95 per cent. alcohol is continued until no more color is given off and the tissue assumes its original color. If it is simply desired to find the bacteria, the section can be dehydrated in absolute alcohol for a moment,

cleared in xylol, and mounted in Canada balsam. If it is necessary to study the relation of the bacteria to the tissue elements, a nuclear stain, such as alum-carmin or Bismarck brown, may be previously or subsequently used. Should a nuclear stain requiring acid for its differentiation be desirable, the process of staining must precede the Gram stain, so that the acid shall not act upon the stained bacteria.

Gram's method rests upon the fact that *the combination of bacterial substance, anilin dye, and the iodids forms a compound insoluble in alcohol.*

The process described may be summed up as follows:

Stain in Ehrlich's anilin-water gentian violet five to thirty minutes;
Wash in water;
Immerse two to three minutes in Gram's solution;
Wash in 95 per cent. alcohol until no more color comes out;
Dehydrate in absolute alcohol;
Clear in xylol;
Mount in Canada balsam.

No matter how carefully the method is performed, an unsightly precipitate is sometimes deposited upon the tissue, obscuring both its cells and contained bacteria. Muir and Ritchie obviate this (1) by making the staining solution with 1 : 20 aqueous solution of carbolic acid instead of the saturated anilin solution, and (2) by clearing the tissue with oil of cloves after dehydration with alcohol. The oil of cloves, however, is itself a powerful decolorant and must be washed out in xylol before the section is mounted in Canada balsam.

Gram's method is also employed to aid in differentiating similar species of bacteria in culture. A thin layer of a suspension of the bacteria to be examined is spread upon a slide or cover-glass, dried, and fixed; then flooded with the anilin-oil gentian violet or other staining solution. The solution is kept warm by holding the glass flooded with the stain over a small flame. The process of staining is continued from two to five minutes. If the heating causes the stain to evaporate, more of it must be added so that it does not dry and incrust the glass.

The stain is poured off, and replaced by Gram's solution, which is allowed to remain from one-half to two minutes, and gently agitated.

The smear is next washed in 95 per cent. alcohol until the blue color is wholly or almost lost, after which it can be counterstained with pyronin, eosin, Bismarck brown, vesuvin, etc., washed, dried, and mounted in Canada balsam. Given briefly, the method is:

Stain with Ehrlich's solution two to five minutes;
Gram's solution for one-half to two minutes;
Wash in 95 per cent. alcohol until decolorized;
Counterstain if desired; wash off the counterstain with water;
Dry;
Mount in Canada balsam.

Nicolle* suggests the following modification of the technic:

(*a*) *For Cover-glass Specimens:*

1. Stain for one to five minutes in a warm solution made as follows: 10 cc. of saturated alcoholic solution of gentian violet, 100 cc. of a 1 per cent. aqueous solution of carbolic acid.
2. Immerse from four to six seconds in the iodine-iodide of potassium solution.
3. Decolorize in a mixture of 3 parts of absolute alcohol and 1 part of acetone.
4. Counterstain if desired.

(*b*) *For Sections:*

1. Stain the nuclear elements of the tissue with carmine. For this Nicolle prefers Orth's carmine solution (5 parts of Orth's carmine with 1 part of 95 per cent. alcohol).
2. Stain in the carbol-gentian violet, as indicated above.
3. Immerse for four to six seconds in the iodine-iodide of potassium solution.
4. Differentiate with absolute alcohol containing 0.33 per cent. (by volume) of acetone.
5. Treat with 95 per cent. alcohol containing some picric acid until the tissue is greenish yellow (one to five seconds).
6. Dehydrate with absolute alcohol.
7. Clear with xylol or other appropriate reagent.
8. Mount in balsam.

The Gram-Weigert Stain can be employed with beautiful results for staining many micro-organisms. It differs from the Gram method in that anilin oil instead of alcohol is used for decolorizing. To secure the most brilliant results it is best first to stain the tissue with alum, borax, or lithium carmin, and then—

1. Stain in Ehrlich's anilin-oil-water gentian violet, five to twenty minutes;
2. Wash off excess with normal salt solution;
3. Immerse in dilute iodin solution (iodin 1, iodid of potassium 2, water 100) for one minute;
4. Drain off the fluid and blot the section spread out upon the slide, with absorbent paper;
5. Decolorize with a mixture of equal parts of anilin and xylol;
6. Wash out the anilin with pure xylol.
7. Mount in xylol balsam.

Gram's method does not stain all bacteria, hence can be used to aid in the differentiation of species:

Gram-negative	Gram-positive
Bacillus anthracis symptomatici;	Bacillus aërogenes capsulatus;
Bacillus coli (whole group);	Bacillus anthracis;
Bacillus ducreyi;	Bacillus botulinus;
Bacillus dysenteriæ	Bacillus diphtheriæ;
Bacillus icteroides;	Bacillus subtilis (whole group);
Bacillus influenzæ;	Bacillus tetani;
Bacillus mallei;	Bacillus tuberculosis (whole acid-fast group);
Bacillus œdematis maligni;	Diplococcus pneumoniæ;
Bacillus pestis bubonica;	Micrococcus tetragenus;
Bacillus pneumoniæ (Friedländer);	

* "Ann. de l'Inst. Pasteur," 1895, IX.

Gram-negative	Gram-positive
Bacillus proteus vulgaris;	Staphylococcus pyogenes albus;
Bacillus pyocyaneus;	Staphylococcus pyogenes aureus;
Bacillus rhinoscleromatis;	Streptococcus pyogenes.
Bacillus suipestifer;	
Bacillus suisepticus;	
Bacillus typhosus (whole group);	
Diplococcus intracellularis meningitidis;	
Micrococcus catarrhalis;	
Micrococcus gonorrhœæ (Neisser)	
Micrococcus melitensis;	
Spirillum choleræ asiaticæ;	
Spirillum choleræ gallinarum;	
Spirillum choleræ nostras;	
Spirillum metschnikovi;	
Spirillum tyrogenum;	
Spirochæte duttoni;	
Spirochæte obermeieri;	
Spirochæte refringens;	
Treponema pallidum;	
Treponema pertenue.	

Eosin and Methylene-blue (Mallory) make a beautiful contrast tissue stain for routine work, and also demonstrate the presence of most bacteria. The success of the method seems to depend largely upon the quality of the reagents used and a careful study of their effects. Hardening in Zenker's fluid is highly recommended as a preliminary. The details as given by Mallory are as follows:

1. Stain paraffin sections in a 5 to 10 per cent. aqueous solution of eosin from five to twenty minutes or longer;
2. Wash in water to get rid of the excess of eosin;
3. Stain in Unna's alkaline methylene-blue solution (methylene-blue 1, carbonate of potassium 1, water 100), diluted 1 : 10 with water, from one-half to one hour, or use a stronger solution and stain for a few minutes only;
4. Wash in water.
5. Differentiate and dehydrate in 95 per cent. alcohol, followed by absolute alcohol until the pink color returns in the section;
6. Clear with xylol;
7. Mount in xylol balsam.

The nuclei and micro-organisms will be colored blue, the cytoplasm, etc., red.

Zieler* recommends for the staining of the typhoid, glanders and other difficultly stainable bacteria, the following method of demonstration in the tissues:

1. Fix and harden in Müller-formol solution.
   Paraffin imbedding.
2. Staining overnight in
   - Orcein D. 0.1
   - Officinal sulphuric acid. . . . . 2.
   - 70 per cent. alcohol. . . . . 100.
3. Washing in 70 per cent. alcohol for a short time to remove the excess of orcein.
4. Washing in water.
5. Staining in polychrome methylene-blue ten minutes to two hours.
6. Washing in distilled water.
7. Thorough differentiation in glycerin-ether 1 : 2–5 water until the tissues become pale blue.

* "Centralbl. f. allg. Path. u. path. Anat." Bd. XIV, No. 14, p. 561.

8. Washing in distilled water.
9. Seventy per cent. alcohol.
10. Absolute alcohol.
11. Xylol.
12. Balsam.

Glanders bacilli appear dark violet on a colorless background; typhoid bacilli intense dark red violet.

Method of Staining Spores.—It has already been pointed out that the peculiar quality of the spore capsules protects them to a certain extent from the influence of stains and disinfectants. On this account they are much more difficult to color than the adult bacteria. Several methods are recommended, the one generally employed being as follows: Spread the thinnest possible layer of material upon a cover-glass, dry, and fix. Have ready a watch-crystalful of Ehrlich's solution, preferably made of fuchsin, and drop the cover-glass, prepared side down, upon the surface, where it should float. Heat the stain until it begins to steam, and allow the specimen to remain in the hot stain for from five to fifteen minutes. The cover is then transferred to a 3 per cent. solution of hydrochloric acid in absolute alcohol for about one minute. Abbott recommends that the cover-glass be submerged, prepared side up, in a dish of this solution and gently agitated for exactly one minute, removed, washed in water, and counterstained with an aqueous solution of methyl or methylene-blue.

In such a specimen the spores should appear red, and the adult organisms blue.

A good simple method is to place the prepared cover-glass in a test-tube half full of carbol-fuchsin:

Fuchsin	1
Alcohol	10
Five per cent. aqueous solution of phenol crystals	100

and boil it for at least fifteen minutes, after which it is decolorized, either with 3 per cent. hydrochloric or 2–5 per cent. acetic acid, washed in water, and counterstained blue.

Muir and Ritchie* recommend that cover-films be prepared and stained as for tubercle baciili (*q.v.*), decolorized with a 1 per cent. sulphuric acid solution in water or methyl alcohol, then washed in water and counterstained with a saturated aqueous methylene-blue solution for half a minute, washed again with water, dried, and mounted in Canada balsam.

Abbott's method of staining spores is as follows:

1. Stain deeply with methylene-blue, heating repeatedly until the stain reaches the boiling-point—one minute.
2. Wash in water.
3. Wash in 95 per cent. alcohol containing 0.2 to 0.3 per cent. of hydrochloric acid.
4. Wash in water.

* "Manual of Bacteriology," London, 1897.

5. Stain for eight to ten seconds in anilin-fuchsin solution.
6. Wash in water.
7. Dry.
8. Mount in balsam.

The spores are blue; the bacteria, red.

Möller* finds it advantageous to prepare the films, before staining, by immersion in chloroform for two minutes, following this by immersion in 5 per cent. chromic acid solution for one-half to two minutes.

The exact technic is as follows:

1. Treat the spread with chloroform for two minutes.
2. Wash with water.
3. Treat with 5 per cent. solution of chromic acid for one-half to two minutes.
4. Wash in water.
5. Stain with carbol-fuchsin, slowly heating until the fluid boils.
6. Decolorize in 5 per cent. aqueous sulphuric acid.
7. Wash well with water.
8. Stain in a 1 : 100 aqueous solution of methylene-blue for thirty seconds.

The spores should be red and the bacilli blue.

Anjeszky† recommends the following method of staining spores, which is said always to give good results even with anthrax bacilli: A cover-glass is thinly spread with the spore-containing fluid and dried. While it is drying, some 0.5 per cent. hydrochloric acid is warmed in a porcelain dish over a Bunsen flame until it steams well and bubbles begin to form. When the solution is hot and the smear dry, the cover-glass is dropped upon the fluid, which is allowed to act upon the unfixed smear for three or four minutes. The cover is removed, washed with water, dried, and fixed for the first time, then stained with Ziehl's carbol-fuchsin solution, which is warmed twice until fumes arise. The preparation is allowed to cool, decolorized with a 4–5 per cent. sulphuric acid solution, and counterstained for a minute or two with malachite green or methylene-blue. The whole procedure should not take longer than eight or ten minutes.

Fiocca‡ suggests the following rapid method: "About 20 cc. of a 10 per cent. aqueous solution of ammonium are poured into a watch-glass, and 10 to 20 drops of a saturated solution of gentian violet, fuchsin, methyl blue, or safranin added. The solution is warmed until vapor begins to rise, then is ready for use. A very thinly spread cover-glass, carefully dried and fixed, is immersed for three to five minutes (sometimes ten to twenty minutes), washed in water, washed momentarily in a 20 per cent. solution of nitric or sulphuric acid, washed again in water, then counterstained with an aqueous solution of vesuvin, chrysoidin, methyl blue, malachite green, or safranin, according to the color of the preceding stain. This whole process is said to take only from eight to ten minutes, and to give remarkably clear and beautiful pictures."

* "Centralbl. f. Bakt. u. Parasitenk.," Bd. X, p. 273.
† Ibid., Feb. 27, 1898, XXIII, No. 8, p. 329.
‡ "Centralbl. f. Bakt. u. Parasitenk.," July 1, 1893, XIV, No. 1.

Method of Staining Flagella.—This is more difficult than the staining of the bacteria or the spores.

*Löffler's Method.**—This is the original and best method, though somewhat cumbersome, and hence rarely employed at the present time. Three solutions are used:

(A)—Twenty per cent. aqueous solution of tannic acid..... 10
Cold saturated aqueous solution of ferrous sulphate..... 5
Alcoholic solution of fuchsin or methyl violet..... 1

(B) One per cent. aqueous solution of caustic soda.

(C) An aqueous solution of sulphuric acid of such strength that 1 cc. will exactly neutralize an equal quantity of solution B.

Some of the culture to be stained is mixed upon a cover-glass with a drop of distilled water making a first dilution, which is still too rich in bacteria to permit the flagella to show well, so that it is recommended to prepare a second by placing a small drop of distilled water, upon a cover and taking a loopful from the first dilution to make the second, and spreading it over the entire surface without much rubbing or stirring. The film is allowed to dry, and is then fixed by passing it three times through the flame. When this is done with forceps there is some danger of the preparation becoming too hot, so Löffler recommends that the glass be held in the fingers while the passes through the flame are made.

The cover-glass is now held in forceps, and the mordant, solution A, dropped upon it until it is well covered, when it is warmed until it begins to steam. The mordant must be replaced as it evaporates. It must not be heated too strongly: above all things, must not boil. This solution is allowed to act from one-half to one minute, is then washed off with distilled water, and then with absolute alcohol until all traces of the solution have been removed. The real stain—Löffler recommends an anilin-water fuchsin (Ehrlich's solution)—which should have a neutral reaction, is next dropped on so as to cover the film, and heated for a minute until vapor begins to rise, after which it is washed off carefully, dried, and mounted in Canada balsam. To obtain the neutral reaction of the stain, enough of the 1 per cent. sodium hydrate solution is added to an amount of the anilin-water-fuchsin solution having a thickness of several centimeters to begin to change the transparent into an opaque solution.

A specimen thus treated may or may not show the flagella. If not, before proceeding further it is necessary to study the chemic products of the micro-organism in culture media. If by its growth the organism elaborates alkalies, from 1 drop to 1 cc. of solution C in 16 cc. must be added to the mordant A, and the staining repeated. It may be necessary to stain again and again until the proper amount is determined by the successful demonstration of the flagella. On the other hand, if the organism by its growth produces acid, solution B must be added, drop by drop, and numerous stained specimens examined to see with what addition of alkali the flagella will appear. Löffler fortunately worked out the amounts required for some species, and of the more important ones the following solutions of B and C must be added to 16 cc. of solution A to attain the desired effect:

Cholera spirillum..... ½–1 drop of solution C
Typhoid fever..... 1 cc. of solution B
Bacillus subtilis..... 28–30 drops of solution B
Bacillus of malignant edema..... 36 or 37 drops of solution B

Part of the success of the staining depends upon using a very young culture and having the bacteria thinly spread upon the glass, so as to be as free from albuminous and gelatinous materials as possible. The cover-glass must be cleaned most painstakingly; too much heating in fixing must be avoided. After using and washing off the mordant, the preparation should be dried before the application of the anilin-water-fuchsin solution.

* Ibid., 1890, Bd. VII, p. 625.

Pitfield's Method.—Pitfield* has devised a single solution, at once mordant and stain. It is made in two parts, which are filtered and mixed:

(A)—

Saturated aqueous solution of alum	10 cc.
Saturated alcoholic solution of gentian violet	1 "

(B)—

Tannic acid	1 gram
Distilled water	10 cc.

The solutions should be made with cold water, and immediately after mixing the stain is ready for use. The cover-slip is carefully cleaned, the grease being burned off in a flame. After it has cooled, the bacteria are spread upon it, well diluted with water. After drying thoroughly in the air, the stain is gradually poured on and by gentle heating brought almost to a boil; the slip covered with the hot stain is laid aside for a minute, then washed in water and mounted.

Smith's Modification of Pitfield's Method.†—A boiling saturated solution of bichlorid of mercury is poured into a bottle in which crystals of alum have been placed in quantity more than sufficient to saturate the fluid. The bottle is shaken and allowed to cool; 10 cc. of this solution are added to the same volume of freshly prepared tannic acid solution and 5 cc. of carbol fuchsin added. Mix and filter. The filtrate, which is the mordant, is caught directly upon the spread (the liquid must always be filtered at the time of use) and heated gently for three minutes, but not permitted to boil. Wash with water and then stain in the following:

Saturated alcoholic solution of gentian violet	1 cc.
Saturated solution of ammonium alum	10 "

Filter the stain directly upon the slide at the time of using, and heat it for three to four minutes. Wash thoroughly in water, dry, and mount in balsam.

Van Ermengem's Method.—Van Ermengem‡ has devised a somewhat complicated method of staining flagella, which has given great satisfaction. Three solutions, which he describes as the *bain fixateur*, *bain sensibilisateur*, and *bain reducteur et reinforçateur*, are to be used as follows:

1. *Bain fixateur:*

2 per cent. solution of osmic acid	1 part
10–25 per cent. solution of tannin	2 parts

The cover-glasses, which are very thinly spread, dried, and fixed, are placed in this bath for one hour at the room temperature, warmed until steam arises, and then kept hot for five minutes. They are next washed with distilled water, then with absolute alcohol, then again with distilled water. All three washings must be very thorough.

* "Medical News," Sept. 7, 1895.

† "British Medical Journal," 1901, 1, p. 205.

‡ "Travaux du Lab. d'hygiene et des bact. de Gand.," t. I, p. 3. Abstracted in the "Centralbl. f. Bakt. u. Parasitenk.," 1894, Bd. xv, p. 969.

2. *Bain sensibilisateur:*

5 per cent. solution of nitrate of silver in distilled water.

The films are allowed to remain in this for a few seconds, and are then immediately transferred to the third bath.

3. *Bain reducteur et reinforçateur:*

Gallic acid	5 grams
Tannin	3 "
Fused potassium acetate	10 "
Distilled water	350 cc.

The preparations are kept in this solution for a few seconds, then returned to the nitrate of silver solution until they begin to turn black. They are then washed, dried, and mounted.

Mervyn Gorden modifies the method by allowing the preparations to remain in the second bath for two minutes, transferring to the third bath for one and a half to two minutes, and then washing, drying, and mounting without returning to the second bath.

Muir and Ritchie find it advantageous to use a fresh supply of the third solution for each specimen.

Rossi* gives the following directions for staining flagella:

The culture to be examined should be a young culture, not more than ten, eighteen, or twenty-four hours old. It should be made upon freshly prepared agar-agar, or upon the reagent after it has been melted and then congealed, as it is of the utmost importance that the surface be moist. The culture should be examined by the hanging-drop method to see that the organisms are actively motile before the staining is attempted.

The staining should be done only after the greatest care has been taken to see that all the conditions are favorable. For this reason the cover-glasses employed in making the spreads must be carefully cleaned with alcohol, then immersed in steaming sulphuric acid for ten to fifteen minutes. They are then washed in water, then placed in a mixture of alcohol and benzine (equal parts), wiped with a clean soft cloth, and passed through the colorless Bunsen flame forty to fifty times, and then that side of the glass utilized for the "spread" that has been in direct contact with the flame.

A platinum loopful of the appropriate culture is placed in a drop of distilled water upon a clean slide and slightly stirred. If conditions are favorable, it forms a homogeneous emulsion. If clumps appear, the cultural conditions are not favorable.

If favorable, a loopful of this dilution is added to 1 cc. of distilled water in a clean cover-glass and thoroughly stirred. From the center of the surface of this fluid a platinum loopful is next taken and placed upon each of the prepared cover-glasses and, without spreading or stirring, allowed to dry in the air or in an exsiccator.

The staining solutions are made as follows:

(A) A solution of 50 grams of pure crystalline carbolic acid in 1000 cc. of distilled water, to which 40 grams of pure tannin are added, the whole being warmed on a water-bath until solution is complete.

(B) Basic fuchsin (rosanilinchlorhydrate)	2.5 grams
Absolute alcohol	100.0 cc.
(C) Potassium hydrate	1.0 gram
Distilled water	100.0 grams

Mix solutions A and B and preserve in a well-closed bottle. Place solution C in a bottle with a pipette stopper. When the staining is to be done, one pours 15 to 20 cc. of the A B mixture into a glass-stoppered test-tube and adds 2 or 3

* "Centralbl. f. Bakt. u. Parasitenk.," Orig., 1903, XXXIII, p. 572

drops of solution C. A precipitate forms, but quickly dissolves on shaking. More of solution C is added, and the tube shaken until the solution becomes brown and clouded and one can see a fine precipitate in a thin layer of the fluid. The fluid is next filtered several times through the same filter and caught in the same glass until it will remain clear for several minutes. Then it is poured on the filter a last time and 4 or 5 drops allowed to fall upon each of the prepared cover-glasses. In a short time a sheen is observed upon the surface of the fluid on the cover-glasses, showing that a fine precipitate has formed. When this has occurred, a little experience will show when the proper moment arrives to throw off the fluid and wash the cover in distilled water. It is the precipitate that clings to the flagella and renders them distinctly visible. If no precipitate occurs, the flagella will not be seen.

L. Smith* offers the following modification of Newman's method† as being a simple and excellent method of staining flagella:

The material and cover-glasses are prepared with care as for the foregoing methods, after which one proceeds as follows:

1. Transfer a loopful of the bacillary emulsion to the clean slide or cover-glass and allow it to dry in the air.
2. Expose to a mild degree of heat, holding the glass in the *fingers*—this is rather *drying* than actual *heating*.
3. Allow the stain to drop from a filter upon the film and remain in contact five to ten minutes.

   The formula for the stain is

   I. Tannic acid ..... 1 gram
   Potassium alum ..... 1 "
   Distilled water ..... 40 cc.

   Dissolve by shaking or allow to stand overnight in the incubator.

   II. "Night blue"‡ ..... 0.5 gram
   95 per cent. or absolute alcohol ..... 20.0 cc.

   Mix I and II thoroughly and remove the heavy precipitate by filtration. If not used at once, drop from a filter upon the film. The stain does not keep more than a few days.
4. Wash carefully but thoroughly in water.
5. Apply a saturated aqueous solution of gentian violet for about two minutes to stain the bodies of the bacteria.
6. Wash thoroughly in water, dry with smooth blotting-paper, and mount in balsam.

To secure a perfectly clean background for photomicrography, it is best to stain on a slide. The stain is then poured into a Petri dish, the slide inverted, the end of the slide used to push aside the film on the surface of the stain, and the film then immersed downward, one end of the slide supported, during staining, on a match-stick or bit of glass rod. In this way the adherence of the precipitate to the slide can be avoided.

THE OBSERVATION OF LIVING PROTOZOA

When protozoa are to be examined in transparent fluids, such as pond-water or culture fluids in which they have been artificially nourished, use can be made of a "live-box" or of the "hanging drop." Ordinarily, however, the organisms to be examined are contained in blood, in pus, in sputum, in feces, or in some other more or less opaque fluid, of which an extremely thin layer must be prepared in order that the formed elements may be separated sufficiently for the individual cells and organisms to be seen.

* "Jour. Med. Research," 1901, VI, p. 341.
† "Bacteria," John Murray, London, 2d edition.
‡ James Strong & Son, Glasgow and Manchester.

Such a thin layer is usually easily obtained by the use of a slide and cover-glass, and the careful preparation of a good film.

The slide and the cover-glass should be thoroughly cleansed and freed from fat and grit and well polished. A comparatively small drop of blood—let us say, for example—is placed upon the center of the slide and immediately covered with the cover-glass. If the drop is not too large and the glasses are clean, the weight of the cover-glass causes the drop to spread, and capillary attraction completes the formation of a very thin film. The quantity of blood used should not be sufficient to reach the edges of the cover-glass, else sometimes the glass is pressed up instead of being drawn down and moves about too freely. If the examination is to take enough time to cause the drop to dry, a match-stick dipped in thin vaselin and drawn about the edge of the cover will prevent it.

Such a film is usually best examined at or near the center, where the formed elements are not widely separated.

The living protozoa in preparations of this kind may be examined by ordinary illumination by transmitted light, or with lateral illumination by means of the "dark-field illuminator." The latter serves better for the discovery of the very small transparent organisms —spirochæta and treponema—and for the observation of the cilia and flagella.

STAINING PROTOZOA

It is through the study of stained protozoa that we arrive at most of our knowledge of their structural details. They can be stained in blood or fluids upon a slide or in sections of tissue.

As in the case of the bacteria, it is first necessary to prepare satisfactory spreads for the purpose. In order that the description shall be as practical as possible, we will suppose that the micro-organisms to be stained are in blood—spirochæta, plasmodium, etc.

As pointed out above, the protozoa, under such circumstances, are distributed among or in cellular elements that interfere with satisfactory observation unless precautions are taken to separate them as widely as may be required.

1. *Cover-glasses.*—The glasses should be perfectly clean and freed from fat, either by washing in alcohol and ether and wiping with a clean soft cotton cloth or Chinese rice paper, or by flaming. The drop of blood should be small and should be placed upon the center of one glass and immediately covered by another, so held that the corners do not coincide. As soon as the drop is fairly well distributed the glasses are gently slid apart.
2. *Slides.*—The slides, like the cover-glasses, must be perfectly clean. The drop of blood is placed upon one slide at about one-fourth the length of the slide from its end, touched with the end (it must have ground edges) of the second slide, and then gently pushed along until the fluid is exhausted.

If the covers are to be stained, they can most conveniently be held in the Stewart forceps. If the slides are used, they can be held in the fingers.

The stain most useful is that of Romanowsky. It has many modifications, of which the most used and best known are Giemsa's, Jenner's, Leishman's, Wright's, and Marino's. These stains can be bought either in solution or in tablet form ready for solution.

Those most highly to be recommended are Wright's and Marino's.

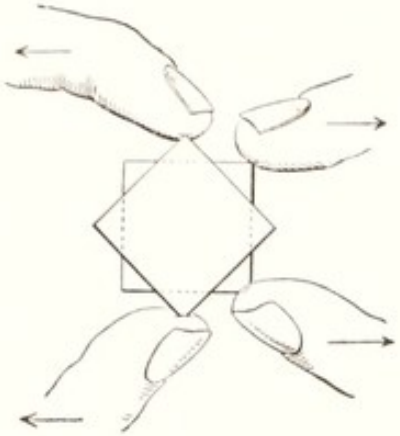


Fig. 35.—Method of making dry film with two cover-glasses (from Daniels' "Laboratory Studies in Tropical Medicine").

Wright's Blood-stain.—This is a modification of Leishmann's stain, to which it is to be preferred because it can be made in a few hours instead of eleven days. It combines the methylene-blue-eosin combination of Romanowsky with the methyl-alcohol fixation of Jenner.

It is prepared as follows:*

"To a 0.5 per cent. aqueous solution of sodium bicarbonate add methylene-blue (B. X. or "medicinally pure") in the proportion of 1 gm. of the dye

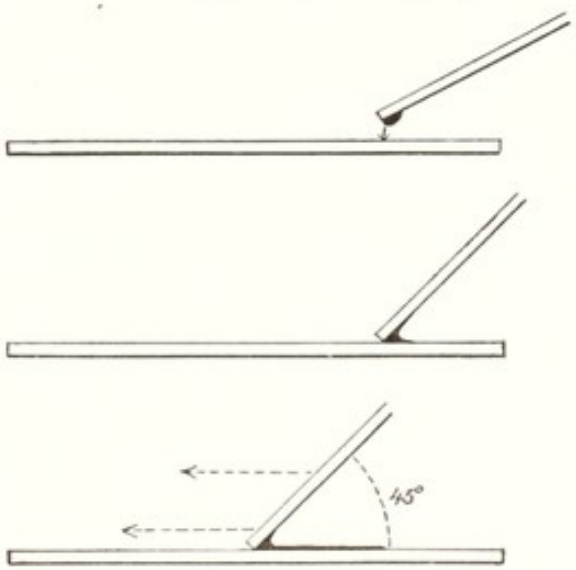


Fig. 36.—Method of making dry films with two slides (from Daniels' "Laboratory Studies in Tropical Medicine").

to 100 cc. of the solution. Heat the mixture in a steam sterilizer at 100°C. for one full hour, counting the time after the sterilizer has become thoroughly heated. The mixture is to be contained in a flask of such size and shape that it forms a layer not more than 6 cm. deep. After heating, the mixture is allowed to cool, placing the flask in cold water if desired, and

* Mallory and Wright, "Pathological Technique," 1911, p. 364.

is then filtered, to remove the precipitate which has formed in it. It should, when cold, have a deep purple-red color when viewed, in a thin layer, by transmitted yellowish artificial light. It does not show this color while it is warm. To each 100 cc. of the filtered mixture add 500 cc. of a 0.1 per cent. aqueous solution of "yellowish, water-soluble" eosin and mix thoroughly. Collect the abundant precipitate which immediately appears on a filter. When the precipitate is dry, dissolve it in methylic alcohol (Merck's "reagent") in the proportion of 0.1 gr. to 60 cc. of the alcohol. In order to facilitate the solution the precipitate is to be rubbed up with the alcohol in a porcelain dish or mortar with a spatula or pestle.

"This alcoholic solution of the precipitate is the staining fluid. It should be kept in a well-stoppered bottle because of the volatility of the alcohol. If it becomes too concentrated by evaporation, and thus stains too deeply or forms a precipitate on the blood-smear, the addition of a suitable quantity of methylic alcohol will quickly correct such fault. It does not undergo any other spontaneous change than that of concentration by evaporation."

Method of Staining.—The blood-films are permitted to dry in the air (not heated):

1. Cover the film with a noted quantity of the staining fluid by means of a medicine dropper.
2. After one minute add to the staining fluid the *same quantity* of distilled water by means of the medicine dropper, and allow it to remain for two or three minutes, according to the intensity of the staining desired. A longer period of staining may produce a precipitate.
3. Wash the preparation in water for thirty seconds or until the thinner portions of the preparation become yellow or pink in color.
4. Dry and mount in balsam.

Films more than an hour old do not stain so well as fresh ones. Old films show bluish instead of pink erythrocytes.

*Marino's stain** is extremely delicate and gives still more beautiful results where parasites are present. It is an azur-eosin combination, prepared as follows:

Solution I:

Methylene-blue (medicinal)	0.5 gram
Azur II	0.5 "
Water (distilled)	100.0 cc.

Solution II:

Sodium carbonate	0.5 gram
Water	100.0 cc.

Pour the two solutions together and stand the mixture in the thermostat for forty-eight hours at 37°C.; then add 0.2 per cent. aqueous solution of eosin ("yellowish aqueous eosin"). The quantity of this solution must be varied according to the blue dyes employed, so as to secure the maximum precipitation. The exact quantity can only be determined by titration. A precipitate now forms in the course of twenty-four hours. This is caught upon a filter-paper and dried.

The precipitate, dissolved in methylic alcohol, in the proportion of 0.04 gm. of the powder to 20 cc. of the methylic alcohol, forms the stain.

Method.—The stain is dropped upon the spread so as to cover it, the number of drops being counted. It is permitted to act for exactly three minutes for purposes of fixation, then, without pouring off the stain, twice the number of drops of a 1 : 100,000 aqueous eosin solution are added.† The two fluids gradually mix, transfusion currents are formed, and the specimen is allowed to stand for exactly two minutes longer. It is during this

* "Ann. de l'Inst. Pasteur," 1904, XVIII, 761.

† Marino used a 1 : 20,000 aqueous solution of eosin, but the 1 : 100,000 solution is less apt to cause objectionable precipitation of the dye and gives equally good results.

time that the staining takes place. A precipitate usually forms upon the surface of the fluid, so that it must not be poured off, but splashed off by dropping distilled water upon it from a height. The distilled water is added until it no longer shows any color, when the specimen is drained, dried, and mounted in balsam.

The student may also try staining with hematoxylin and eosin, thionin and eosin, methylene-blue and eosin, or any other dyes, some of which sometimes bring out special details of structure. The protozoa do not show the same reaction to Gram's stain that makes it so useful for differentiating the bacteria.

STAINING PROTOZOA IN TISSUE

For this purpose the sections should be embedded in paraffin, cut very thin, and cemented to the slides.

Ordinary staining with hematoxylin and eosin is rarely of much use. Methylene-blue and eosin is better, but still more useful are the Romanowsky methods, and both the Wright stain and the Marino stain can, with some modification of the time of staining and washing, be employed with good results.

Still better and more satisfactory for certain protozoa are the iron-hematoxylin and the Biondi stain.

*Heidenhain's Iron-hematoxylin.**—Fix the tissue, by preference, in Zenker's solution, though alcohol fixation will do. Embed in paraffin, cut very thin, and fix to the slide.

1. Stain from three to twelve hours in 2.5 per cent. solution of violet iron-alum (sulphate of iron and ammonium). The sections should be stood vertically in the solution, so that no precipitate may form upon them.
2. Wash quickly in water.
3. Stain in a 0.5 per cent. ripened alcoholic solution of hematoxylin for from twelve to thirty-six hours.
4. Wash in water.
5. Differentiate in the iron-alum solution, controlling the results under the microscope. The section should be well washed in a large dish of tap-water before each examination to stop decolorization.
6. Wash in running water for a quarter of an hour.
7. Pass through alcohol, xylol, and mount in xylol balsam.

A counterstain with Bordeau R. before or with rubin S. after the iron stain is sometimes useful.

Biondi-Heidenhain Stain.†—The tissues must be fixed in Zenker's or corrosive sublimate solutions. Embed in paraffin, cut very thin, fix to the slide.

Stain	I. Orange G.	8 grams
	Water	100 cc.
	II. Acid fuchsin or Rubin S.	20 grams
	Water	100 cc.
	III. Methyl-green	8 grams
	Water	100 cc.

Let the solutions stand for several days, occasionally shaking the bottles to make sure that a saturated solution of each is secured. At the end of the time set, mix the solutions in the following proportions:

* Mallory and Wright, "Pathological Technique," 1911, p. 309.

† Modified from Mallory and Wright, "Pathological Technique," 1911, p. 289.

I.	100 parts
II.	20 "
III.	50 "

At the time of staining dilute the mixture 1 : 60 or 1 : 100 with water. To test the solution: (1) Acetic acid makes it redder. (2) A drop of the solution on filter-paper should make a blue spot with a green center and an orange border. If a red zone appears outside of the orange, too much acid fuchsin is present.

1. Stain the sections from six to twenty-four hours.
2. Wash out a little in 90 per cent. alcohol.
3. Dehydrate in absolute alcohol.
4. Xylol.
5. Xylol balsam.

It is important to place the sections directly from the staining fluid into the alcohol, because water instantly washes out the methyl-green.

Ross' Thick Blood-spreads.—In case the number of parasites in the blood is very small, so that they would be scattered sparingly over a large area of the ordinary blood spread, Ross* has suggested a modification of the technic by which they can be more readily found. To do this a *very thick* spread is prepared and dried. As soon as it is dry, and without fixing, the slide is stood vertically in a vessel filled with distilled water. The red corpuscles at once begin to hemolyze and the process is carried on to completion. When all of the hemoglobin has been removed, the slide is taken out, dried, and then fixed and stained. There now being no red corpuscles to distract the attention or obscure the vision, the stained parasites can quickly be found.

Measurement of Micro-organisms.—They can best be measured by an eyepiece micrometer. As these instruments vary somewhat in construction, the unit of measurement for each objective magnification and the method of manipulating the instruments must be learned from dealers' catalogues.

Photographing Micro-organisms.—This requires special apparatus and methods, for which it is necessary to refer to special text-books.†

* "Lancet," Jan. 10, 1903.

† See the excellent chapter upon Photomicrography in Aschoff and Gaylord's "Pathological Histology," Philadelphia, 1900.

CHAPTER VI

STERILIZATION AND DISINFECTION

BEFORE considering the methods employed for the artificial cultivation of micro-organisms and for the preparation of media for that purpose, it is necessary to have a thorough knowledge of the principles of sterilization and disinfection in order intelligently to apply the methods to the elimination or destruction of micro-organisms whose accidental presence might ruin the experiments.

The dust of the atmosphere, almost invariable in its micro-organismal contamination, constantly settles upon our glassware, pots, kettles, funnels, etc., and would certainly ruin every culture-medium with which we experiment did we not take appropriate measures for its purification and protection.

To get rid of these undesirable "weeds" we make use of our knowledge of the conditions destructive to bacterial life, and subject the articles contaminated by them to the action of heat beyond their known enduring power, or to the action of chemic agents known to destroy them, or remove them from fluids into which they have entered by passage through unglazed porcelain. By all of these methods the articles are made *sterile*. Anything is sterile when it contains no germs of life.

Sterilization is the act of making sterile by destroying or removing all micro-organismal life, whether infectious or non-infectious. *Disinfection* signifies the destruction of the infectious agents, taking no account of those that are non-infectious. A *germicide* is any substance that will kill germs. It may be used for disinfection and for sterilization. An *antiseptic* is a substance that will inhibit the growth of micro-organisms. It does not necessarily kill them.

The following table will serve to outline the methods used for effecting sterilization or the complete destruction or removal of living organisms:

I. The Sterilization and Protection of Instruments and Glassware.—Sterilization may be accomplished by either moist or dry heat. For the perfect sterilization of objects capable of withstanding it, tubes, flasks, dishes, etc., dry heat is always to be preferred, because of its more certain action. If we knew just what organisms we had to deal with, we might be able in many cases to save time and gas; but though some non-spore-producing forms are killed at a temperature of 60°C., spore-bearers may withstand 100°C. for an hour;

- **Sterilization**
  - **By heat**
    - **By dry heat**
      - By passing through the flame. Used for platinum wires, needles, glass pipets, etc.
      - By baking in the hot-air oven at 175°C. for one-half hour. Used for glassware, cotton batting, etc.
    - **By moist heat**
      - At low temperatures. Used for perishable and coagulable substances which are heated in a water-bath at 55° to 60°C. for one-half hour one or more times.
      - At high temperatures
        - By boiling. Used for sterilizing instruments, syringes, etc.
        - By steaming
          - Without pressure. In the Koch or Arnold sterilizer, by the intermittent method; 100°C. for one-half hour on three consecutive days. Used for culture-media.
          - Under pressure. In the autoclave at 120°C., under 20 pounds' pressure, requiring but one exposure for one-half hour. Used for culture-media.
  - **By filtration**
    - By passage through the Berkefeld filter of diatomaceous earth. Used for thick liquids.
    - By passage through the Pasteur-Chamberland filter of unglazed porcelain. Used for thin liquids.
  - By the addition of chemical agents. This is not suitable for culture-media, as the agent that effects sterility also maintains it. Useful for the preservation of fermentable and putrescible serums and vaccines. Useful in sanitary operations for destroying infectious agents in the atmosphere, in rooms, upon clothing and bed-clothing, fomites, and for the treatment of dead bodies.

it is, therefore, best to employ a temperature high enough to kill all with certainty. The apparatus is known as a "hot-air sterilizer."

Platinum wires used for inoculation are sterilized by being held in the direct flame until they become incandescent. In sterilizing the wires attention must be bestowed upon the glass handle, which should be flamed for least half its length for a few moments. Carelessness in this respect may result in the contamination of the cultures.

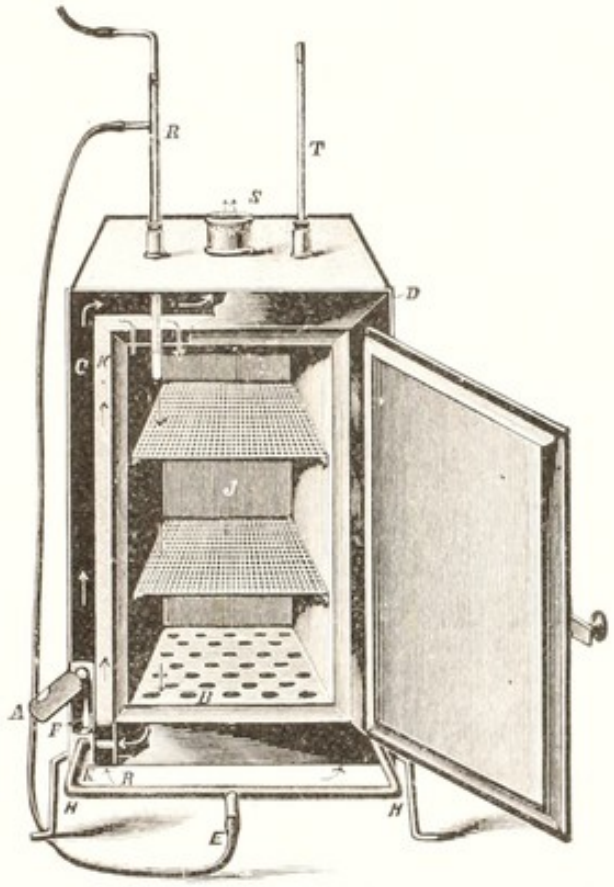


Fig. 37.—Hot-air sterilizer. The gas jets are inclosed within the space between the outer and middle walls, *C*, and can be seen at *F*. That heat ascends, warming the air between the two inner walls, which ascends between the walls, *K*, then descends over the contents, *J*, and escapes through perforations in the bottom, *B*, to supply the draft at *F*, and eventually escapes again at *S*; *R*, gas regulator; *T*, thermometer.

Knives, *scissors*, and *forceps* may be exposed for a very brief time to the direct flame, but as this affects the temper of the steel when continued too long, they are better boiled, steamed or carbolized.

All articles of *glassware* are to be sterilized by an exposure of one-half to one hour to a sufficiently high temperature—150°C. or 302°F.—in the hot-air sterilizer. This temperature is fatal to all forms of microscopic life.

Rubber stoppers, *corks*, *wooden apparatus*, and other objects which are warped, cracked, charred, or melted by so high a temperature

must be sterilized by exposure to streaming steam or steam under pressure, in the steam sterilizer or autoclave, before they can be pronounced sterile.

It must always be borne in mind that after sterilization has been accomplished it is necessary to protect the sterilized objects and media from future contamination.

To Schröder and Van Dusch belongs the credit of having first shown that when the mouths of flasks and tubes are closed with plugs of sterile cotton no germs can filter through. This discovery has been of inestimable value, and has been one of the chief means permitting the advance of bacteriology. If, before sterilizing, flasks and tubes are carefully plugged with ordinary (non-absorbent) cotton-wool, they and their contents will remain free from the access of germs until opened. Instruments may be sterilized wrapped in cotton, to be opened only when ready for use; or instruments and rubber goods sterilized by steam can subsequently be wrapped in sterile cotton and kept for use. It is of the utmost importance to carefully protect every sterilized object, in order that the object of the sterilization be not defeated. As the spores of molds falling upon cotton sometimes grow and allow their mycelia to work their way through and drop into the culture-medium, Roux has employed paper caps, with which the cotton stoppers can be protected from the dust. These are easily made by curling a small square of paper into a "cornucopia," and fastening by turning up the edge or putting in a pin. The paper is placed over the stopper before the sterilization, after which no contamination of the cotton can occur.

II. Sterilization and Protection of Culture-media.—As almost all of the culture-media contain about 80 per cent. of water, which would evaporate in the hot-air closet, and so destroy the material, hot-air sterilization is inappropriate for them, sterilization by streaming steam being the only satisfactory method. The prepared media are placed in previously sterilized flasks or tubes, carefully plugged with cotton-wool, and then sterilized in an Arnold's steam sterilizer.

The temperature of boiling water, 100°C., does not kill the spores, so that one exposure of the culture-media to streaming steam is of little use. The sterilization must be applied in a systematic manner—*intermittent sterilization*—based upon a knowledge of sporulation.

In carrying out intermittent sterilization the culture-medium is exposed for fifteen minutes to the passage of streaming steam or to some temperature judged to be sufficiently high, so that the adult micro-organisms contained in it are killed. As the spores remain uninjured, the medium is stood aside in a cool place for twenty-four hours, and the spores allowed slowly to develop into adult organisms.

When the twenty-four hours have passed, the medium is again

exposed to the same temperature until these newly developed bacteria are also killed. Eventually, the process is repeated a third time, lest a few spores remain alive. When properly sterilized in this way culture-media will remain free from contamination indefinitely.

A prolonged single exposure to lower temperatures (60°–70°C.), known as *pasteurization*, is employed for the destruction of bacteria in milk and other fluids that are injured or coagulated by exposure

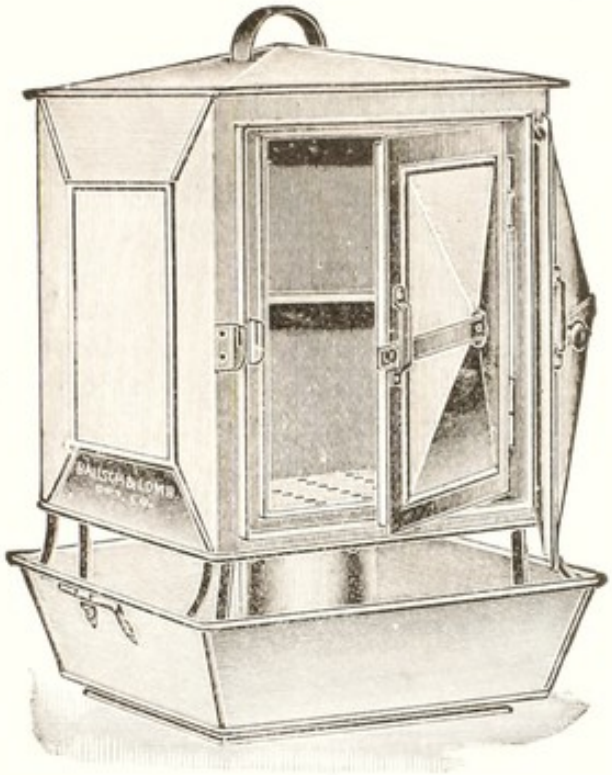


Fig. 38.—Arnold's steam sterilizer (Boston Board of Health form).

to 100°C. It is appropriate only when the organisms to be killed are without spores and without marked resisting powers.

Sterilization in the Autoclave.—If it should be desirable to sterilize a medium at once, not waiting the three days required by the intermittent method, it may be done by superheated steam under pressure, sufficient heat being generated to immediately destroy the spores.

Because of its convenience many laboratory workers habitually use the autoclave for the sterilization of all media not injured by the high temperature. The sterilization, to be complete, requires that the exposure shall be for fifteen minutes at 110°C. (six pounds' pressure).

The media to be sterilized should be placed in the autoclave, the top firmly screwed down, but the escape-valve allowed to remain open until steam is freely generated within and replaces the hot air. The valve is then closed, and the temperature maintained for fifteen minutes or longer if the media be in bulk in flasks. The apparatus should be permitted to cool before the valve is opened,

and the vacuum be slowly relieved. If the valve be opened suddenly the fluids boil rapidly and the cotton plugs may be forced into the tubes or flasks by the air pressure. The chief objection to the use of the autoclave is that the high temperature sometimes brings about chemic changes in the media by which the reaction is altered.

Sterilization by Filtration.—Liquids that cannot be subjected to heat without the loss of their most important qualities may be sterilized by filtration—*i.e.*, by passing them through unglazed porcelain or some other material whose interstices are sufficiently fine to resist the passage of bacteria. This method is largely employed for the sterilization of the unstable bacterial toxins that are destroyed by heat. Various substances have been used for filtration, as diatomaceous earth (Berkefeld filters), stone, sand, powdered glass, etc., but experimentation has shown unglazed porcelain to be the only reliable filtering material by which to remove bacteria. Even this material, whose interstices are so small as to allow the liquid to pass through with great slowness, is only certain in its action for a time, for after it has been repeatedly used the bacteria seem able to work their way through. To be certain of the efficacy of any filter, the fluid first passed through must be tested by cultivation methods to prove that all the bacteria have been removed.

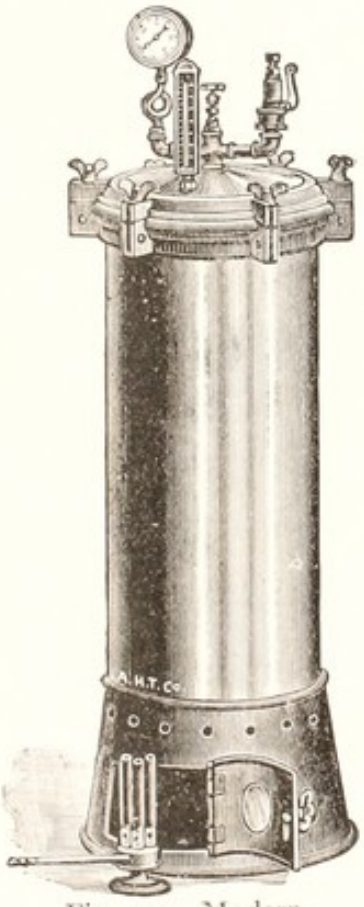


Fig. 39.—Modern autoclave.

The porcelain bougies as well as their attachments must be thoroughly sterilized before use.

After having been used, a porcelain filter must be disinfected, scrubbed, *dried thoroughly*, and then heated in a Bunsen burner or blowpipe flame until all the organic matter is consumed. In this firing process the filter first turns black as the organic matter chars, then becomes white again as it is consumed. The porcelain must be dry before entering the fire, or it is apt to crack.

It should not be forgotten that the filtrate must be received in sterile receivers and handled with care to prevent subsequent contamination.

The filtration of water, peptone solution, and bouillon is comparatively easy, but gelatin and blood-serum pass through with great difficulty, and speedily gum the filter.

III. The Disinfection of Instruments, Ligatures, Sutures, the Hands, etc.—There are certain objects used by the surgeon that cannot well be rendered incandescent, exposed to dry heat at 150°C.,

or steamed continuously, or intermittently heated without injury. For these objects disinfection must be practised. Ever since Sir Joseph Lister introduced antisepsis, or disinfection, into surgery there has been a struggle for the supremacy of this or that highly recommended germicidal substance, with two results—viz., that a great number of feeble germicides have been discovered, and that belief in the efficacy of all germicides has been somewhat shaken;

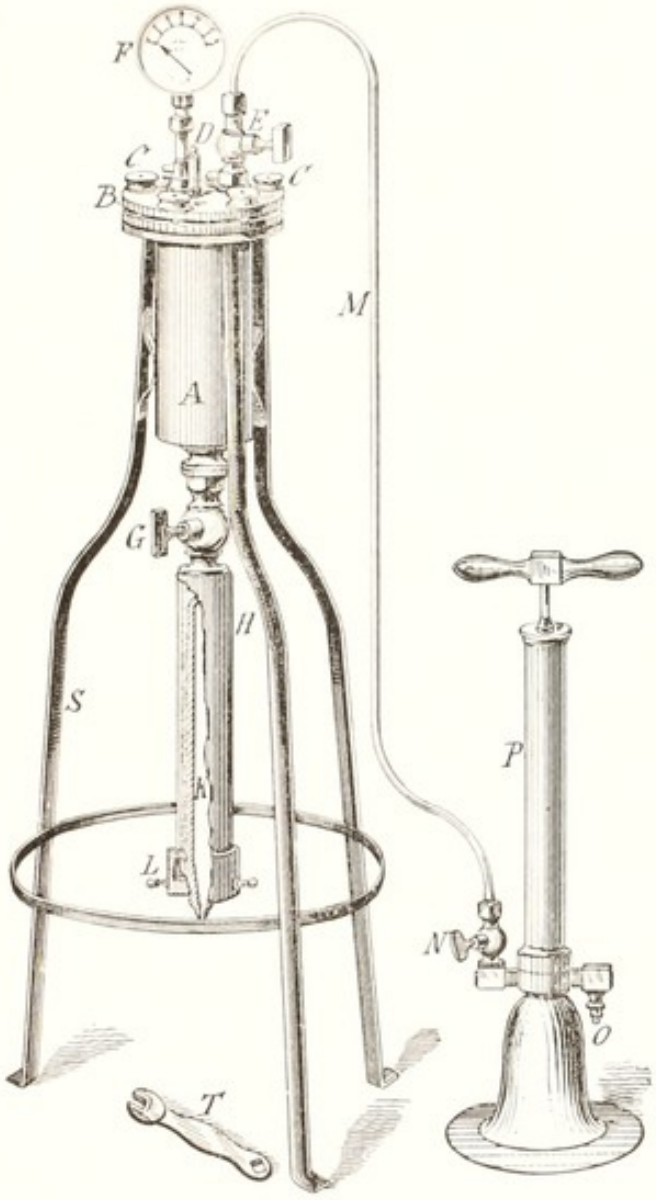
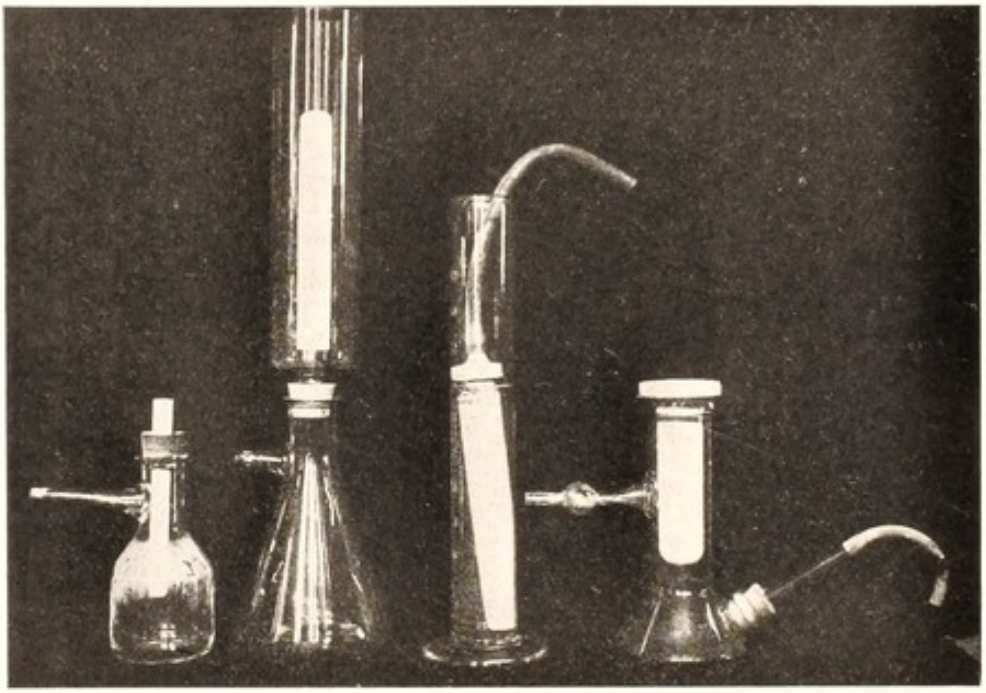


Fig. 40.—Pasteur-Chamberland filter arranged to filter under pressure.

hence the *aseptic* surgery of the present day, which strives to *prevent the entrance of germs into the wound rather than their destruction after admission to it.*

For a complete discussion of the subject of antiseptics in relation to surgery the reader must be referred to text-books of surgery.

The Disinfection of the Hands, etc.—The disinfection of the skin—both the hands of the surgeon and the part about to be incised—is a matter of the utmost importance. Washing the hands with soap, which has marked germicidal properties, will in many cases suffice to destroy or remove bacteria from smooth skins. This method, which is regarded by some surgeons as adequate, is not, however, commonly regarded as sufficient protection to the patient who might be infected by any remaining micro-organisms. To overcome this, many surgeons prefer the use of sterilized gloves



a *b* *c* *d*

Fig. 41.—Different types of bacteriologic filters: *a*, Kitasato; *b*, Berkefeld; *c*, Chamberland; *d*, Reichel.

of thin rubber to all other means of preventing manual infections. Others prefer to use detergent and disinfectant measures. The method at present generally employed, and recommended by Welch and Hunter Robb, is as follows:

The nails must be trimmed short and perfectly cleansed. The hands are washed thoroughly for ten minutes in water of as high a temperature as can comfortably be borne, soap and a previously sterilized brush being freely used, and afterward the excess of soap washed off in clean hot water. The hands are then immersed for from one to two minutes in a warm saturated solution of permanganate of potassium, then in a warm saturated solution of oxalic acid, until complete decolorization of the permanganate occurs, after which they are washed free from the acid in clean warm water or salt solution. Finally, they are soaked for two minutes in a 1 : 500 solution of bichlorid of mercury.

Lockwood,* of St. Bartholomew's Hospital, recommends, after the use of the scissors and penknife, scrubbing the hands and arms for three minutes in hot water and soap to remove all grease and dirt. The scrubbing brush ought to be steamed or boiled before use, and kept in 1 : 1000 biniodid of mercury solution. When the soapsuds have been thoroughly washed away with plenty of clean water, the hands and arms are thoroughly washed and soaked for not less than

* "Brit. Med. Jour.," July 11, 1896.

two minutes in a solution of biniodid of mercury in methylated spirit; 1 part of the biniodid in 500 of the spirit. Hands that cannot bear 1 : 1000 bichlorid and 5 per cent. carbolic solutions bear frequent treatment with the biniodid. After the spirit and biniodid have been used for not less than two minutes, the solution is washed off in 1 : 2000 or 1 : 4000 biniodid of mercury solution.

It is a mistake to insist upon the employment of disinfecting solutions of a strength injurious to the skin. It must be obvious to every one that rough skins with numerous hang-nails and fissures offer greater difficulties to be overcome in disinfection, and more readily convey micro-organisms into the wound than smooth, soft skins.

Sterilization of Ligatures, etc.—Catgut cannot be sterilized by boiling without deterioration. The present method of treatment is to dry it in a hot-air chamber and then boil it in cumol, which is afterward evaporated and the skeins preserved in sterile test-tubes or special receptacles plugged with sterile cotton. Cumol was first introduced for this purpose by Krönig, as its boiling-point is 168°–178°C., and thus sufficiently high to kill spores. The use of cumol for the sterilization of catgut has been carefully investigated by Clarke and Miller.*

Catgut may also and equally well be sterilized by the use of chemical agents. This subject has been carefully reviewed by Bertarelli and Bocchia,† who regard the method of Claudius and the modification of it by Rogone as the best. The method of Claudius is to roll the catgut into skeins and, without taking any precautions to remove any fat it may contain, place it in a mixture of iodin 1, iodid of potassium 1, and distilled water 100. After immersion for eight days the catgut is removed, under aseptic precautions, to alcohol or to 3 per cent. carbolic solution, in which it is indefinitely preserved for use.

Ligatures of silk and silkworm gut are boiled in water immediately before using, or are steamed with the dressings, or placed in test-tubes plugged with cotton and steamed in the sterilizer.

Sterilization of Surgical Instruments, etc.—In most hospitals instruments are boiled, before using, in a 1 to 2 per cent. soda (sodium carbonate, sodium bicarbonate, or sodium biborate) solution, as plain water has the disadvantage of rusting them. During the operation they are either kept in the boiled water or in a carbolic solution, or are dried with a sterile towel. Andrews makes special mention of the fact that the instruments must be completely immersed to prevent rusting.

Disinfection of the Wound.—Cleansing solutions (normal salt solution) and disinfecting solutions (such as 1 : 10,000 to 1 : 1000 bichlorid of mercury) are only applied to septic wounds.

IV. The Disinfection of Sick-chambers, Dejecta, etc.—The Air of the Sick-room.—It is impossible to sterilize or disinfect the

* "Bull. of the Johns Hopkins Hospital," Feb. and March, 1896.
† "Centralbl. für Bakt. u. Parasitenk.," Orig. I, 620.

atmosphere of a room during its occupancy by the patient. It is entirely useless to place beneath the bed or in the corner of a room small receptacles filled with carbolic acid or chlorinated lime. These can serve no purpose for good, and may do harm by obscuring odors emanating from harmful materials that should be removed from the room. The practice is only comparable to the old faith in the virtue of asafetida tied in a corner of the handkerchief as a preventive of cholera and smallpox.

DISINFECTANTS

Before one is able to make a scientific application of any germicidal substance it is necessary to become acquainted with its micro-organism-destroying powers. This may seem at first thought to be a simple matter, but is, in reality, one of great complexity and difficulty, for the various micro-organisms show marked variations in their powers of endurance; different stages in the development of the micro-organisms show different degrees of resisting power, and the conditions under which the germicide meets the micro-organism effect marked variations in action. These factors make it necessary to vary the process of disinfection according to the exact purpose to be achieved.

Let two examples serve to illustrate these requirements: Bichlorid of mercury is one of the most powerful, reliable, and generally useful germicides, but the strength of its solutions must vary according to the purpose for which they are intended. It kills cocci and non-sporogenic bacilli in dilutions of 1:10,000 in from five minutes to twenty-four hours, but to kill anthrax spores requires twenty-four hours' immersion in 1:2000 solution. If albuminous substances are present in the medium containing the micro-organisms they precipitate the salt immediately, diminishing the strength of the solution and so retarding or perhaps preventing the germicidal action. Again, certain micro-organisms are defended from the action of destructive agents, and among them the germicides, through the presence of waxy matter in their substance. Such is the case with the acid-fast organisms, and notably the tubercle bacillus. Antiformin, a combination composed of equal parts of liquor sodæ chlorinatæ and a 15 per cent. solution of caustic soda, immediately dissolves the great majority of micro-organisms, but has no destructive action upon the tubercle bacillus.

The most useful germicidal substances act destructively upon the micro-organisms by forming chemical compounds with their cytoplasm. Thus, the salts of mercury unite with the protoplasm to form an albuminate of mercury. Other germicidal agents dissolve or coagulate the protoplasm; still others oxidize and so completely destroy the cells. In the process of germicidal action many and varied activities are at work, and, as all are not understood, the subject is a difficult one to handle in a limited amount of space.

With the salts, acids, and bases it appears from the researches of Krönig and Paul* that ionization in solution plays an important part in the destruction of micro-organisms. They found that double metallic salts, in which the metal is a constituent of a complex ion in which the concentration of the dissociated metal ions is consequently very low, have very little germicidal power, but that simple salts, in which the condition is reversed, have correspondingly higher germicidal power. Dissociation, therefore, seems to have much to do with the matter.

Inorganic Disinfectants.

Acids.—These agents are seldom employed, since the concentration required makes them objectionable.

Alkalis.—The same holds good with regard to these agents.

Salts.—In this group we find some of the most powerful and most useful germicidal substances.

Copper Sulphate.—It is curious and interesting that while this salt is highly destructive to algæ and other low forms of vegetable life, it is not of much value for the destruction of bacteria. Its chief use is for the destruction of the green algæ that sometimes render the water of reservoirs dirty and offensive. Some of the salt contained in a gunny-sack and permitted to drag to and fro over the surface of the water behind a slowly rowed boat usually accomplishes the end, the actual quantity dissolving in the water being almost infinitesimal.

Mercuric Chlorid ($HgCl_2$).—This is probably the most generally useful as well as one of the strongest germicides.

A study of its activity under varying conditions is instructive as exemplifying the varying behavior of germicides under the varying conditions under which they may be employed.

First, it makes great difference whether the mercuric chlorid is added to the substratum containing the bacteria, or whether the bacteria are added to solutions of the germicide.

Thus, when the salt is dissolved in gelatin in a concentration of 1 : 1,000,000, anthrax bacilli cannot grow. If it is dissolved in blood-serum, the concentration must be increased to 1 : 10,000 to prevent their growth.

When the anthrax spores are dropped in solutions of the salt, Krönig and Paul found that they were killed in twelve to fourteen minutes by 1 : 65 solutions; in eighty minutes by 1 : 500 solutions, and in two hours by 1 : 1000 solutions. When the reaction takes place in albuminous media Behring and Nocht† found that much more time was required. Thus, the destruction of the spores by a 1 : 200 solution required eighty minutes, and a 1 : 1000 solution twenty-four hours to completely kill all of the spores.

Laplace‡ and Panfili§ found that the addition of 5 per cent. of tartaric or hydrochloric acid facilitated the germicidal action through the prevention of albuminate of mercury formation. Lübbert and Schneider and Behring have used sodium chlorid and ammonium chlorid. Both of these salts diminish the germicidal action of the mercuric salt about one-half. Notwithstanding this, however, the "antiseptic tablets" in common use for surgical and household purposes contain one or both of these salts, added for the purpose of preventing the precipitation of the mercuric compounds formed in the presence of alkaline albuminous materials, such as blood, pus, sputum, feces, etc.

The addition of about 25 per cent. of alcohol to the solution of the mercuric salt greatly enhances its value. Strong alcoholic solutions are, however, less useful than aqueous solutions, for the 95 or 100 per cent. alcohol dehydrates the micro-organisms and prevents the diffusion currents by which the mercury is carried into their substance.

* "Zeitschrift für Hygiene," 1897, XXV, 1.

† Ibid., IX, 432.

‡ "Deutsche med. Wochenschrift," 1887, 866; 1888, 121.

§ "Ann. Ig. Roma," 1893, III, 527.

For most purposes a 1 : 2000 solution of the mercuric chlorid is to be recommended.

Silver Nitrate ($AgNO_3$).—The solutions of this salt are probably more useful than the frequency of their employment might suggest. They have, however, the disadvantages of decomposing when kept in the light and of making black stains when applied in concentrated form to the skin or dressings.

The germicidal power of the salt in aqueous solution is less than that of the mercuric chlorid, but the power in albuminous fluids is greater. Anthrax spores in blood-serum are killed in seventy hours in a 1 : 12,000 solution. The addition of other salts, as ammonium salts, interferes with the germicidal activity by inhibiting ionization.

Combinations of the silver nitrate with albuminous compounds, and variously known as argonin, argentum casein, argyrol, protargol, etc., have been used where the disinfecting power of the silver is sought for with the least amount of irritation and the deepest degree of penetration, as in the treatment of gonorrhea.

Potassium Permanganate ($KMnO_4$).—Solutions of this salt seem to act by virtue of a strong oxidizing power. In 2 per cent. solutions anthrax spores are killed in forty minutes; in 4 per cent. solutions, within fifteen minutes. Koch's experiments showed less activity of the germicidal power against anthrax spores. In his hands a 5 per cent. solution seemed to require about a day to effect complete destruction. A 1 per cent. solution kills the pus cocci in ten minutes; a 1 : 10,000 solution kills plague bacilli in five minutes.

The chief difficulty is that the salt is quickly reduced and its strength destroyed by the organic substrata in which the bateria are contained.

Halogens and Compounds.—Those with the lowest atomic weight have the greatest disinfecting power.

Chlorin.—This is usually employed in the form of chlorinated lime. It seems to be a mixture of calcium hypochlorite, $Ca(ClO_2)$, and calcium chlorid, $CaOCl_2$. The addition of any acid, including the atmospheric CO_2, causes the evolution of Cl. The powder is readily soluble and solutions of 1 : 500 kill vegetative forms of most bacteria in a few minutes (not, however, resisting spores).

A proprietary compound known as "electrozone," made by electrolyzing sea-water in such a manner that magnesia and chlorin are liberated and magnesium hypochlorite and magnesium chlorid formed, is a cheap and useful chlorin disinfectant. Nissen found that 1.5 per cent. of it killed typhoid bacilli in a few minutes; Rideal, that 1 : 400 to 500 dilutions of it disinfected sewage in fifteen minutes; and Delépine, that 1 : 50 (equal to 0.66 per cent. of chlorin) rapidly killed the tubercle bacillus and 1 : 10 (equal to 3.3 per cent. chlorin) killed anthrax spores.

Iodin Terchlorid (ICl_3).—This compound, which is so unstable that it only keeps in an atmosphere of Cl-gas, has great germicidal action, that probably depends upon the readiness with which it decomposes. In solutions of 1 : 1000 it kills vegetative bacteria in a few minutes, and in 1 : 100 it kills anthrax spores with equal rapidity. The presence of organic and albuminous materials does not interfere with the germicidal action.

Organic Disinfectants.

Carbolic Acid (C_6H_5OH) is the most important and generally useful of these. It has the advantage of being cheap and easily kept and handled. In the pure state it consists of colorless acicular crystals. When exposed to the atmosphere it takes up water and gradually becomes a brownish-yellow oily fluid. The crystals and deliquesced crystals have powerful escharotic properties and cannot be touched without destruction of the skin. In 2 to 3 or 5 per cent. solutions carbolic acid destroys most bacteria within a few minutes. Anthrax and other powerfully resisting spores, however, require prolonged exposure. Tetanus spores are said not to be killed in less than fifteen hours. There is no ionization; the reagent seems to act by coagulating the bacterial protoplasm.

Though carbolic acid has been for a quarter of a century a favorite surgical disinfectant, the application of 5 per cent. solution to the skin has so frequently caused gangrene that it is at present in some merited disfavor.

Closely related to carbolic acid and other products of coal-tar distillation are orthocresol, metacresol, and paracresol. "Trikresol," a much used antiseptic, is a commercial product consisting of a mixture of all three of the cresols. It is more strongly germicidal than carbolic acid, but is less soluble in water. It is or has been largely used for addition to therapeutic serums in the proportion of 0.4 per cent. as an antiseptic. Such addition causes the formation of an albuminous precipitate in which, doubtless, much of the antiseptic is lost, for upon its removal or even upon its sedimentation resisting forms of bacteria may grow in the serum. It cannot, therefore, be looked upon as a reliable preservative.

"Lysol" is said to be a solution of coal-tar cresol in potassium soap. It has the advantage of forming a lather-like soap, so that it can be employed both as a cleanser and disinfectant. In 1 per cent. solutions it is capable of destroying cocci, typhoid bacilli, and other microorganisms of low resisting power.

"Creolin" is also a combination of cresols with potassium soap. When added to water it immediately forms an emulsion. It has been much used in obstetric practice, where it has earned more reputation than it deserves.

"*Formalin.*"—This is Schering's commercial denomination of a 30 to 40 per cent. aqueous solution of formaldehyd gas (H—COH) or formic aldehyd. The solution is highly germicidal so long as it is fresh. When exposed for long to the atmosphere it polymerizes into trioxymethylene and paraformaldehyde and greatly loses its power. A 10 per cent. solution of formalin kills pus cocci in half an hour. A 5 per cent. solution kills cholera spirilli in three minutes; anthrax bacilli, in fifteen minutes; anthrax spores, in five hours. Pure formalin kills anthrax spores in ten to thirty minutes. Strong solutions are extremely irritating and so not applicable in surgery. They are, however, of great use for household disinfection. Formalin and formaldehyd gas find their chief usefulness for the aërial disinfection of sick chambers and domiciles, where they are either used as a spray or the gas evolved by chemical means or by heat, as will be shown below.

Peroxid of hydrogen (H_2O_2) is germicidal through its power to liberate the nascent O. It quickly decomposes when brought into contact with organic matter, and, therefore, has a very limited sphere of usefulness.

The following tables, compiled by Hiss from Flügge, will show the comparative values of the commonly employed antiseptics and germicides:

Certain fundamental principles govern the rationale of disinfection, and must be kept in mind: (1) the reagent employed should be known to act destructively upon bacteria; (2) it must be applied to the bacteria to be killed; (3) it must be applied in sufficiently concentrated form, and (4) it must be left in contact with the bacteria long enough to accomplish the effect desired.

During the period of illness the chamber in which the patient is confined should be freely ventilated. An abundance of fresh, pure air is a comfort to the patient and a protection to the doctor and nurse.

After recovery or death one should rely less upon fumigation than upon disinfection of the walls and floor, the similar disinfection of the wooden part of the furniture, and the sterilization of all else.

The fumes of sulphur do some good, especially when combined with steam, but are greatly overestimated in action and are *very destructive to furnishings*, so that they are rapidly giving way to the more satisfactory, less destructive, and equally germicidal formaldehyd vapor.

INHIBITION STRENGTHS OF VARIOUS ANTISEPTICS
(Adapted from Flügge, Leipzig, 1902)

	Anthrax Bacilli	Other Bacteria	Putrefactive Bacteria in Bouillon
ACIDS			
Sulphuric	1:3000	Chol. spir. 1:6000	
Hydrochloric	1:3000	B. diph. 1:3000 B. mallei 1:700 B. typh. 1:500	
Sulphurous	.....	Chol. spir. 1:1000	1:6000
Arsenous	.....	.....	1:200
Boric	1:800	.....	1:100
ALKALIES			
Potass. hydrox	1:700	B.diph. 1:600 Chol. spir. 1:400 B. typh. 1:400	
Ammon hydrox	1:700	Chol. spir. 1:500 B. typh. 1:500	
Calcium hydrox	.....	Chol. spir. 1:1100 B. typh. 1:1100	
SALTS			
Copper sulphate	.....	.....	1:1000
Ferric sulphate	.....	.....	1:90
Mercuric chlorid	1:100,000	B. typh. 1:60,000	1:20,000
Silver nitrate	1:60,000	Chol. spir., B. typhosus 1:50,000	
Potass. perman	1:1000	.....	1:500
HALOGENS AND COMPOUNDS			
Chlorin	1:1500	.....	1:4000
Bromin	1:1500	.....	1:2000
Iodin	1:5000	.....	1:5000
Potass. iodid	.....	.....	1:7
Sodium chlor	1:60		
ORGANIC COMPOUNDS			
Ethyl alcohol	1:12	.....	1:10
Acetic and oxalic acids	.....	B. diph. 1:500	1:40
Carbolic acid	1:800	B.typh. 1:400 Chol. spir. 1:600	
Benzoic acid	1:1000		
Salicylic acid	1:1500		
Formalin (40% formaldehyd)	.....	Chol. spir. 1:20,000 Staphylo. 1:5000	1:1000
Camphor	1:1000		
Thymol	1:10,000	.....	1:3500
Oil mentha pip	1:3000		
Oil of terebinth	1:8000		
Peroxid of hydrogen	.....	.....	1:2000

Formaldehyd is probably the best germicide that has yet been recommended. Its use for the disinfection of rooms and hospital wards was first suggested by Trillat* in 1892, but it did not make

* "Compte rendu de l'Acad. des Sciences," Paris, 1892.

much stir in the medical world until a year or more had passed and a 40 per cent. solution of the gas, under the name of "Formalin," had been placed upon the market. Care must be exercised in handling the fluid, that the hands do not become wet with it, as it hardens the skin and deadens sensation. The vapor is exceedingly irritating to the mucous membrane of the eyes and nose.

BACTERICIDAL STRENGTH OF COMMON DISINFECTANTS

(Adapted from Flügge, Leipzig, 1902)

	Streptococci and Staphylococci	Anthrax and Typhoid Bacilli, Cholera Spirillum		Anthrax Spores
	5 minutes	5 minutes	2 to 24 hours	
ACIDS				
Sulphuric	1:10	1:100	1:1500	1:50 in 10 days
Hydrochloric	1:10	1:100	1:1500	1:50 in 10 days
Sulphurous	.....	.....	Typhoid 1:700	
Sulphurous	.....	.....	1:300 (Gas 10 vol. %)	
Boric	.....	.....	1:30	Conc. sol. incomplete disinfection.
ALKALIES				
Potass. hydrox	1:5	1:300		
Ammon. hydrox	.....	1:300		
Calcium	.....	1:1000		
SALTS				
Copper sulphate	.....	.....	.....	1:20 (5 days)
Mercuric chlor	1:10,000 to 1000	1:2000	1:10,000	1:2000 (26 hrs.)
Silver nitrate	1:1000	.....	1:4000	
Potass. permang	1:200	.....	.....	1:20 (1 day)
"Calc. chlorid."	.....	1:500	.....	1:20 (1 hr.)
HALOGENS AND COMPOUNDS				
Chlorin	1%	1%	.....	2% (in 1 hr.)
Trichlorid of iodin	1:200	1:1000	.....	1:1000 (in 12 hours)
ORGANIC COMPOUNDS				
Ethyl alcohol	70%—15 minutes	70%—10 minutes	1:200 to 300	Alcol. 50% for 4 months without killing spores (Koch*)
Acetic and oxalic acids.				
Carbolic acid	1:60	Cholera 1:200 Typh. 1:50	1:300	1:20 (4 to 45 days) (at 40° in 3 hours)
Lysol	1:300	1:300		
Creolin	.....	1:100	1:3000	(10% in 5 hrs.)
Salicylic acid	1:1000			
Formalin (40% formaldehyd)	1:10	1:20	1:1000	1:20 (in 6 hrs.)
Peroxid of hydrogen	Conc.	1:200	1:500	1:100 (in 1 hr.) 3:100 (in 1 hr.)

The solution can be employed to spray the walls and floors of

* Koch, Arb. a. d. kais. Gesundheitsamt, 1881, 1.

rooms, though Rosenau* finds that unless the spray discharged from a large atomizer be very fine, its action is uncertain.

The original method of disinfection, suggested by Robinson,† consisted of the evolution of the gas by volatilizing methyl alcohol, and passing the vapor over heated asbestos. Shortly many efficient forms of apparatus were placed upon the market, for the evolution of the gas or for discharging it from the solution.

It is not necessary to use a special apparatus in order to disinfect with formaldehyd; one can, in an emergency, hang up a number of sheets, saturated with the 40 per cent. solution, in the room to be disinfected. The number of sheets must vary with the size of the room, as each is able to evolve but a certain amount of the gas, and the quantity necessary for disinfection varies with the size of the room.

One of the best methods of evolving the gas for purposes of disinfection is that devised by Evans and Russell† who combine the 40 per cent. solution of formaldehyd with permanganate of potassium, when an almost explosive liberation of the gas takes place.

Frankforter‡ found that a good method of escaping the undesirable features of the gaseous evolution was to mix the powder of permanganate of potassium with an equal volume of sand, so that the formaldehyd solution is brought more slowly into contact with the permanganate, under conditions unfavorable to the formation of oxids of manganese, such as otherwise tend to coat the grains of permanganate and prevent further reaction between the formaldehyd solution and the permanganate.

The employment of calcium carbide for the same purpose is suggested by Evans.§ The best results were obtained when the calcium carbide was in lumps about the size of a pea; when the formaldehyd solution was diluted with an equal volume of water, and when the diluted formaldehyde was added to the carbide in the proportion of 5 cc. of the former to 3 grams of the latter. In the permanganate method the quantity of formalin (or 37–40 per cent. formaldehyd in water) should equal 200 cc. to 1000 cubic feet of space, but in the carbide method 500 cc. must be used to achieve the same result. Evans, therefore, prefers the permanganate method.

To disinfect with formaldehyd or any gaseous disinfectant, the room must be carefully closed, the cracks of the windows and doors being sealed by pasting strips of paper over them. If an apparatus is used, it is set in action, the discharged vapor entering the room through the keyhole or some other convenient aperture,

* "Disinfection and Disinfectants," P. Blakiston's Son & Co., Philadelphia, 1902.

† "Ninth Report of the State Board of Health of Maine," 1896.

‡ "Reports and Papers of the American Public Health Association," 1906, vol. XXXII, part II, p. 114.

§ Ibid., p. 108.

the gas being allowed to act undisturbed for some hours, after which the windows and doors are all thrown open to fresh air and sunlight.

If sheets are hung up, or the permanganate method employed, the windows and doors, other than that by means of which the operator is to escape, are closed and sealed. If the permanganate of potassium or calcium carbide methods are to be employed, the cracks about the doors and windows are sealed with paper, a dish-pan or wash-tub is placed in the center of the room, and in it the can containing the permanganate or carbide and sand. The formaldehyd solution is poured on, the operator making his escape, closing and sealing the door behind him. Any closets in the room must be left open so that they and their contents may be disinfected with the room.

So far as is known at present, superficial disinfection by formaldehyd leaves little to be desired. Care must, however, be exercised to see that the required volume of gas is generated to disinfect the apartment. *A sufficient concentration of the gas is absolutely necessary* and the method selected should be one capable of discharging the gas in a short time, so that it immediately pervades the atmosphere.

Disinfection with formaldehyd is, however, only superficial, its penetrating powers being limited. The discharge of gas into the room should only be preliminary to other and more thorough disinfection and sterilization of its contents by the application of solutions of disinfectants to the woodwork, and the baking of the mattresses and pillows and the boiling of the linen, etc.

The Dejecta.—In diphtheria the expectoration and nasal discharges are highly infectious and should be received in old rags or in Japanese paper napkins—not handkerchiefs or towels—and

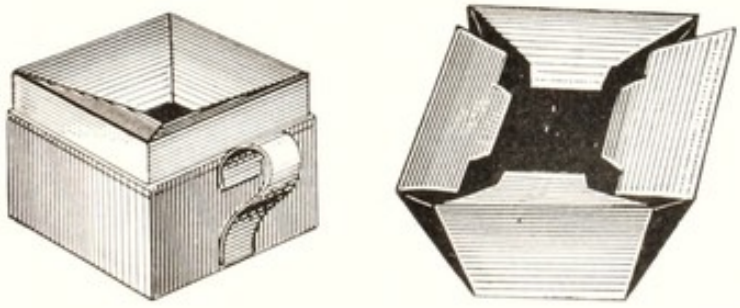


Fig. 42.—Pasteboard cup for receiving infectious sputum. When used the pasteboard can be removed from the iron frame and burned.

should be burned. The sputum of tuberculous patients should either be collected in a glazed earthen vessel which can be subjected to boiling and disinfection, or, as is an excellent plan, should be received in Japanese rice-paper napkins, which can at once be burned. These napkins are not quite so good as the small pasteboard boxes recommended by some city boards of health, because, being highly absorbent, the sputum is apt to soak through and soil the fingers.

For the fastidious patients, cut-glass bottles with tightly fitting lids are used to collect the sputum, and as these are not unsightly the patients make no objection to carrying them about with them. Tuberculous patients should be provided with rice-paper instead of handkerchiefs, and should have their napkins, towels, knives, forks, spoons, plates, etc., kept strictly apart from the others of the household and carefully sterilized by boiling after using. Patients with sensitive dispositions need never be told of these arrangements.

The excreta from cases of typhoid fever and cholera require particular attention. These, and indeed all alvine matter the possible source of infection or contagion, should be received in glazed earthen vessels and immediately and intimately mixed with a 5 per cent. solution of chlorinated lime (containing 25 per cent. of chlorin) if semi-solid, or with the powder if liquid, and allowed to stand for an hour before being thrown into the drain.

Thoughtful consideration should always be given the germicides used to disinfect the discharges, lest combination of the chemical with ingredients of the discharge produce inert compounds. Thus, bichlorid of mercury cannot be used because it forms an inert compound with albumin.

The Clothing, etc.—The bed-clothing, towels, napkins, handkerchiefs, night-robes, underclothes, etc., used by a patient suffering from an infectious disease, as well as the towels, napkins, handkerchiefs, caps, aprons, and outside dresses worn by the nurse, should be regarded as infective and carefully sterilized. The only satisfactory method of doing this is by prolonged subjection to steam in a special apparatus; but, as this is only possible in hospitals, the next best thing is boiling for some time in the ordinary wash-boiler. In drying, the wash should hang longer than usual in the sun and wind. Woolen underwear can be treated exactly as if made of cotton. The woolen outer clothing of the patient, if infective, requires special treatment. Fortunately, the infection of the outer garments is unusual. The only reliable method for their sterilization is prolonged exposure to hot air at 110°C. In private practice it often becomes a grave question what shall be done with these articles. Prolonged exposure to fresh air and sunlight will, however, aid in rendering them harmless; and can be practised when it is not certain that they are actually infective. Infective articles of wool may be sent to the city hospital and baked.

The doctor visiting a case of dangerous infection or a hospital for infectious diseases should cover his clothing with a linen or cotton gown, and protect his hair with a cap, these articles being disinfected after the visit. By such precautions he will avoid spreading infection among his patients or carrying it to his own family.

The Furniture, etc.—The destruction of infective furniture is unnecessary. The doctor treating a case of infectious disease, if he properly perform his functions, will save much trouble and money

for his patient by ordering his immediate isolation in an uncarpeted, scantily and simply furnished room the moment an infectious disease is *suspected*. However, if before his removal the patient has occupied another bed, its clothing should be promptly disinfected.

After the recovery or death of the patient the walls and ceiling of the room should be sprayed with a formaldehyd solution, or the room sealed and filled with the vapor. If they are hung with paper, they should be dampened with 1:1000 bichlorid of mercury solution before new paper is hung.

Strehl has demonstrated that when 10 per cent. formalin solution is sponged upon artificially infected curtains, etc., the bacteria are killed. This is an important adjunct to our means of disinfecting the furniture of the sick-chamber.

The floor should be scoured with 40 per cent. formaldehyd solution, 5 per cent. carbolic acid solution, or 1:1000 bichlorid of mercury solution (no soap being used, as it destroys the bichlorid of mercury and prevents its action), and all the wooden articles wiped off two or three times with one of the same solutions. If a straw mattress was used it should be burned and the cover boiled. If a hair mattress was used, it can be steamed or baked by the manufacturers, who usually have ovens for the purpose of destroying moths, but which answer for sterilizing closets. Curtains, shades, etc., should receive proper attention; but, of course, the greater the precautions exercised in the beginning, the fewer the articles that will need attention in the end.

The Patient, whether he live or die, may be a means of spreading the disease unless specially cared for. After convalescence the body should be scoured with biniodide of mercury soap, bathed with a weak bichlorid of mercury solution or with a 2 per cent. carbolic acid solution, or with 25–50 per cent. alcohol, before the patient is allowed to mingle with society, and the hair should either be cut off or carefully washed with the disinfecting solution or an antiseptic soap. In desquamative diseases it seems best to have the entire body anointed with cosmolin once daily, beginning before desquamation begins and having the unguent well rubbed in, in order to prevent the particles of epidermis, in which the specific contagium probably occurs, being distributed through the atmosphere. Carbolated may be better than plain cosmolin, not because of the very slight antiseptic value it possesses, but because it helps to allay the itching which may accompany the desquamative process.

After the patient is about again, common sense will prohibit the admission of visitors until the suggested disinfective measures have been adopted, and after this, touching, and especially kissing him, should be avoided for some time.

The bodies of those that die of infectious diseases should be washed in a strong disinfectant solution, and given a strictly private funeral.

If this be impossible, the body should be embalmed, sealed in the coffin, and the face viewed through a plate of glass; the body is best disposed of by cremation, though it is not rarely necessary as a dead body cannot remain a source of infection for an indefinite period. Esmarch,* who made a series of laboratory experiments to determine the fate of pathogenic bacteria in the dead body, found that in septicemia, cholera, anthrax, malignant edema, tuberculosis, tetanus, and typhoid fever the pathogenic bacteria all die sooner or later, more rapidly during active decomposition than during preservation of the tissues.

* "Zeitschrift für Hygiene," 1893.

CHAPTER VII

CULTURE-MEDIA AND THE CULTIVATION OF MICRO-ORGANISMS

In order to observe them accurately micro-organisms must be separated from their natural surroundings and artificially cultivated upon certain prepared media of standard composition. The effects of one organism upon the growth of another, by neutralizing its metabolic products, by changing the reaction of the medium in which it grows so as to inhibit further multiplication, by dissolving the other species through its enzymes, etc., suffice to show how impossible it is to determine the natural history of any organism unless it be kept strictly away from other species.

Fortunately the same general principles apply equally for the cultivation of all forms of micro-organismal life, and much the same media apply in all cases. What is said, therefore, about the bacteria may be regarded as appropriate for all.

BACTERIA

Various organic and inorganic mixtures have been suggested for the cultivation of bacteria, but few have met with particular favor and become standards. At the present time certain standard media are used in every laboratory in the world; all systematic study of the organisms depends upon the behavior of bacteria upon them, and no study of micro-organisms can be regarded as complete unless the behavior of the bacteria upon them has been carefully considered.

Our studies of the biology of the bacteria have shown that they grow best in mixtures containing at least 80 per cent. of water, of neutral or feebly alkaline reaction, and of a composition which, for the pathogenic forms at least, should approximate the juices of the animal body. It might be added that transparency is a very desirable quality, and that the most generally useful culture-media are those that can be liquefied and solidified at will.

All accurate bacteriologic culture experiments require that an exact knowledge of the chemistry of the media used shall be at hand. The importance of this knowledge is suggested by the pains taken to arrive at it. The best bacteriologists of America have agreed upon certain details that are explained in the following excerpts from the Report of the Committee of Bacteriologists of the American Public Health Association.*

* "Jour. Amer. Public Health Assoc.," Jan., 1898, p. 72.

"The first thing to obtain is a standard 'indicator' which will give uniform results. These requirements are best fulfilled by phenolphthalein."

"The question of the proper reaction of media for the cultivation of bacteria and the method of obtaining this reaction have been discussed in a valuable paper by Mr. George W. Fuller, published in the 'Journal of the American Public Health Association,' Oct., 1895, vol. XX, p. 321."

"Method of determining the degree of reaction of culture-media: For this most important part in the preparation of culture-media, burets graduated into one-tenth c.c. and three solutions are required—

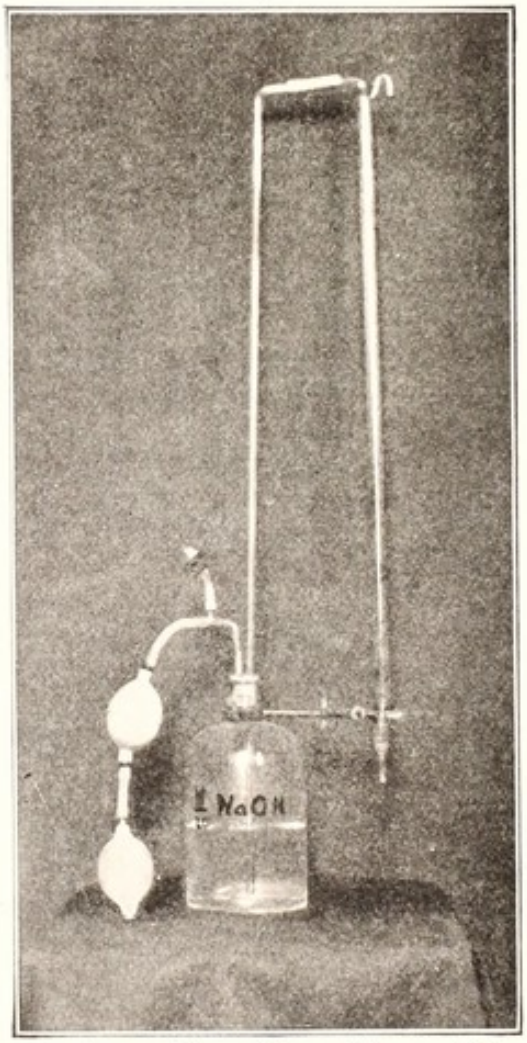


Fig. 43.—Buret for titrating media. (From Hiss and Zinsser, "Text-Book of Bacteriology," D. Appleton & Co., Publishers.)

"1. A 0.5 per cent. solution of commercial phenolphthalein, in 50 per cent. alcohol.

"2. A $\frac{n}{20}$ solution of sodium hydroxid.

"3. A $\frac{n}{20}$ solution of hydric chlorid.

"Solutions 2 and 3 must be accurately made and must correspond with the normal solutions soon to be referred to.

"Solutions of sodium hydroxid are prone to deterioration from the absorption of carbon dioxid and the consequent formation of sodium carbonate. To prevent as much as possible this change, it is well to place in the bottle containing the stock solution a small amount of calcium hydroxid, while the air entering the burets or the supply bottles should be made to pass through a U-tube containing caustic soda, to extract from it the carbon dioxid."

"The medium to be tested, all ingredients being dissolved, is brought to the

prescribed volume by the addition of distilled water to replace that lost by boiling, and after being thoroughly stirred, 5 cc. are transferred to a 6-inch porcelain evaporating-dish. To this 45 cc. of distilled water are added and the 50 cc. of fluid are boiled for three minutes over a flame. One cubic centimeter of the solution of phenolphthalein (No. 1) is then added, and by titration with the required reagent (No. 2 or No. 3) the reaction is determined. In the majority of instances the reaction will be found to be acid, so that the $\frac{n}{20}$ sodium hydroxid is the reagent most frequently required. This determination should be made not less than three times and the average of the results obtained taken as the degree of the reaction.

"One of the most difficult things to determine in this process is exactly when the neutral point is reached as shown by the color developed, and to be able in every instance to obtain the same shade of color. To aid in this regard, it may be remarked that in bright daylight the first change that can be seen on the addition of alkali is a very faint darkening of the fluid, which, on the addition of more alkali, becomes a more evident color and develops into what might be described as an Italian pink. A still further addition of alkali suddenly develops a clear and bright pink color, and this is the reaction always to be obtained. All titrations should be made quickly and in the hot solutions to avoid complications arising from the presence of carbon dioxid.

"The next step in the process is to add to the bulk of the medium the calculated amount of the reagent, either alkali or acid, as may be determined. For the purpose of neutralization a normal solution of sodium hydroxid or of hydric chlorid is used, and after being thoroughly stirred the fluid thus neutralized is again tested in the same manner as at first, to insure the proper reaction of the medium being attained. When neutralization is to be effected by the addition of an alkali, it not infrequently happens that after the calculated amount of normal solution of sodium hydroxid has been added, the second test will show that the medium is acid to phenolphthalein, to the extent sometimes of 0.5 to 1 per cent. This discrepancy is perhaps due to side reactions which are not understood. The reaction of the medium, however, must be brought to the desired point by the further addition of sodium hydroxid, and the titrations and additions of alkali must be repeated until the medium has the desired reaction (*i.e.*, 0.0 per cent. to 0.005 per cent.; see below).

"After the prescribed period of heating, it is frequently found that the medium is again slightly acid, usually about 0.5 per cent. Without correcting this, the fluid is to be filtered and the calculated amount of acid or alkali is to be added to change the reaction to the one desired. A still further change in reaction is not infrequently to be observed after sterilization, the degree of acidity varying apparently with the composition of the media and the degree and continuance of the heat."

"Manner of expressing the reaction: Since at the time the reaction is first determined culture-media are more often acid than alkaline, it is proposed that acid media be designated by the plus sign and alkaline media by the minus sign, and that the degree of acidity or alkalinity be noted in parts per hundred. Thus, a medium marked + 1.5 would indicate that the medium was acid, and that 1.5 per cent. of $\frac{n}{1}$ sodium hydroxid is required to make it neutral to phenolphthalein; while − 1.5 would indicate that the medium was alkaline and that 1.5 per cent. of $\frac{n}{1}$ acid must be added to make it neutral to the indicator."

"*Standard reaction of media (provisional)*:

"Experience seems to vary somewhat as to the optimum degree of reaction which shall be uniformly adopted in the preparation of standard culture-media. To what extent this is due to variation in natural conditions as compared with variations of laboratory procedure it seems impossible to state. Somewhat different degrees of reaction for optimum growth are required, not only in or upon the media of different composition and by bacteria of different species, but also by bacteria of the same species when in different stages of vitality. The bulk of available evidence from both Europe and America points to a reaction of +1.5 as the optimum degree of reaction for bacterial development in inoculated culture media. While this experience is at variance with that in several of our own laboratories, it has been deemed wisest to adopt +1.5 as the provisional

standard reaction of media, but with the recommendation that the optimum growth reaction be always recorded with the species."

BOUILLON

This is one of the most useful and most simple media. It can be prepared from meat or from meat extract, and is the basis of most of the culture-media. The addition of 10 per cent. of gelatin makes it "gelatin;" that of 1 per cent. of agar-agar makes it "agar-agar."

I. To Prepare Bouillon from Fresh Meat.—To 500 grams of finely chopped lean, boneless beef, 1000 cc. of clean water are added and allowed to stand for about twelve hours on ice. At the end of this time the liquor is decanted, that remaining on the meat expressed through a cloth, and then, as the entire quantity is seldom regained, enough water added to bring the total amount up to 1000 cc. This liquid is called the *meat-infusion*. To it 10 grams of Witte's or Fairchild's dried beef-peptone and 5 grams of sodium chlorid are added, and the whole boiled until the albumins of the meat-infusion coagulate, titrated or otherwise corrected for acidity, boiled again for a short time, and then filtered through a fine filter paper. It should be slightly yellow and perfectly clear and limpid. Smith,* referring to bouillon intended for the culture of diphtheria bacilli for toxin, says that when the peptones are added before boiling most of them are lost, and therefore recommends that the meat-infusion be boiled and filtered and the solid ingredients added and dissolved subsequently. The reaction, which is strongly acid, is then carefully corrected by titration according to the directions already given.

For rough work in students' classes litmus paper may be used as an indicator for determining and correcting the acidity resulting from the sarcolactic and other acids in the meat-infusion, the alkaline solution being added drop by drop until a faint blue appears on the red paper; or phenolphthalein can be employed, the addition of the alkaline solution being continued until a drop of the bouillon produces a red spot upon phenolphthalein paper, made, as suggested by Timpe, by saturating bibulous paper cut into strips with a solution of 5 grams of phenolphthalein to 1 liter of 50 per cent. alcohol. Acids do not change the appearance of the paper, but small traces of alkali turn it red.

If the bouillon is to be employed for exact work, these crude methods should not be adopted, but chemical titration according to the method already given should be performed. After titration the bouillon must again be boiled for a few minutes, in order to precipitate the acid albumins, as much water added as has been lost by evaporation, and the fluid filtered through a pharmaceutic filter.

The filtered fluid is dispensed in previously sterilized tubes with cotton plugs—about 10 cc. to each—or in flasks, and is then sterilized

* "Trans. Assoc. Amer. Phys.," 1896.

by steam three successive days for fifteen to twenty minutes each, according to the directions already given for intermittent sterilization, or superheated in the autoclave.

The loss of water during boiling is an important matter to bear in mind, as unless properly replaced it is the cause of disproportion between the fluids and solids of the media. The quantity must therefore be measured before filtration and enough water added to replace what has been lost. Measuring before filtration is comparatively easy with bouillon, but difficult with heavy liquids, like the gelatin and agar-agar solutions. To overcome this difficulty it is

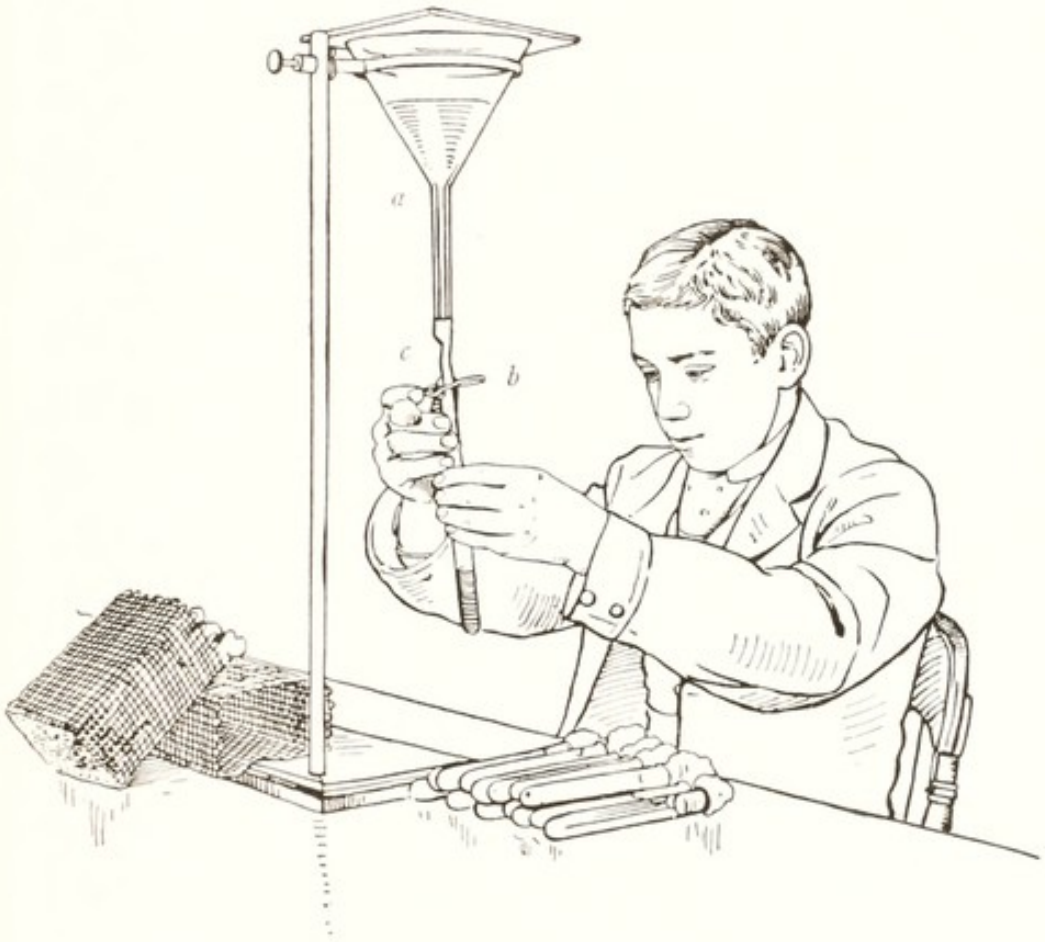


Fig. 44.—Funnel for filling tubes with culture media (Warren): *a*, Funnel containing the culture media in liquid condition; *b*, pinch-cock by which the flow of fluid into the test-tube is regulated; *c*, rubber tubing.

best to make the entire preparation by weight and not by volume. A pair of platform scales with sliding indicators will first balance the empty kettle and then show the correct quantity of each added ingredient. After boiling, the kettle can be returned to the scale and the exact quantity of water to be added determined.

II. To Prepare Bouillon from Meat Extract.—When desirable, the bouillon may also be prepared from beef-extract, the method being very simple: To 1000 cc. of clean water 10 grams of Witte's dried beef-peptone, 5 grams of sodium chlorid, and about 2 grams of beef-

extract are added. The solution is boiled until the constituents are dissolved, titrated, and filtered *when cold*. If it be filtered while hot, there is always a subsequent precipitation of meat-salts, which clouds it.

Bouillon and other liquid culture media are best dispensed and kept in small receptacles—test-tubes or flasks—in order that a single contaminating organism, should it enter, may not spoil the entire quantity. A convenient, simple apparatus for filling tubes with liquid media consists of a funnel to which a short glass pipet is attached by a bit of rubber tubing. A pinch-cock controls the outflow of the liquid. The apparatus need not be sterilized before using, as the culture medium must subsequently be sterilized either by the intermittent method or in the autoclave after the tubes are filled. The test-tubes and flasks into which the culture medium is filled must, however, be previously sterilized by dry heat, unless the subsequent sterilization is to be performed in the autoclave, when it may be unnecessary.

Sugar bouillon is bouillon containing in solution known percentages of such sugars as glucose, lactose, saccharose, etc. As Smith* has pointed out, if the quantity of sugar in the bouillon is to be accurately known, it is necessary to first destroy the muscle sugars in the meat-infusion. This can be done by adding a culture of the colon bacillus to the meat-infusion and permitting fermentation to continue overnight, then finishing the bouillon and adding the known quantity of whatever sugar is desired. About 1 per cent. of dextrose, lactose, saccharose or galactose is all that is required. More may be injurious. If the bouillon be made from meat extract, fermentation may not be necessary.

The sugar bouillons should not be sterilized in the autoclave, as the high temperatures chemically alter the sugars.

GELATIN

The culture-medium known as gelatin is bouillon to which 10 per cent. of gelatin is added. It has the decided advantage over bouillon that it is not only an excellent food for bacteria, and, like the bouillon, transparent, but also is *solid* at the room temperature. Nor is this all: it is a transparent solid that can be made liquid or solid at will. Leffmann and LaWall have examined commercial gelatins and found that many of them contain sulphur dioxid in quantities as great as 835 parts per million. As the varying quantity of this impurity may modify the growth of the culture, *pure* gelatin should be demanded, and all gelatin should be washed for some hours in cold running water after being weighed and before being added to the bouillon. It is prepared as follows:

To 1000 cc. of meat-infusion or to 1000 cc. of water containing

* "Jour. of Exp. Med.," II, No. 5, p. 546.

2 grams of beef-extract in solution, 10 grams of peptone, 5 grams of salt, and 100 grams of gelatin ("Gold label" is the best commercial article) are added, and heated until the ingredients are dissolved. The solution reacts strongly acid and must be corrected by titration, as already described. It must then be returned to the fire and boiled for about an hour. As gelatin is apt to burn when boiled over the direct flame, double boilers have been suggested, but unless the outer kettle is filled with brine or saturated calcium chlorid solution, they are very slow, and when proper care is exercised there is really no great danger of the gelatin burning. It must be stirred occasionally, and the flame should be so distributed by wire gauze or by placing a sheet of asbestos between it and the kettle as not to act upon a single point. At the end of the hour the albumins of the meat-infusion will be coagulated and the gelatin thoroughly dissolved. Günther has shown that the gelatin congeals better if allowed to dissolve slowly in warm water before boiling. As much water as has been lost by vaporization during the process of boiling should be replaced. It is well to cool the liquid to about 60°C., add the water mixed with the white of an egg to clear the liquid, boil again for half an hour, and filter.

If the filter paper be of good quality, properly folded (pharmaceutic filter), wet with boiling water, and if the gelatin be properly dissolved, the whole quantity should pass through before cooling too much. Should only half go through before cooling, the remainder must be returned to the pot, heated to boiling once more, and then passed through a new filter paper. As a matter of fact, gelatin usually filters readily. A wise precaution is to catch the first few centimeters in a test-tube and boil them, so that if cloudiness show the presence of uncoagulated albumin, the whole mass can be boiled again. The finished gelatin, which is *perfectly transparent* and of an amber color, is at once distributed into sterilized tubes and sterilized like the bouillon by the intermittent method. The sterilization can also be satisfactorily performed by the use of the autoclave at 110°–115°C. for fifteen minutes, but this method is probably less well adapted to the sterilization of gelatin than of the other media, as the high degree of heat injures its subsequent solidifying power.

Gelatin becomes liquid at 37°C. It cannot, therefore, be used with advantage for cultures that must be kept at body temperatures.

AGAR-AGAR

Agar-agar is the commercial name of a preparation made from a Ceylonese sea-weed. It reaches the market in the form of long shreds of semi-transparent, isinglass-like material, less commonly in long bars of compressed flakes, rarely in the form of powder. It dissolves slowly in boiling water with a resulting thick jelly when

cold. The jelly, which solidifies between 40° and 50°C., cannot again be melted except by the elevation of its temperature to the boiling-point. The culture-medium made from agar-agar is *nearly* transparent. In addition to its ability to liquefy and solidify, it has the advantage of remaining solid at comparatively high temperatures so as to permit keeping the cultures grown upon it at the incubation temperature,—*i.e.*, 37°C.,—at which temperature gelatin is always liquid.

The preparation of agar-agar is commonly described in the text-books as one "requiring considerable patience and much waste of filter paper." In reality, it is not difficult if a good heavy filter paper be obtained and no attempt made to filter the solution until the agar-agar is perfectly dissolved.

It is prepared as follows: To 1000 cc. of bouillon made as described above, preferably of meat instead of beef-extract, 10 to 15 grams of agar-agar are added. The mixture is boiled vigorously for an hour in an open pot over the direct gas flame or in the double boiler with saturated calcium chlorid solution in the outside pot. After being cooled to about 60°C., and after the correction of the reaction by titration, an egg beaten up in water is added, and the liquid again boiled until the egg-albumen is entirely coagulated.

After the second boiling and the replacement of the volatilized water, the agar-agar is filtered through a carefully folded pharmaceutic filter wet with boiling water. It may expedite matters to pour in about one-half of the solution, keep the remainder hot, and subsequently add it.

The formerly much employed hot-water and gas-jet filters are unnecessary. If properly prepared, the whole quantity will filter in from fifteen to thirty minutes.

Ravenel* prepares agar-agar by making two solutions, one representing the meat-infusion, but twice the usual strength, the other the agar-agar dissolved in one-half the usual quantity of water. The agar-agar is dissolved by exposure to superheated steam in the autoclave, after which the two solutions are poured together and boiled until all of the albumins are precipitated. The coagulation of the albumins of the meat-infusion serves to clarify the agar-agar.

If agar-agar is to be made with beef-extract, the bouillon should be made first and filtered *when cold*, to exclude the uratic salts which otherwise precipitate in the agar-agar when cold and form an unsightly cloud.

The finished agar-agar should be a colorless, nearly transparent, firm jelly. It is dispensed in tubes like the gelatin and bouillon, sterilized by steam, either by the intermittent process or in the autoclave, and after the last sterilization, before cooling, each tube is inclined against a slight elevation, so as to permit the jelly to solidify obliquely and afford an extensive flat surface for the culture.

* "Journal of Applied Microscopy," June, 1898, vol. 1, No. 6, p. 106.

After the agar-agar jelly solidifies it retracts so that a little water collects at the lower part of the tube. This should not be removed, as it keeps the jelly moist, and also distinctly influences the character of the growth of the bacteria.

Glycerin Agar-agar.—Certain bacteria among which is the tubercle bacillus, will not grow upon agar-agar prepared as described above, but will do so if 3 to 7 per cent. of glycerin be added after filtration. This fact was discovered by Roux and Nocard.

Blood Agar-agar was recommended by R. Pfeiffer for the cultivation of the influenza bacillus. It is ordinary agar-agar whose surface is coated with a little blood secured under aseptic precautions from the finger-tip, ear-lobule, etc., of man, or from the vein of one of the lower animals. Some bacteriologists prepare a hemoglobin agar-agar by spreading a little powdered hemoglobin upon the surface of the agar-agar. As powdered hemoglobin is not sterile, the medium must be sterilized after its addition.

The blood agar-agar should be kept in the incubator a day or two before use so as to insure perfect sterility.

BLOOD-SERUM

The advantage possessed by this medium is that it is primarily a constituent of the animal body, and hence offers conditions favorable for the development of the parasitic forms of bacteria. If the blood-serum is to be employed fresh, it must either be heated or kept sufficiently long to lose its natural germicidal properties. The statement that serum represents the normal body-juice is erroneous, as it is minus the fibrin factors and some of the salts, and contains new bodies liberated from the destroyed leukocytes. Solidified blood-serum, exposed to the heat of the sterilizing apparatus, in no sense resembles the body-juices.

It is one of the most difficult media to prepare. The blood must be obtained either by bleeding some good-sized animal, or from a slaughter-house, in appropriate receptacles, the best things for the purpose being 1-quart fruit jars with tightly fitting lids. The jars are sterilized by heat, closed, and carried to the slaughter-house, where the blood is permitted to flow into them from the severed vessels of the animal. It seems advisable to allow the first blood to escape, as it is likely to become contaminated from the hair. By waiting until a coagulum forms upon the hair the danger of contamination is diminished. The jars, when full, are allowed to stand undisturbed until firm coagula form within them, after which they are carried to the laboratory and stood upon ice for forty-eight hours, by which time the clots will have retracted considerably, and a moderate amount of clear serum can be removed by sterile pipets and placed in sterile tubes. If the serum obtained be red and clouded from the presence of corpuscles, it may be pipetted into

sterile cylinders and allowed to sediment for twelve hours, then repipetted into tubes.

As the demand for serum has been considerable during the last few years, commercial houses dealing in biologic products now market fresh horse serum, preserved with chloroform, in liter bottles. This can be employed with great satisfaction, the chloroform being driven off during coagulation and sterilization.

If it be desirable to use the serum as a liquid medium, it is exposed to a temperature of 60°C. for one hour upon each of five consecutive days. To coagulate the serum and make a solid culture medium, it may be exposed twice, for an hour each time—or three times if there be reason to think it badly contaminated—to a temperature

Fig. 45.—Koch's apparatus for coagulating and sterilizing blood-serum.

just short of the boiling-point. During the process coagulation occurs, and the tubes should be inclined, so as to offer an oblique surface for the growth of the organisms. The serum thus prepared should be white, but may have a reddish-gray color if many red corpuscles be present. It is always opaque and cannot be melted; once solid, it remains so.

Koch devised a special apparatus for coagulating blood-serum. The bottom should be covered with wet cotton, a single layer of tubes placed upon it, the glass lid closed and covered with a layer of felt, and the temperature elevated until coagulation occurs. The repeated sterilizations may be conducted in this same apparatus, or may be done equally well in a steam apparatus, the cover of which is not completely closed, for if the temperature of the serum be raised too rapidly it is certain to bubble, so that the desirable smooth surface, upon which the culture is to be made, is ruined.

Like other culture-media, blood-serum and its combinations may be sterilized in the autoclave and much time thus saved. The serum should, however, first be coagulated, else bubbling is apt to occur

and ruin its surface. The autoclave temperature unfortunately makes the preparation very firm and hard, considerable fluid being pressed out of it.

It is said that considerable advantage is secured from the addition of *neutrose* to blood-serum, which prevents its coagulating when heated. It can then be sterilized like bouillon and can subsequently be solidified, when desired, by the addition of some agar-agar.

Fresh blood-serum can be kept on hand in the laboratory, in sterile bottles, by adding an excess of chloroform. In the process of coagulation and sterilization the chloroform is evaporated; the serum is unchanged by its presence.

Löffler's Blood-serum Mixture, which seems rather better for the cultivation of some species than the blood-serum itself, consists of 1 part of a beef-infusion bouillon containing 1 per cent. of glucose and 3 parts of liquid blood-serum. After being well mixed the fluid is distributed in tubes, and sterilized and coagulated like the blood-serum itself. As prepared by Löffler it was soft, semi-gelatinous and semi-transparent, not firm and white; therefore should be sterilized at low temperatures. Many organisms grow more luxuriantly upon it than upon either plain blood-serum or other culture media. Its especial usefulness is for the cultivation of Bacillus diphtheriæ, which grows rapidly and with a characteristic appearance.

Alkaline Blood-serum.—According to Lorrain Smith, a very useful culture medium can be prepared as follows: To each 100 cc. of blood-serum add 1–1.5 cc. of a 10 per cent. solution of sodium hydrate and shake it gently. Put sufficient of the mixture into each of a series of test-tubes, and, laying them upon their sides, sterilize like blood-serum, taking care that their contents are not heated too quickly, as then bubbles are apt to form. The result should be a clear, solid medium consisting chiefly of alkali-albumins. It is especially useful for Bacillus diphtheriæ.

Deycke's Alkali-albuminate.—One thousand grams of meat are macerated for twenty-four hours with 1200 cc. of a 3 per cent. solution of potassium hydrate. The clear brown fluid is filtered off and pure hydrochloric acid carefully added while a precipitate forms. The precipitated albuminate is collected upon a cloth filter, mixed with a small quantity of liquid, and made distinctly alkaline. To make solutions of definite strength it can be dried, pulverized, and redissolved.

The most useful formula used by Deycke was a 2.5 per cent. solution of the alkali-albuminate with the addition of 1 per cent. of peptone, 1 per cent. of NaCl, and gelatin or agar-agar enough to make it solid.

Potatoes.—Without taking time to review the old method of boiling potatoes, opening them with sterile knives, and protecting them in the moist chamber, or the much more easily conducted method of Esmarch in which the slices of potato are sterilized in the small dishes in which they are afterward kept and used, we will at once pass to what seems the most simple and satisfactory method—that of Bolton and Globig.*

With the aid of a cork-borer or Ravenel potato cutter a little smaller in diameter than the test-tube ordinarily used, a number of cylinders are cut from potatoes. Rather large potatoes should

* "The Medical News," 1887, vol. L, p. 138.

be used, the cylinders being cut transversely, so that a number, each about an inch and a half in length, can be cut from one potato. The skin is removed from the cylinders by cutting off the ends, after which each cylinder is cut in two by an oblique incision, so as to leave a broad, flat surface. The half-cylinders are placed each in a test-tube previously sterilized, and are exposed three times, for half an hour each, to the streaming steam of the sterilizer. This steaming cooks the potato and also sterilizes it. Such potato cylinders are apt to deteriorate rapidly, first by turning very dark, second by drying so as to be useless. Abbott has shown that if the cut cylinders be allowed to stand for twelve hours in running water before being dispensed in the tubes, they are not so apt to turn dark. Drying may also be prevented by adding a few drops of clean water to each tube before sterilizing. Some workers insert a bit of glass or a pledget of glass wool into the bottom of the tube so as to support the potato and keep it up out of the water. It is not necessary to have a special small chamber blown in the tube to contain this water, only a small quantity of which need be added. The special reservoir increases the trouble of cleaning the tubes.

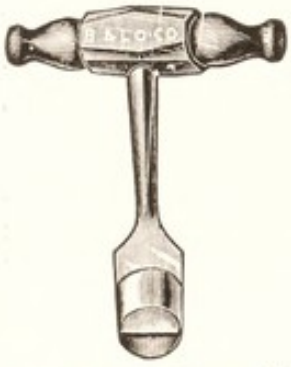


Fig. 46.—Ravenel's potato cutter.

If the work to be done with potatoes is to be accurate, it is necessary to correct their variable reaction, especially if the acids have not been sufficiently removed by the washing in running water already described.

To do this the cut cylinders are placed in a measured quantity of distilled water and steamed for about an hour. The reaction of the water is then determined by titration and the desired amount of sodium hydroxid added to correct the reaction, after which the potatoes are steamed in the corrected solution for about thirty minutes before being placed in the tubes.

A *potato-juice* has also been suggested, and is of some value. It is made thus: To 300 cc. of water 100 grams of grated potato are added, and allowed to stand on ice over night. Of the pulp, 300 cc. are expressed through a cloth and cooked for an hour on a water-bath. After cooking, the liquid is filtered, titrated if desired, and receives an addition of 4 per cent. of glycerin. Upon this medium the tubercle bacillus grows well, especially when the reaction of the medium be acid.

Milk.—Milk is a useful culture-medium. As the cream which rises to the top is a source of inconvenience, it is best to secure fresh milk from which the cream has been removed by a centrifugal machine. It is given the desired degree of alkalinity by titration, dispensed in sterile tubes, and sterilized by steam by the intermittent method or in the autoclave. The opaque nature of this culture-me-

dium often permits the undetected development of contaminating organisms. A careful watch should therefore be kept lest it spoil.

Litmus Milk.—This is milk to which just enough of a saturated watery solution of pulverized litmus is added to give a distinct blue color after titration. Litmus milk is probably the best reagent for determining acid and alkali production by bacteria.

The watery solution of litmus, being a vegetable infusion, is likely to be spoiled by micro-organismal growth, hence must be treated like the culture media and sterilized by steam every time the receptacle in which it is kept is opened.

An excellent method of preparing litmus is given by Prescott and Winslow* and is as follows:

> To one-half pound of litmus cubes add enough water to more than cover, boil, decant off the solution. Repeat this operation with successive small quantities of water until 3 to 4 liters of water have been used and the cubes are well exhausted of coloring matter. Pour the decantations together and allow them to settle over night. Siphon off the clear solution. Concentrate to about 1 liter and make the solution decidedly acid with glacial acetic acid. Boil down to about ½ liter and make exactly neutral with caustic soda or potash. To test for the neutral point, place one drop of the solution in a test-tube, while one drop of $\frac{n}{20}$ HCl should turn it red, one drop of $\frac{n}{20}$ NaOHO should turn it blue. Filter the solution and sterilize at 110°C. This solution should be added to the media just before use in the proportion of about ¼ cc. to 5 cc. of medium.

If litmus be added to the milk before sterilization, it is apt to be browned or decolorized, so that it is better to sterilize the two separately and pour them together subsequently. It is said that *lacmoid* is never thus changed, and many workers prefer it to litmus on that account.

Petruschky's Whey.—In order to differentiate between acid and alkali producers among the bacteria, Petruschky has recommended a neutral whey colored with litmus. It is made as follows:

To a liter of fresh skimmed milk 1 liter of water is added. The mixture is violently shaken. About 10 cc. are taken out as a sample to determine how much hydrochloric acid must be added to produce coagulation of the milk, and, having determined the least quantity required for the whole bulk, it is added. After coagulation the whey is filtered off, exactly neutralized, and boiled. After boiling it is found clouded and acid in reaction. It is therefore filtered again, and again neutralized. Litmus is finally added to the neutral liquid, so that it has a violet color, changed to blue or red by alkalies or acids.

Peptone Solution, or Dunham's solution, is a perfectly clear, colorless solution, made as follows:

Sodium chlorid	0.5
Witte's dried peptone	1.0
Water	100.0

Boil until the ingredients dissolve; filter, fill into tubes and sterilize.

* "Elements of Water Bacteriology," John Wiley & Sons, New York, 1904, p. 126.

It was for a long time used for the detection of indol. Garini* found that many of the peptones upon the market were impure, and on this account failed to show the indol reaction in cultures of bacteria known to produce it. He recommends testing the peptone to be employed by the use of the biuret reaction. The reagent employed is Fehling's copper solution, with which pure peptone strikes a violet color not destroyed upon boiling, while impure peptone gives a red or reddish-yellow precipitate. Both the peptone and copper solutions should be in a dilute form to make successful tests.

The addition of 4 cc. of the following solution—

Rosolic acid	0.5
Eighty per cent. alcohol	100.0

makes the peptone solution a reagent for the detection of acids and alkalies. The solution is of a pale rose color. If the organisms cultivated produce acids, the color fades; if alkalies, it intensifies. As the color of rosolic acid is destroyed by glucose, it cannot be used in culture-media containing it.

Theobald Smith† has called attention to the fact that many bacteria fail to grow in Dunham's solution, and recommends that, for the detection of indol, bouillon free of dextrose be used instead. All bacteria grow well in it, and the indol reaction is pronounced in sixteen-hour-old cultures. His method of preparation is as follows: Beef-infusion, prepared either by extracting in the cold or at 60°C., is inoculated in the evening with a rich fluid culture of some acid-producing bacterium (Bacillus coli) and placed in the thermostat. Early next morning the infusion, covered with a thin layer of froth, is boiled, filtered, peptone and salt added, and the neutralization and sterilization carried on as usual.

This method is subject to error caused by the presence in the medium of indol produced by the colon bacillus. This can be demonstrated if the tests for indol be sensitive. Selter‡ finds that the method of Smith gives inferior results to a simple culture-medium consisting of water, 90 parts; Witte's peptone, 10 parts; sodium phosphate, 0.5 part, and magnesium sulphate, 0.1 part.

Other culture-media employed for special purposes will be mentioned as occasion arises.

* "Centralbl. f. Bakt. u. Parasitenk.," XIII, p. 790.
† "Journal of Exp. Medicine," Sept. 5, 1897, VI, p. 546.
‡ "Centralbl. f. Bakt. u. Parasitenk.," Orig. LI, p. 465.

CHAPTER VIII

CULTURES, AND THEIR STUDY

THE purposes for which culture-media are prepared are numerous. Through their aid it is possible to isolate the micro-organisms, to keep them in healthy growth for considerable lengths of time, during which their biologic peculiarities can be observed and their metabolic products collected, and to introduce them free from contamination into the bodies of experiment animals.

The isolation of bacteria was next to impossible until the fluid media of the early observers were replaced by the solid culture-media introduced by Koch, and exceedingly difficult until he devised the well-known "plate cultures."

A growth of artificially planted micro-organisms is called a *culture*. If such a growth contains but one kind of organism, it is known as a *pure culture*.

It has at present become the custom to use the term "culture" rather loosely, so that it does not always signify an artificially planted growth of micro-organisms, but may signify a growth taking place under natural conditions; thus, the typhoid bacillus is said to occur in "pure culture" in the spleens of patients dead of typhoid fever, because no other bacteria are associated with it; and sometimes, when the tubercle bacilli are very numerous and unmixed with other bacteria, in the expectorated fragments of cheesy matter from tuberculosis pulmonalis, they are said to occur in "pure culture."

The culture manipulations are performed either with a sterilized platinum wire or with a capillary pipet of glass.

The platinum wire is so limber that it is scarcely to be recommended, and a wire composed of platinum and iridium, which is elastic in quality, is to be preferred. The wires are about 5 cm. in length, of various thicknesses according to the use for which they are employed, and are usually fused into a thin glass rod about 17 cm. in length. The wires may be straight or provided with a small loop at the end so as to conveniently take up small drops of fluid. Heavy wires used for securing diseased tissue from animals may be flattened at the ends by hammering, and may thus be fashioned into miniature knives, scrapers, harpoons, etc., as desired.

Ravenel has invented a convenient form for carrying in the pocket. It consists of the platinum wire fastened in a heavier aluminium wire which in turn fits into a piece of glass tubing. When carried in the

pocket, the position of the platinum wire is reversed in the glass tubing and protected by it.

Immediately before and immediately after use, the platinum wire is to be sterilized by heating to incandescence in a flame, in order that it convey nothing undesirable into the culture, and in order that it scatter no micro-organisms about the laboratory.

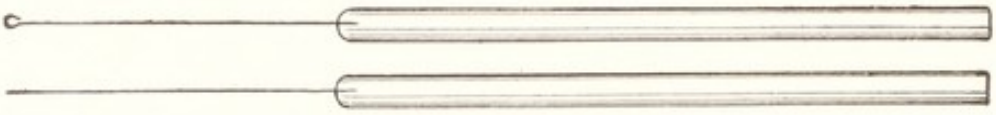


Fig. 47.—Platinum needles for transferring bacteria; made from No. 27 platinum wire inserted in glass rods.

Capillary glass tubes are employed by the French for many of the manipulations. They are made of ¼- or ⅜-inch glass tubing cut into 25 cm. lengths, heated at the center, and drawn out to capillary ends about 5 cm. long. They are sealed at one end and plugged with cotton at the other, and a number of them, prepared at the same time, sterilized. They can be used for all the purposes for which the

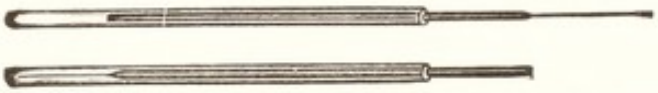


Fig. 48.—Ravenel's platinum wires for bacteriologic use.

platinum wire is employed, and in addition can be used as containers for small quantities of fluids sealed in them. When about to use such a tube, its sealed capillary end should be broken off with forceps, and the tube sterilized by flaming.

Technic of Culture Manipulation.—Containers of stored culture-media should be kept in an upright position, that the cotton stoppers

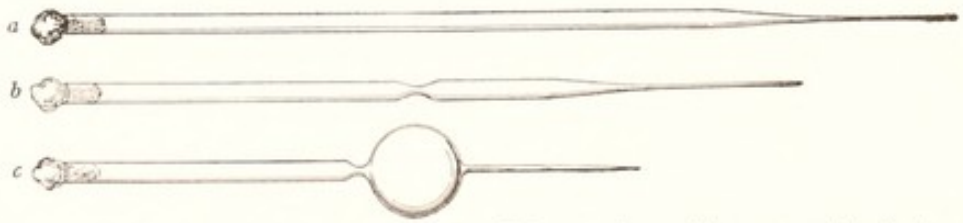


Fig. 49.—Capillary glass tubes. *a*, Pipette for ordinary manipulations; *b*, constricted pipette in which small quantities of cultures, etc., can be sealed by fusing the glass; *c* bulbous pipette in which larger quantities of fluids may be sealed.

are not moistened or soiled. If moistened with the culture-media, molds whose spores fall upon the surface of the stoppers may gradually work their mycelial threads between the fibers until they appear upon their inner surface and drop newly formed spores into the contained media.

In handling tubes care must be taken to stand them up in tum-

blers, racks, or other contrivances, and not lay them upon the table so that the contents touch the stoppers.

When the cotton plugs are removed in order that the contents of the tubes or flasks may be inoculated or otherwise manipulated, the removal and replacement should be done as quickly as convenient, and the mouth of the tube should be flamed before removal. The plugs should be held between the fingers, by that part which projects above the glass, not laid upon the table, from which dust, and incidentally bacteria, may be taken up and subsequently dropped into the medium; nor must they be touched with the fingers at that part which enters the neck of the container lest they take up micro-organisms from the skin. The stoppers thus require careful consideration lest they become the source of future contamination.

So soon as the cotton stopper is removed, the medium is left without protection from whatever micro-organisms happen to be in the air, so that it should be replaced as soon as possible, and every manipulation requiring its removal performed expeditiously. During the time the stopper is withdrawn it is wise to hold the tubes or other containers in an oblique or horizontal position that will aid in excluding the micro-organisms of the air. Some bacteriologists make inoculations with the tubes reversed in all cases in which solid media are employed, but it is not necessary. If the tubes are held *obliquely*, the danger of contamination is reduced to a minimum. It is well to adopt some method of handling the tubes that has given satisfaction to others and is found convenient to one's self and habitually practise it until it becomes second nature and can be done without thought.

Fig. 50.—Method of holding tubes during inoculation.

The usual method of making a transplantation of bacteria from culture-tube to culture-tube, is, in detail, as follows:

In order that any bacteria loosely scattered over the surface of the cotton stopper, and upon the glass near the mouth of the tube, may be destroyed and prevented from entering the medium as the stopper is withdrawn, both the tube containing the culture and the fresh tube to which it is to be transferred should be held for a moment in a flame and rolled from side to side so that all parts are flamed. The cotton ignites and blazes actively, but the flame can be extinguished by forcibly blowing upon it and any smoldering remains extinguished by pinching with the fingers. The tubes are now placed side by side

between the thumb and upward-directed palm of the left hand, the stoppers toward the operator. The position of the tubes should be such as to permit one to see the contained media without the fingers being in the way. The stopper of the tube toward the left is removed by a gentle twist and placed between the index and middle fingers of the left hand; the stopper of the next tube similarly removed and placed between the middle and ring fingers of the same hand. If three or four tubes are to be held, the third stopper can be placed between the ring and little fingers of the left hand and the fourth retained in the right hand. The part of each stopper that enters the tube must not be touched.

The necessary manipulation is usually made with the platinum wire, which is sterilized by heating to incandescence before using. The wire must not be used while hot, but cools in a moment or two. The culture is touched, the wire entering and exiting without touching the tube, and the bacteria adhering to the wire are applied to the medium in the other tube, the same care being exerted not to have the platinum wire touch the glass. After the transfer is made, the wire is made incandescent in the flame before being returned to the table or stand made to hold it, and the stoppers returned one after the other, each to its own tube, that part entering the tube not being touched. Each stopper is given a twist as it enters the mouth of the tube.

Modifications of these directions can be made to suit the different forms of containers used, but the essential features must be maintained.

When any manipulation requires that a tube or flask be permitted to remain open an unusual length of time, its contamination from the air can be prevented for some minutes by heating its neck quite hot. The air about it, being heated by the hot glass, ascends, forming a current that carries the bacteria away from, rather than into, the receptacle.

Isolation of Bacteria.—Three principal methods are, at present, employed for securing pure cultures of bacteria. Before beginning a description of them it is well to observe that the peculiarities of certain pathogenic micro-organisms enable us to use special means for their isolation, and that these general methods are chiefly useful for the isolation of non-pathogenic organisms.

Plate Cultures.—All the methods depend upon the observation of Koch, that when bacteria are equally distributed throughout some liquefied nutrient medium that is subsequently solidified in a thin layer, they grow in scattered groups or families, called *colonies*, distinctly isolated from one another and susceptible of transplantation.

The plate cultures, as originally made by Koch, require considerable apparatus, and of late years have given place to the more simple and ready methods. So great is their historic interest, how-

ever, that it would be a great omission not to describe the original method in detail.

Apparatus.—Half a dozen glass plates, measuring about 6 by 4 inches, free from bubbles and scratches and ground at the edges, are carefully cleaned, placed in a sheet-iron box made to receive them, and sterilized in the hot-air closet. The box is kept tightly closed, and in it the sterilized plates can be kept indefinitely before use.

A moist chamber, or double dish, about 10 inches in diameter and 3 inches deep, the upper half being just enough larger than the lower to allow it to close over it, is carefully washed. A sheet of bibulous paper is placed in the bottom, so that some moisture can be retained, and a 1 : 1000 bichlorid of mercury solution poured in and brought in contact with the sides, top, and bottom by turning the dish in all directions. The solution is emptied out, and the dish, which is kept closed, is ready for use.

A leveling apparatus is required. It consists of a wooden tripod with adjustable screws, and a glass dish covered by a flat plate of glass upon which a low bell-jar stands. The glass dish is filled with broken ice and water, covered with the glass plate, and then exactly leveled by adjusting the screws under the legs of the tripod. When level, the cover is placed upon it, and it is ready for use.

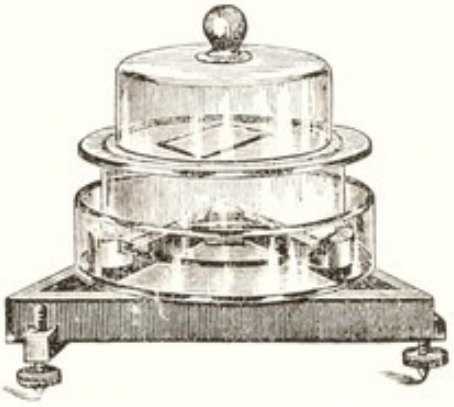


Fig. 51.—Complete leveling apparatus for pouring plate cultures, as taught by Koch.

Method.—A sterile platinum loop is dipped into the material to be examined, a small quantity secured, and stirred about so as to distribute it evenly throughout the contents of a tube of melted gelatin. If the material under examination be very rich in bacteria, one loopful may contain a million individuals, which, if spread out in a thin layer, would develop so many colonies that it would be impossible to see any one clearly; hence further dilation becomes necessary. From the first tube, therefore, a loopful of gelatin is carried to a second and stirred well, so as to distribute the organisms evenly throughout its contents. In this tube we may have no more than ten thousand organisms, and if the same method of dilution be used again, the third tube may have only a few hundred, and a fourth only a few dozen colonies.

After the tubes are thus inoculated, one of the sterile glass plates is caught by its edges, removed from the iron box, and placed beneath the bell-glass upon the cold plate covering the ice-water of the leveling apparatus. The plug of cotton closing the mouth of tube No. 1 is removed, and to prevent contamination during the outflow of the gelatin the mouth of the tube is held in the flame of a Bunsen burner for a moment or two. The gelatin is then cautiously poured out upon the plate, the mouth of the tube, as well as the plate, being covered by the bell-glass to prevent contamination by germs in the air. The apparatus being level, the gelatin spreads out in an even, thin layer, and, the plate being cooled by the ice beneath, it immediately solidifies, and in a few moments can be removed to the moist chamber prepared to receive it. As soon as plate No. 1 is prepared, the contents of tube No. 2 are poured upon plate No. 2, allowed to spread out and solidify, and then superimposed on plate No. 1 in the moist chamber, being separated from the plate already in the chamber by small glass benches made for the purpose and previously sterilized. After the contents of all the tubes are thus distributed, the moist chamber and its contents are stood away to permit the bacteria to grow. Where each organism falls a colony develops, and the success of the whole method depends upon the isolation of a colony and its transfer to a tube of new sterile culture-media, where it can grow unmixed and undisturbed.

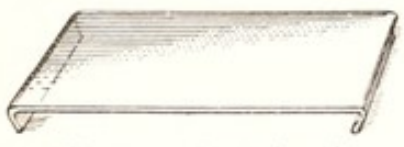


Fig. 52.—Glass bench.

From the description it must be evident that only those culture-media that

can be melted and solidified at will can be used for plate cultures—viz., gelatin, agar-agar, and glycerin agar-agar. Blood-serum and Löffler's mixture are entirely inappropriate.

The chief drawbacks to this excellent method are the cumbersome apparatus required and the comparative impossibility of making plate cultures, as is often desirable, in the clinic, at the bedside, or elsewhere than in the laboratory. The method therefore soon underwent modifications, the most important being that of Petri, who invented special dishes to be used instead of plates.

Petri's Dishes.—These are glass dishes, about 4 inches in diameter and ½ inch deep, with accurately fitting lids. They were first

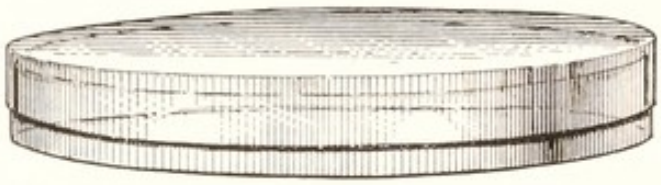


Fig. 53.—Petri dish for making plate cultures.

recommended by Petri* and greatly simplify bacteriologic technic by dispensing with the plates and plate-boxes, the moist chambers and benches, and usually with the levelling apparatus of Koch, though this is still employed in some laboratories, and must always be employed when an even distribution of the colonies is necessary in order that they can be accurately counted.

The method of using the Petri dishes is very simple. They are carefully cleaned, polished, closed and sterilized by hot air, care

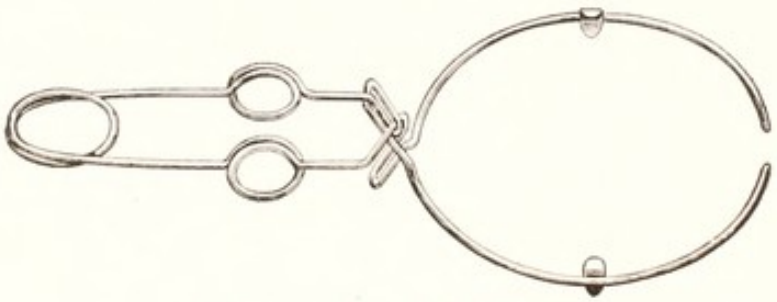


Fig. 54.—Petri dish forceps.

being taken that they are placed in the hot-air closet right side up, and after sterilization are kept covered and in that position. They should be sterilized immediately before using, or if they must be kept for a time should be wrapped in tissue paper and then sterilized. The tissue paper protects the accidental entrance of dust between dish and lid, keeps the dish closed, and need not be removed until the last moment before using.

Time can be saved by sterilizing the dish and cover in the direct

* "Centralbl. f. Bakt. u. Parasitenk.," 1887, I, No. 1, p. 279.

flame, instead of in the hot-air closet, special forceps adapted to holding them having been devised by Rosenberger.*

The dilution of the material under examination is made with gelatin or agar-agar tubes in the manner above described, the plug is removed, the mouth of the tube cautiously held for a moment in the flame, and the contents poured into one of the sterile dishes, whose lid is just sufficiently elevated to permit the mouth of the tube to enter. The gelatin is spread over the bottom of the dish in an even layer, allowed to solidify, labeled, inverted, so that the water of condensation may not drop from the lid upon the culture film and spoil the cultures, and stood away for the colonies to develop.

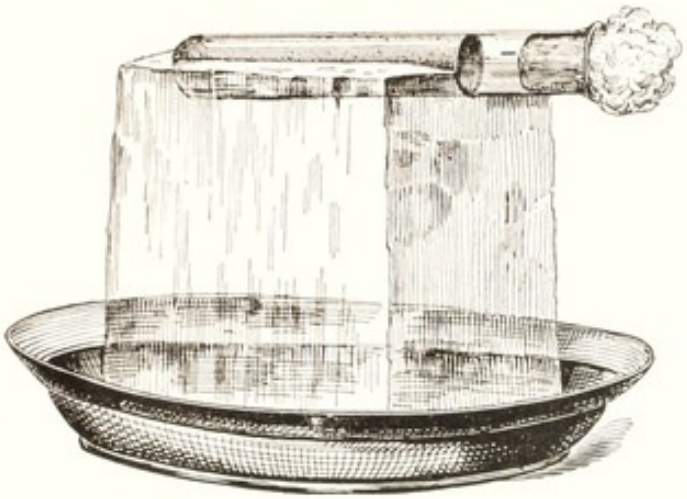


Fig. 55.—Esmarch tube on block of ice (redrawn after Abbott).

To overcome the difficulty of excessive water of condensation Hill has introduced lids made of porous clay, by which the moisture is absorbed. These can be obtained from most laboratory purveyors.

Among the other advantages of the Petri dish is the convenience with which colonies can be studied with a low-power lens. To do this with the Koch plates meant to remove them from the sterile chamber to the stage of a microscope and so expose them to the air, and to contamination, but to examine colonies in the Petri dish, one simply examines through the thin glass of the bottom dish without any exposure to contaminating organisms.

Esmarch's Tubes.—This method, devised by Esmarch, converts the wall of the test-tube into the plate and dispenses with all other apparatus. The tubes, which are inoculated and in which the dilutions are made, should contain less than half the usual amount of gelatin or agar-agar. After inoculation the cotton plugs are pushed into the tubes until even with their mouths, and then covered with a rubber cap, which protects them from wetting. A groove is next cut in a block of ice, and the tube, held almost horizontally, is rolled in this until the entire surface of the glass is covered with a thin layer of the soldified medium. Thus the wall of the tube becomes the plate upon which the colonies develop.

In carrying out Esmarch's method, the tube must not contain too much of the culture medium, or it cannot be rolled into an even layer; the contents should not touch the cotton plug, lest it be glued to the glass and its subsequent usefulness injured, and no water must be admitted from the melted ice.

* "Phila. Med. Jour.," Oct. 20, 1900, vol. VI, No. 16, p. 760.

Colonies.—The progeny of each bacterium form a mass which is known as a *colony*. When these are separated from one another, each is spoken of as a *single colony*, and different characteristics belonging to different micro-organisms enable us at times to recognize by macroscopic and microscopic study of the colony the particular kind of micro-organism from which it has grown. The illustrations show the various types of colonies and the legends the terms used in describing them.

Growing colonies should be observed from day to day, as it not infrequently happens that unexpected changes, such as pigmentation and liquefaction, develop after the colony is several days old and indeed sometimes not until much later. Again, many colonies make their first appearance as minute, sharply circumscribed points, and later spread upon the surface of the culture-medium, either in the form of a thin, homogeneous layer or a filamentous cluster. It is particularly important that in describing new species of bacteria an account of the appearance of the colonies from day to day, comparing all of their variations for at least two weeks, should be included.

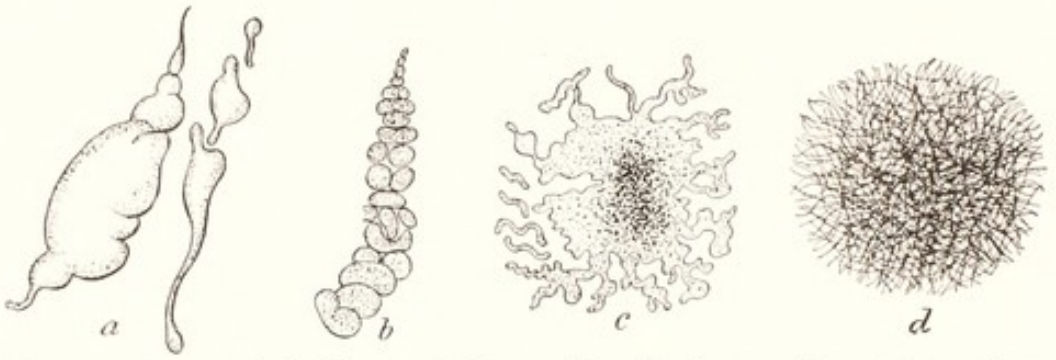


Fig. 56.—Types of colonies: *a*, Cochleate (B. coli, abnormal form); *b*, conglomerate (B. zopfii); *c*, ameboid (B. vulgatus); *d*, filamentous (Frost).

Fig. 57.—Surface elevations of growths: *a*, Flat; *b*, raised; *c*, convex; *d*, pulvinate; *e*, capitate; *f*, umbilicate; *g*, umbonate (Frost).

Pure Cultures.—Single colonies also subserve a second very important purpose, that of enabling us to secure pure cultures of bacteria from a mixture. For this purpose an isolated colony is selected and carefully examined to see that it is single and not a mixture of two closely approximated colonies of different kinds, and then transplanted to a tube of an appropriate culture-medium. If the colonies are few and of good size, each is picked up with a sterile platinum wire and transplanted to a tube of appropriate culture-medium. If, however, the colonies are numerous, of small size, and close to-

gether, it may be necessary to do it under a dissecting microscope or even a low power of the ordinary bacteriologic microscope. This operation of transplantation is familiarly known as *fishing*.

Fishing.—It requires considerable practice and skill to fish successfully, and the student should early begin to practise it. The colony to be transplanted, selected because of its isolation, its typical appearance, and convenient position on the plate, is brought to the center of the field and the plate firmly held in position with the left hand. A sterile platinum wire is held in the right hand, the little finger, comfortably fixed upon the stage of the microscope, being used to support the hand. As the operator looks into the microscope the point of the platinum wire is carefully brought into the field of vision without touching either the lens of the microscope or any part of the plate beneath. Of course, the wire and the colony cannot be simul-

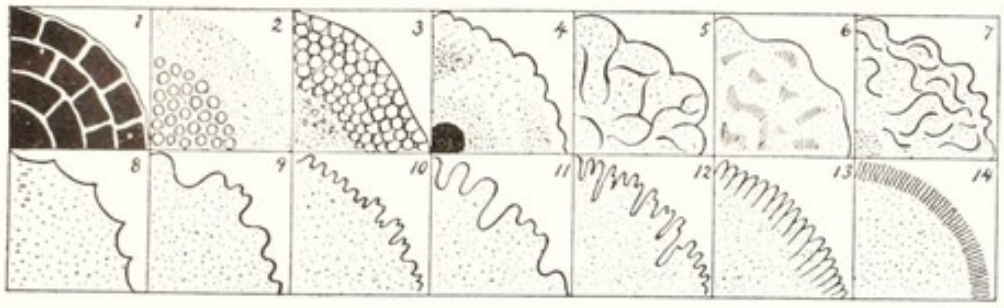


Fig. 58.—Microscopic structure of colonies: 1, Areolate; 2, grumose; 3, moruloid; 4, clouded; 5, gyrose; 6, marmorated; 7, reticulate, 8, repand; 9, lobate; 10, erose; 11, auriculate; 12, lacerate; 13, fimbricate; 14, ciliate (Frost).

taneously focussed upon. When the colony is distinctly seen the platinum wire appears as a shadow, but the endeavor should be to make the end of the shadow which corresponds to the point of the wire appear exactly over the colony. It is then gradually depressed until it touches the colony and can be seen to break up and remove some of its substance; or should the colony be tough and coherent, to tear it away from the culture-medium. It requires almost as much skill to withdraw the wire from the colony without touching anything as to successfully approach the colony in the first place. The bacterial mass adhering to the wire is now spread upon the surface of agar-agar or stabbed in gelatin or stirred in fluid medium, as the case may be. The higher the magnification under which this operation is done, the more difficult it is. Therefore only low-power lenses should be employed.

The Gelatin Puncture or "Stab" Culture.—To make satisfactory puncture cultures, the gelatin must be firm but not old or dry. Should the gelatin be soft and semi-fluid at the time the puncture is made, the bacteria diffuse themselves and the typical appearance of the growth may be masked. On the other hand, if the gelatin be old, dry, or retracted, it is very apt to crack after the culture has been

made and thus entirely destroy the characteristics of the growth. The wire used in the operation should be perfectly straight, and the puncture should be made from the center of the surface directly down to the bottom of the tube and then withdrawn, so that a simple puncture is made. The appearances presented as the growth progresses are subject to striking variations according to the liquefying or non-liquefying tendency of the micro-organisms. Various types of gelatin cultures are shown in the accompanying diagrams,

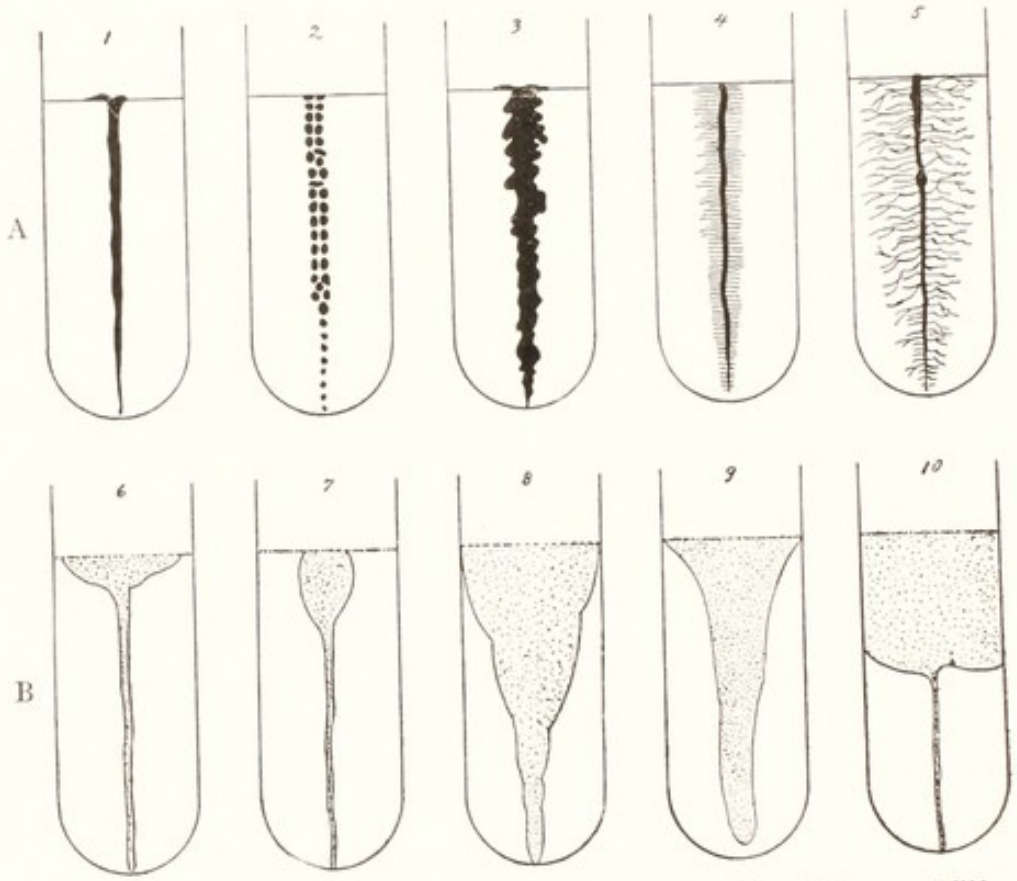


Fig. 59.—Types of growth in stab cultures. A, Non-liquefying: 1, Filiform (B. coli); 2, beaded (Str. pyogenes); 3, echinate (Bact. acidi-lactici); 4, villous (Bact. murisepticum); 5, arborescent (B. mycoides). B, Liquefying: 6, Crateriform (B. vulgare, 24 hours); 7, napiform (B. subtilis, 48 hours); 8, infundibuliform (B. prodigiosus); 9, saccate (Msp. finkleri); 10, stratiform (Ps. fluorescens) (Frost).

and it is rather important that the student should familiarize himself with the terms by which these different growths are described, in order that uniformity of description may be maintained. Gelatin cultures may not be kept in the incubating oven, as the medium liquefies at such temperatures. On the other hand, they must not be kept where the temperature is too low, else the bacterial growth may be retarded. The temperature of a comfortably heated room, not subject to excessive variations, such as are caused by steam heat and the burning of gas, etc., is about the most appropriate. Like the colonies, the cultures must be carefully examined from day to day, as it not infrequently happens that a growth which shows no

signs of liquefaction to-day may begin to liquefy to-morrow or a week hence, or even as late as two weeks hence.

The Agar-agar Culture.—In most cases, the culture is planted by a simple stroke made from the bottom of the tube in which the agar-agar has been obliquely solidified, and where it is fresh and moist, to the upper part, where it is thin and dry. In addition to this, it is advisable to make a puncture from the center of the oblique surface to the bottom of the tube. This enables us to tell whether the bacteria can grow as readily below the surface as above. Some workers always make a zigzag stroke upon the surface of the agar-agar. This does not seem to have any particular advantage except in cases where it is desired to scatter the transplanted organisms as much as possible, in order that a large bacterial mass may be secured.

The growth upon agar-agar is in many ways less characteristic than in gelatin, but as the medium does not liquefy except at a high temperature (100°C.), it has the advantage that cultures may be

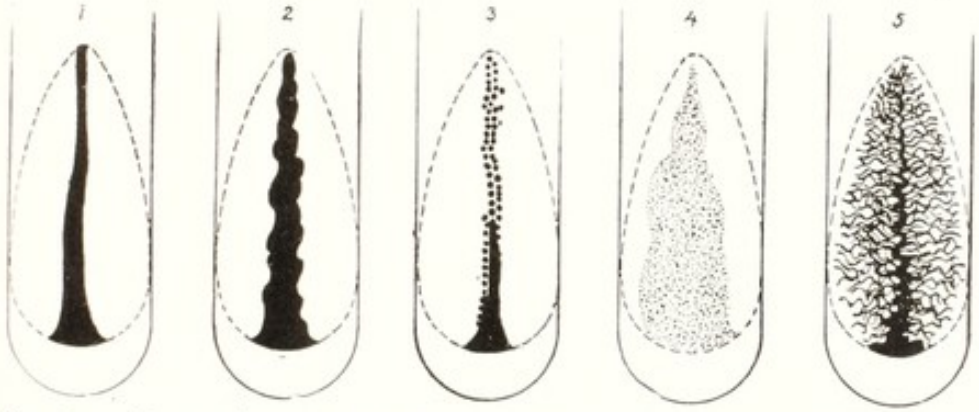


Fig. 60.—Types of streak cultures: 1, Filiform (B. coli); 2, echinulate (Bact. acidi-lactici); 3, beaded (Str. pyogenes); 4, effuse (B. vulgaris); 5, arborescent (B. mycoides) (Frost).

kept in the incubating oven. The colorless or almost colorless condition of the preparation also aids in the detection of chromogenesis.

The growth may be filamentous, or simply a smooth, shining band. Occasionally the bacterium does not grow upon agar-agar unless glycerin be added (tubercle bacillus); sometimes it will not grow even then (gonococcus).

Cultures upon Blood-serum.—Bacteria are planted upon coagulated blood serum and blood-serum preparations as upon agar-agar.

Blood-serum is liquefied by some bacteria, but the majority of organisms have no characteristic reaction upon it. A few, as the bacillus of diphtheria, are, however, characterized by rapid development at given temperatures.

Cultures upon Potato.—These are made by simply stroking the surface of the culture-medium, the density and opacity of the potato making it impracticable to puncture it.

Most bacteria produce smooth, shining, irregularly extending growths upon potato, that may show characteristic colors.

Cultures in Fluid Media.—Here, as has already been stated, transplantation consists in simply stirring in the bacteria so as to distribute them fairly well throughout the medium.

In milk and litmus milk one should observe change in color from the occurrence of acid or alkali production, coagulation, gelatinization, and digestion of the coagulum.

Adhesion Preparations.—Sometimes it is desirable to preserve an entire colony as a permanent microscopic specimen. To do this a perfectly clean cover-glass, not too large in size, is momentarily warmed, then carefully laid upon the surface of the gelatin or agar-agar containing the colonies. Sufficient pressure is applied to the surface of the glass to exclude bubbles, but not to destroy the integrity of the colony. The cover is gently raised by one edge, and if successful the whole colony or a number of colonies, as the case may be, will be found adhering to it. It is treated exactly as any other cover-glass preparation—dried, fixed, stained, mounted, and kept as a permanent specimen. It is called an *adhesion preparation*—"*Klatschpräparat.*"

Special Methods of Securing Pure Cultures.—Pure cultures from single colonies may also be secured by a very simple manipulation suggested by Banti.* The inoculation is made into the water of condensation at the bottom of an agar-agar tube, without touching the surface. The tube is then inclined so that the water flows over the agar, after which it is stood away in the vertical position. Colonies will grow where bacteria have been floated upon the agar-agar, and may be picked up later in the same manner as from a plate.

When the bacterium to be isolated (gonococcus, etc.) will not grow upon media capable of alternate solidification and liquefaction, the blood-serum, potato, or other medium may be repeatedly stroked with the platinum wire dipped in the material to be investigated. Where the first strokes were made, confluent impure cultures occur; but as the wire became freer of organisms by repeated contact with the medium, the colonies become scattered and can be studied and transplanted.

In some cases pure cultures may be most satisfactorily secured by animal inoculation. For example, when the tubercle bacillus is to be isolated from milk or urine which contains bacteria that would outgrow the slow-developing tubercle bacillus, it is better to inject the fluid into the abdominal cavity of a guinea-pig, await the development of tuberculosis in the animal, and then seek to secure pure cultures of the bacillus from the unmixed infectious lesions.

In other cases, as when it is desired to isolate Micrococcus tetragenus, the pneumococcus, and other bacteria that pervade the blood, it is easier to inoculate the animal most susceptible to the infection

*"Centralbl. f. Bakt. u. Parasitenk.," 1895, XVII, No. 16.

and recover it from the blood or organs, than to plate it out and search for the colony among many others similar to it.

Microscopic Study of Cultures.—Some attention has been given to the preparation of microtome sections of gelatin cultures, though

Fig. 61.—Modern incubating oven.

not much practical value has come of it. It can be done by warming the glass of the tube sufficiently to permit the gelatin containing the growth to be removed in a lump and placed in Müller's fluid (bichromate of potassium 2–2.5, sulphate of sodium 1, water 100), where it

is hardened. When quite firm it is washed in water, passed through alcohols ascending in strength from 50 to 100 per cent., embedded in celloidin, cut wet, and stained like a section of tissue.

Winkler* accomplishes the same end by boring a hole in a block of paraffin with the smallest size cork-borer, soaks the block in bichlorid solution for an hour, pours liquid gelatin into the cavity, allows it to solidify, inoculates it by the customary puncture of the platinum wire, allows it to develop sufficiently, and when ready cuts the sections under alcohol, subsequently staining them with much diluted carbol-fuchsin.

Museum Culture Preparations.—Neat museum specimens of plate and puncture cultures in gelatin can be made by simultaneously killing the micro-organisms and fixing the gelatin with formaldehyd, which can either be sprayed upon the gelatin or applied in dilute solution. As gelatin fixed in formaldehyd cannot subsequently be liquefied, such preparations will last a long time.

Standardizing Freshly Isolated Cultures.—This is a matter of some importance, as in bringing bacteria into the new environment of artificial cultivation their biologic peculiarities are temporarily altered, and it takes some time for them to recover themselves. While the appearances of the freshly isolated organism should be carefully noted, too much stress should not be laid upon them, and before beginning the systematic study of any new organism it should be made to grow for several successive generations upon two or three of the most important culture media. Its saprophytic existence being thus established, the characteristics manifested become the permanent peculiarities of the species.

* "Fortschritte der Medicin," 1893, Bd. xi, No. 22.

CHAPTER IX

THE CULTIVATION OF ANAËROBIC ORGANISMS

The presence of uncombined oxygen in ordinary cultures inhibits the development of anaërobic bacteria. When such are to be cultivated, it therefore becomes necessary to utilize special apparatus or adopt physical or chemic methods for the exclusion of the air. Many methods have been suggested for the purpose, an excellent review of which has recently been published by Hunziker,* who divides them as follows, according to the principle by which the anaërobiosis is brought about:

1. By the formation of a vacuum.
2. By the displacement of the air by inert gases.
3. By the absorption of the oxygen.
4. By the reduction of the oxygen.
5. By the exclusion of atmospheric air by means of various physical principles and mechanical devices.
6. By the combined application of any two or more of the above principles.

This classification makes such an excellent foundation for the description of the methods that it has been unhesitatingly adopted.

1. Withdrawal of the Air and the Formation of a Vacuum.—This method was first suggested by Pasteur and was later modified by Roux, Gruber, Zupinski, Novy, and others. It is now rarely employed. The appropriate container, whether a tube, flask, or some special device such as the Novy jar, receives the culture, and then has the air removed by a vacuum pump, the tube either being sealed in a flame or closed by a stop-cock.

2. Displacement of the Air by Inert Gases.—This method is decidedly preferable to the preceding, as it leaves no vacuum. It is easier to displace the oxygen than to withdraw it, and any apparatus permitting a combination of both features, as that designed by Ravenel,† from which the air can be sucked by a pump, to be later replaced by hydrogen, can be viewed with favor.

The most simple apparatus of the kind was suggested by Fränkel who inoculated a culture-tube of melted gelatin or agar-agar, solidified it upon the wall of the tube, as suggested by Esmarch, sub-

* "Journal of Applied Microscopy and Laboratory Methods," March, April and May, 1902; vol. v, Nos. 3, 4, and 5.

† "Bacteria of the Soil," "Memoirs of the National Academy of Sciences," First Memoir, 1896.

stituted for the cotton stopper a sterile rubber cork containing a long entrance and short exit tube of glass, passed hydrogen through the tube until the oxygen had been entirely removed, then sealed the ends in a flame. In this tube the growth of superficial and deep colonies can be observed. Hansen and Liborius constructed special

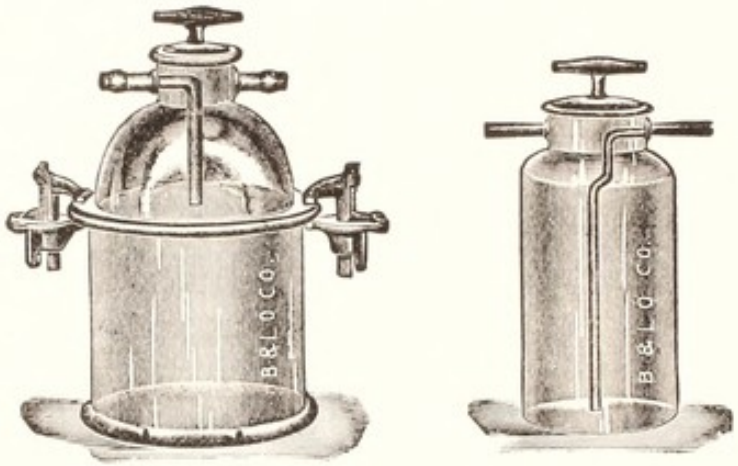


Fig. 62.—Novy's jars for anaërobic cultures.

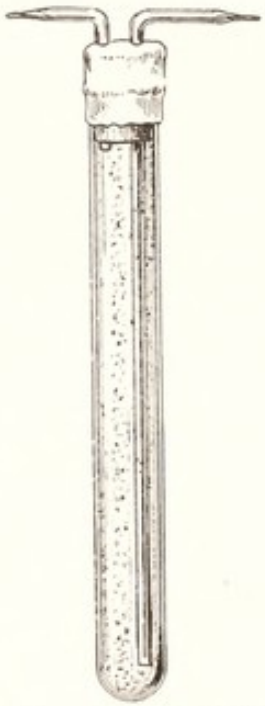


Fig. 63.—Fränkel's method of making anaërobic cultures.

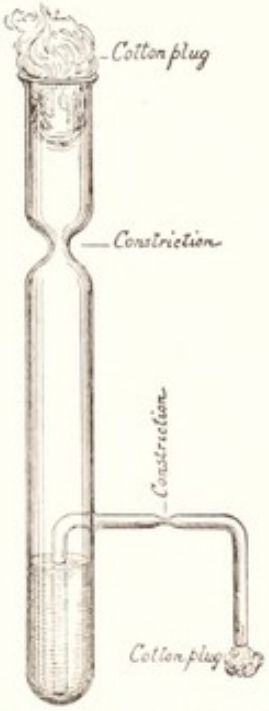


Fig. 64.—Liborius' tube for anaërobic cultures.

tubes by fusing a small glass tube into the wall of a culture-tube, and narrowing the upper part of the tube in a flame. After inoculation, hydrogen is passed into the small tube and permitted to escape through the mouth of the large tube until the air is entirely replaced, after which both tubes are sealed in a flame.

Instead of having a special apparatus for each culture, it is far

better to adapt the principle to some larger piece of apparatus that can contain a number of tubes or Petri dishes at a time. For this purpose the jar invented by Novy or the apparatus of Botkin can be used.

The Novy jar receives as many inoculated tubes as it will contain and has its stopper so replaced that the openings in the neck and stopper correspond. Hydrogen gas is passed through until the air is displaced. This usually takes several hours, as the cotton stoppers retain the air in the test-tubes and prevent rapid diffusion. When the air is all displaced, the stopper is turned so that the tubes are closed. If it be desired to expedite matters a pump can be used to withdraw the air, after which the hydrogen is permitted to enter.

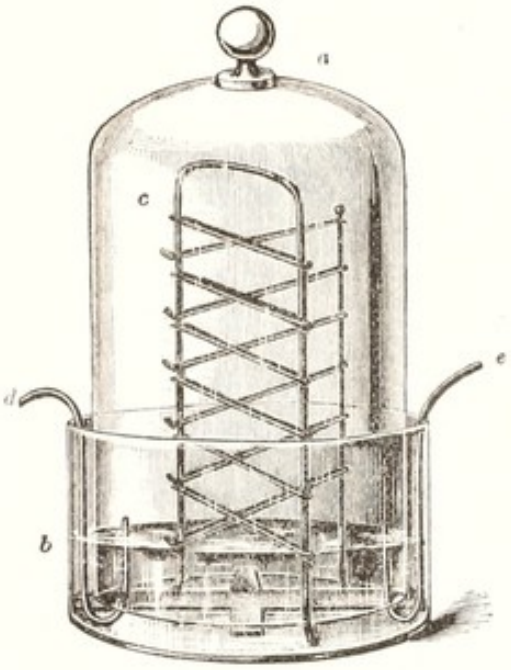


Fig. 65.—Botkin's apparatus for making anaërobic cultures.

Botkin's apparatus is intended for cultures in Petri dishes. It consists of three parts—a deep dish of glass (*b*), a stand to support the Petri dishes to be exposed (*c*), and a bell-glass (*a*) to cover the stand and fit inside of the dish. The prepared dishes are stood uncovered in the rack, which is then placed in the dish forming the bottom of the apparatus, and into which liquid paraffin is poured to a depth of about 2 inches. The bell-glass cover is now stood in place and hydrogen gas is conducted through previously arranged rubber tubes (*d*, *e*). As soon as the air is displaced through tube *d*, both tubes are withdrawn. It is well to place one Petri dish containing alkaline pyrogallic acid in the rack to absorb any oxygen not successfully displaced.

3. The Absorption of the Atmospheric Oxygen.—This method was first suggested by Buchner, whose idea was to absorb the atmospheric oxygen by alkaline pyrogallic acid and permit the bacteria to develop in the indifferent nitrogen. Various methods have been suggested for achieving this end, Buchner's own method consisting in the use of two tubes, a small one to contain the culture and a larger one to contain the absorbing fluid. A fresh solution of pyrogallic acid and sodium hydroxid were poured into the large tube, the smaller tube placed within it, upon some appropriate support, and the whole tightly corked.

Nichols and Schmitter,* at the suggestion of Carroll, have modified

* "Jour. of Medical Research," 1906, XV, p. 113.

the method by connecting the tube containing the inoculated culture medium with a U-shaped tube, to the other end of which is attached a tube to contain the pyrogallic acid solution. The apparatus will at once be understood by a glance at the cut. The mode of employing it is as follows: "After inoculating the culture-tube the plug is pushed in a little below the lips of the tube; the ends of the U tube and the test-tubes are coated externally with vaselin, the rubber tubes are adjusted on the U tube and a connection made with the culture-tube so that the glass ends meet. One or two grams of pyrogallic acid are put in the empty test-tube, and packed down with a little filter-paper over it; ten or twenty cubic centimeters, respectively, of a 10 per cent. solution of sodium hydroxide are then poured into the tube and the second connection made before the acid and alkali react to any extent."

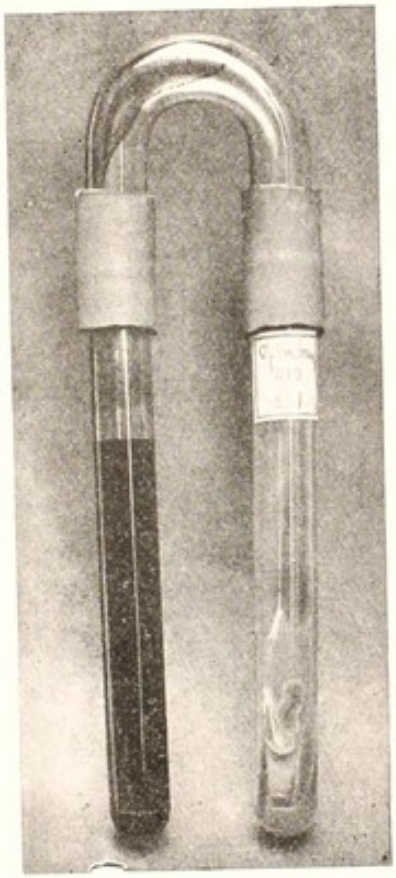


Fig. 66.—Spirillum rubrum. Glucose agar slant culture of five days. Abundant production of pigment on the surface. (The U tube was soiled by the reducing fluid during handling by the photographer.) (Nichols and Schmitter.)

Wright has suggested that the cotton stopper of the ordinary culture-tube have its projecting part cut off and the plug itself pushed down the tube for a short distance. Some alkaline pyrogallic acid solution is poured upon the cotton, to saturate it, and the tube tightly corked.

Zinsser* has recommended the following method as satisfactory for use with Petri dishes. The dishes selected should be rather deeper than ordinary. They are sterilized and inoculated in the ordinary manner and then inverted. The dish is cautiously raised, and some pyrogallic acid carefully poured into the lid and the dish gently dropped into place again. The alkaline solution is then poured into the crevice between the edges of the dish and the lid, and the remainder of the space filled with melted albolene. When these dishes are carefully stood away, the alkaline pyrogallic acid absorbs all of the contained oxygen and the anaërobic cultures develop quite well. The growing colonies can be examined as often as may be necessary through the bottom of the dishes, which must, of course, always be kept in the inverted position.

4. Reduction of Oxygen.—Pasteur and, later, Roux have recommended the cultivation of anaërobic bacteria in association with

* "Journal of Experimental Medicine," 1906, VIII, 542.

aërobic bacteria by which the oxygen was to be absorbed. This method is too crude to be employed at the present time, as it destroys the essential characteristics of the cultures by mixing the products of the bacteria.

Chemic reduction of the oxygen has been attempted by the addition of 2 per cent. of glucose, as suggested by Liborius, 0.3–0.5 per cent. of sodium formate, as suggested by Kitasato and Weil, 0.1 per cent. of sodium sulphate, suggested by the same authors, and various other chemicals. None of these additions has been sufficiently successful to merit continued favor, and at the present time this method is not employed.

5. Exclusion of Atmospheric Oxygen by Means of Various Physical Principles and Mechanical Devices.—This has appealed to the ingenuity of many experimenters, and many means of accomplishing it have been tried with success.

Fig. 67.—Buchner's method of making anaërobic cultures.

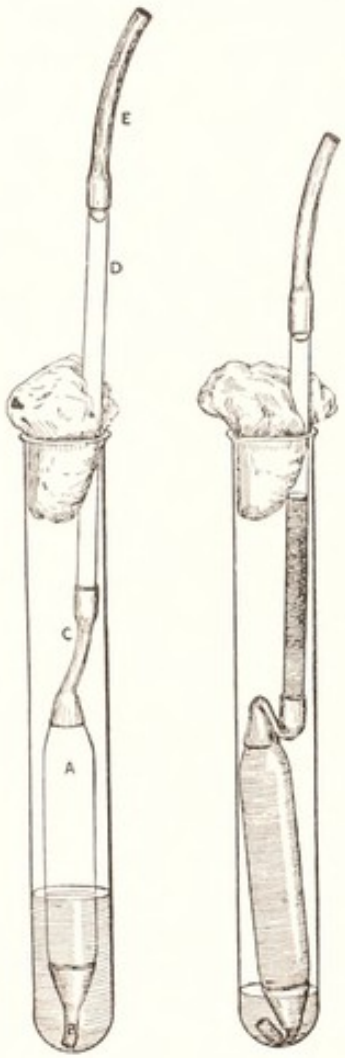
Fig. 68.—Hesse's method of making anaërobic cultures.

The most simple plan is that of Hesse, who made a deep puncture in recently boiled and rapidly cooled gelatin or agar-agar, then covered the surface of the medium with sterile oil. The so-called "shake culture" is another very simple method, suggested by Liborius and Hesse. The medium to be inoculated, contained in a well-filled tube or flask, is boiled to displace the contained air, cooled so as no longer to endanger the introduced bacteria, then inoculated, the inoculated bacteria being distributed by gently shaking. On cooling, the medium "sets," the organisms below the surface remaining under anaërobic conditions.

Kitasato first used paraffin as a covering for the inoculated medium,

his recommendation having recently been revived by Park and made successful for the cultivation of the tetanus bacillus. The paraffin floats upon the surface of the medium, melts during sterilization, but does not mix with it, and "sets" when cool. The inoculation is to be made while the culture medium is warm, after boiling and before the paraffin sets.

Koch studied the colonies of anaërobic organisms by cultivating them upon a film of gelatin covered by a thin sheet of sterilized mica, by which the air was excluded.



Figs. 69, 70.—Wright's method of making anaërobic cultures in fluid media (Mallory and Wright).

Salamonsen has made use of a pipet for making anaërobic cultures. It is made of a glass tube a few millimeters in diameter, drawn out to a point at each end. The inoculated gelatin or agar-agar is drawn in while liquefied and the ends sealed. The tube, of course, contains no air, and perfect anaërobiosis results.

Theobald Smith has found the fermentation-tube and various modifications of it excellently well adapted to the growth of anaërobes, which, of course, grow only in the closed limb.

Hens' eggs have been used for anaërobic cultures, and in them the tetanus bacillus grows remarkably well. Conditions of anaërobiosis are, however, not perfect, as can be shown by the behavior of the egg itself. If oxygen be completely shut out by oiling or varnishing the shell, a fertile egg will not develop.

A quite satisfactory and simple device for routine work with anaërobic organisms has been invented by Wright.* The essential feature consists of a pipet, D, with a rubber tube, E, at the end, and one interruption connected by a rubber tube, C. The device will be made clear at once by a glance at the accompanying illustration. The method of employment is very simple. An ordinary tube of bouillon or other fluid culture-media receives the pipet, the whole being sterilized, the cotton plug in place. The bouillon being inoculated with the culture or secretion to be studied is drawn up in the bulb of the pipet, A, by suction, until it passes the rubber interruption, C. By forcing the upper end of the pipet downward in the

* "Jour. Boston Soc. of Med. Sci.," Jan., 1900.

test-tube, a kink is given each rubber tube and the fluid contained in the bulbous part of the pipet becomes hermetically sealed.

In all cases where the presence of suspected micro-organisms is to be demonstrated, it is necessary to make both aërobic and anaërobic cultures. For routine work of this kind, this method of Wright is probably the most convenient yet suggested.

CHAPTER X

EXPERIMENTATION UPON ANIMALS

THE principal objects of medical bacteriology are to discover the cause, explain the symptoms, and bring about the cure and future prevention of disease. We cannot hope to achieve these objects without experimentation upon animals, in whose bodies the effects of bacteria and their products can be studied.

No one should more heartily condemn wanton cruelty to animals than the physician. Indeed, it is hard to imagine men, so much of whose life is spent in relieving pain, and who know so much about pain, being guilty of the butchery and torture accredited to them by a few of the laity, whose eyes, but not whose brains, have looked over the pages of text-books of physiology, and whose "philanthropy has thereby been transformed to zoölatry."

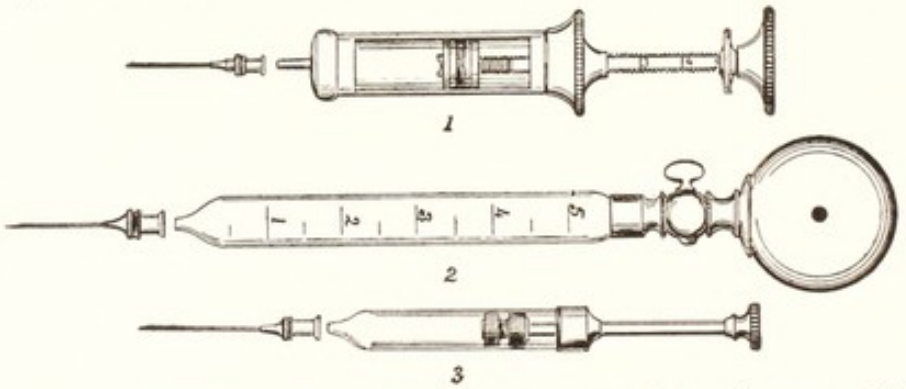


Fig. 71.—1, Roux's bacteriologic syringe; 2, Koch's syringe; 3, Meyer's bacteriologic syringe. Such syringes, because of their complexity and the destructible packings, give very unsatisfactory service and are no longer employed.

It is largely through experimentation upon animals that we have attained our knowledge of physiology, most of our important knowledge of therapeutics, and most of our knowledge of the infectious diseases. Without its aid we would still be without one of the greatest achievements of medicine, the "*blood serum therapy*."

Experiments upon animals, therefore, must be made, and, as the lower animals differ in their susceptibility to diseases, large numbers and different kinds of animals must be employed.

The bacteriologic methods are fortunately not cruel, the principal modes of introducing bacteria into the body being by subcutaneous, intraperitoneal, and intravenous injection.

Hypodermic syringes, expressly designed for bacteriologic work

are shown in the illustration. Those of Meyer and Roux resemble ordinary hypodermic syringes; that of Koch is supposed to possess

Fig. 72.—Altmann syringes for bacteriologic and hematologic work. These are capable of sterilization without injury and are thoroughly satisfactory.

Fig. 73.—Method of making an intravenous injection into a rabbit. Observe that the needle enters the posterior vein from the hairy surface.

the decided advantage of not having a piston to come into contact with the fluid to be injected. This is, however, really disadvantageous, inasmuch as the cushion of compressed air that drives out the

contents is elastic, and unless carefully watched will follow the injection into the body of the animal. In making subcutaneous injections there is no disadvantage or danger from the entrance of air, but in intravenous injections it is extremely dangerous.

Syringes with metal or glass pistons like those shown are to be preferred. All syringes should be disinfected by boiling thoroughly, *before* and *after* using. Syringes with packings to tighten the pistons cannot be boiled with impunity, as it soon ruins them, and new packings may be difficult to obtain or fit. Syringes of such design should be avoided.

The intravenous injection is easy to achieve in a large animal, like a horse, but is very difficult in animals smaller than a rabbit. Such injections, when given to rabbits, are usually made into the ear-veins, which are most conspicuous and accessible. A peculiar and important fact to remember is that the less conspicuous *posterior vein* of the ear is much better adapted to the purpose than the anterior. The introduction of the needle should be made from the *hairy external surface* of the ear where the vein is immediately beneath the skin.

If the ear be manipulated for a moment or two before the injection, vasomotor dilatation occurs and the blood-vessels become larger and more conspicuous. The vein should be compressed at the root of the ear until the needle is introduced, and the injection made as near the root as possible. The fluid should be injected slowly.

By using very fine needles, similar injections may be made into the ear veins of guinea-pigs. By dipping the tails of rats and even mice into warm water so as to cause dilatation of the caudal veins, it may be possible to effect intravenous injections of such animals. Kolmer suggests that the tails be vigorously rubbed with xylol or alcohol, and the epidermal cells softened and scraped off so as to expose the veins better. As the first attempt to get the needle into the caudal vein may fail, and new attempts be required, it is well to begin at a point not too near the body.

Bacteria can be introduced into the lymphatics only by injecting liquid cultures into some organ with comparatively few blood-vessels and large numbers of lymphatics. The testicle is best adapted to this purpose, the needle being introduced deeply into the organ.

Sometimes subcutaneous inoculations are made by introducing the platinum wire through a small opening made in the skin by a snip of the scissors. By this means solid cultures from agar-agar, etc., can be introduced.

Intra-abdominal and intrapleural injections are sometimes made, and in cases where it becomes necessary to determine the presence or absence of the bacilli of tuberculosis or glanders in fragments of tissue it may be necessary to introduce small pieces of the suspected tissue under the skin. To do this the hair is closely cut over the point of election, which is generally on the abdomen near the groin,

the skin picked up with forceps, a snip made through it, and the points of the scissors introduced for an inch or so and then separated. By

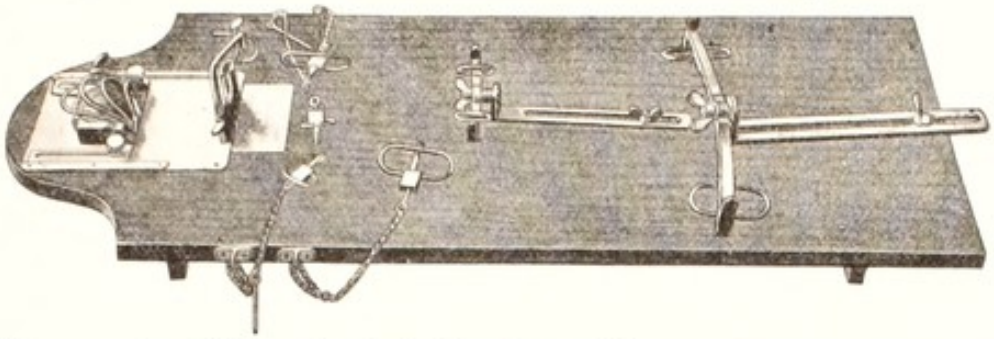


Fig. 74.—Latapie's animal holder for rabbits, guinea-pigs, and other small animals. This form of holder is in general use at the Institute Pasteur in Paris.

this manœuver a subcutaneous pocket is formed, into which the tissue is easily forced. The opening should not be large enough to require subsequent stitching.

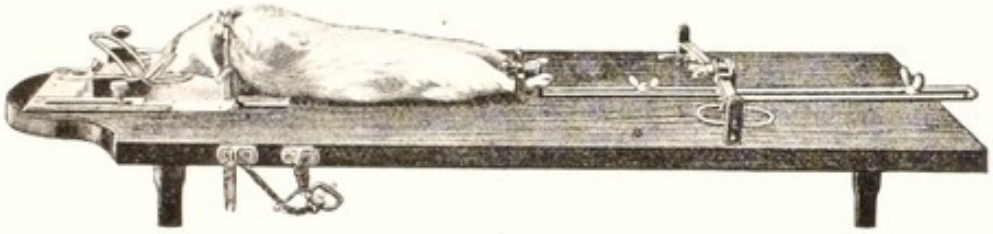


Fig. 75.—Guinea-pig confined in the holder.

When tissue fragments or collodion capsules are to be introduced into the abdominal cavity, the animal should be anesthetized and

Fig. 76.—Mouse-holder.

a formal laparotomy done, the wound being carefully stitched together.

When, in studying Pfeiffer's phenomenon and similar conditions, it is desirable occasionally to withdraw drops of fluid from the abdominal cavity, a small opening can be burned through with a blunt needle. This does not heal readily, and through it, from time to time, a capillary pipet can be introduced and the fluids withdrawn.

Small animals, such as rabbits and guinea-pigs, can be held in the hand, as a rule. Guinea-pig and rabbit-holders of various forms can be obtained from dealers in laboratory supplies. The best of these is undoubtedly that of Latapie, shown in the accompanying illustration. Dogs, cats, sheep, and goats can be tied and held in troughs. A convenient form of mouse-holder, invented by Kitasato, is shown in the figure.

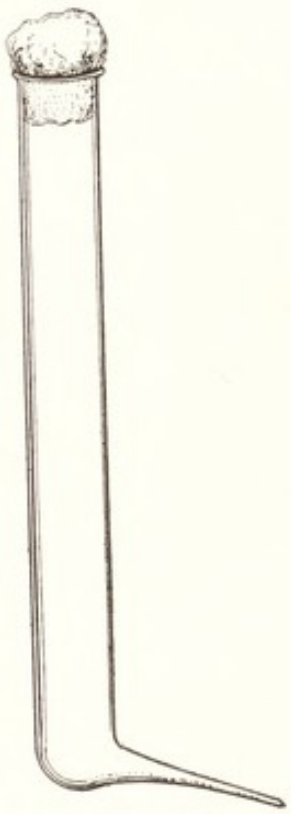


Fig. 77.—Tube for taking blood from the carotid artery of a rabbit or guinea-pig.

In all these experiments one must remember that the amount of material introduced into the animal must be in proportion to its size, and that injection experiments upon mice are usually so crude and destructive as to warrant the comparison drawn by Fränkel, that the injection of a few minims of liquid into the pleural cavity of a mouse is "much the same as if one would inject through a fire-hose three or four quarts of some liquid into the respiratory organs of a man."

Method of Securing Blood from Animals.—For many experimental purposes it becomes necessary to secure blood in larger or smaller quantities from animals. For horses, cattle, calves, goats, sheep, large dogs, etc., this is a simple matter, all that is necessary being to restrain the animal, make a minute incision in the skin over the jugular vein, which is easily found by compressing it at the root of the neck and noting where the vessel expands, and introducing a canula when the vein is well distended. The trocar being withdrawn, the blood at once flows. A sterile tube is slipped over the canula and the blood conducted into a sterile bottle or flask.

For rabbits and guinea-pigs the technic is rather more difficult because of the smaller size of the vessels. Drops and small quantities of blood may be secured by opening one of the ear veins, but when any quantity of blood is required, the neatest operation is done by tapping the common carotid artery by the method employed at the Pasteur Institute at Paris.

The animal is restrained in a Latapie holder, with the neck extended. Anesthesia can be used, but must be employed with great care. The hair on the front of the neck is clipped and the neck

shaved, or, as is easier, the hair is pulled out, leaving a clean surface an inch square. The skin is then washed with a disinfecting solution, an incision one and a half inches long made through the skin and superficial fascia in the middle line of the neck, the tissues carefully separated, the deep fascia cautiously opened, the tissues separated with the point of the forceps and a grooved director, the sheath of the vessels opened, and the artery completely separated from its surrounding tissues for a distance of at least an inch. A ligature is now tightly tied about the artery at the distal end of exposure, and a ligature placed in position and loosely looped ready to tie about the proximal end. A tube with a sharp lateral tubulature, as is shown in the illustration, is now made ready by breaking off

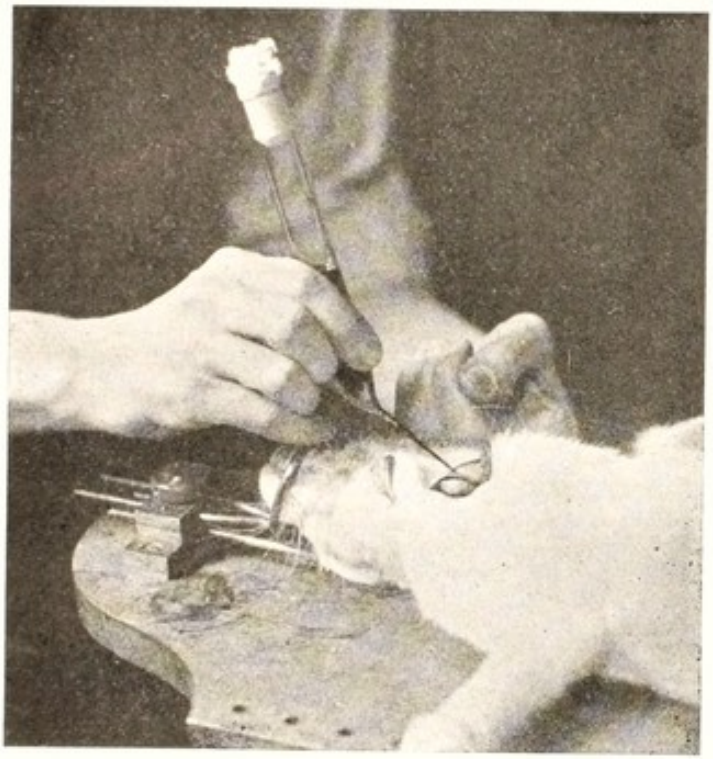


Fig. 78.—Showing the method of taking blood from the carotid artery of a rabbit.

the closed tip, the moistened forefinger of the operator is placed beneath the artery, and the sharp tube inserted (point toward the heart) into the artery, through whose walls it cuts its way easily. The moment the vessel is entered the blood-pressure drives the blood into the tube so that 20 cc. may be collected in about as many seconds. An assistant now ties the artery at its proximal end, the tube is withdrawn, holding it so that the blood does not escape, and the end sealed in a flame. The ends of the ligatures are now cut short and the external wound stitched. The wound usually heals at once, and if subsequent study of the blood is required, the other carotid and the femorals can be similarly employed for obtaining it.

Small quantities of blood (drops) can be secured from mice and rats by cutting off the tip of the tail, but to secure a large quantity

is difficult. One method that has been recommended is to tie the animal to a tray or board, on its back, anesthetize it, and, just before it dies, quickly open the thoracic cavity, and cut through the heart with scissors. The animal at once dies, the blood pouring out into the pleural cavities. After coagulation the serum can be secured by carefully pipetting it from the cavities.

Post-mortems.—Observation of experiment animals by no means ceases with their death. Indeed, he cannot be a bacteriologist who is not already a good pathologist and expert in the recognition of diseased organs.

When an autopsy is to be made upon a small animal, it is best to wash it for a few moments in a disinfecting solution, to kill the germs present upon the hair and skin, as well as to moisten the hair, which can then be much more easily kept out of the incision.

Small animals can be tacked to a board or tied, by cords fastened to the legs, to hooks soldered to the corners of an easily disinfected tray. The dissection should be made with sterile instruments. When a culture is to be made from the interior of an organ, its surface should first be seared with a hot iron, a puncture made into it with a sterile knife, and the culture made by introducing a platinum wire.

If the bacteriologic examination cannot be made at once, the organs to be studied should be removed with aseptic precautions, wrapped in a sterile towel or a towel wet with a disinfecting solution, and carried to the laboratory, where the surface is seared and the necessary incisions made with sterile instruments.

Fragments intended for subsequent microscopic examination should be cut into small cubes (of 1 cc.) and fixed in Zenker's fluid or absolute alcohol.

Collodion capsules are quite frequently employed for the purpose of cultivating bacteria in a confined position in the body of an animal, where they can freely receive and utilize the body-juices without being subjected to the action of the phagocytes. In such capsules the bacteria usually grow plentifully, and not rarely their virulence is increased.

The capsules can be made of any size, though they are probably most easily handled when of about 5–10 cc. capacity. The size is always an objection, because of the disturbance occasioned when they are introduced into the abdominal cavity.

The capsules are made by carefully coating the outside of the lower part of a test-tube with collodion until a sufficiently thick, homogeneous layer is formed. During the coating process the tube must be twirled alternately within and without the collodion, so that it is equally distributed upon its surface. When the desired thickness is attained, and the collodion is sufficiently firm, the tube is plunged under water and the hardening process checked.

A cut is next made around the upper edge of the collodion film, and it is removed by carefully turning it inside out. In this manner

an exact mold of the tube is formed. If a small opening be made at the end of the tube over which the sac is molded, and the tube filled with water after being properly coated with collodion, a small amount of pressure, applied by blowing gently into the tube, will force the water between the collodion and glass and so detach it without inversion. A test-tube of the same size is next constricted to a degree that will not interfere with the future introduction of culture-media in a fine pipet or inoculation with a platinum loop, and that will permit of ready sealing in a flame when necessary; the rounded end is cut off, and the edges are smoothed in a flame. The upper open end of the collodion bag is carefully fitted over the end of the tube, shrunk on by a gentle heating, and cemented fast with a little fresh collodion applied to the line of union. Novy recommends that a thread of silk be wound around the point of union, to hold the collodion in place and to aid in handiing the finished sac. The sac is next filled with distilled water up to the thread, the tube is plugged with cotton, and the whole placed in a larger test-tube containing distilled water, the cotton plug being packed tightly around the smaller tube, so that the collodion sac does not reach the bottom of the large tube, but hangs suspended in the water it contains. The whole is now carefully sterilized by steam.

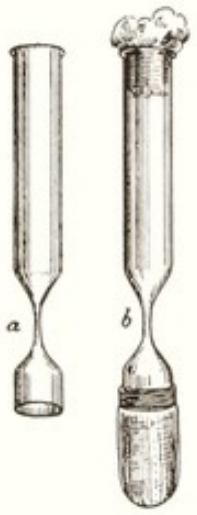


Fig. 79.—Preparation of collodion sacs: *a*, Test-tube constricted and cut; *b*, sac attached to the tube.

When ready for use, a tube of bouillon is inoculated with the culture intended to be placed in the animal, the water in the capsule is pipetted out and replaced by the inoculated bouillon carefully introduced with a pipet, the constricted portion is sealed in a flame, and the capsule picked up with forceps is introduced into the peritoneal cavity by an aseptic operation.

The collodion capsules may be made of any size. Those for rabbit experiments should be of about 10 cc. capacity, those for guinea-pig experiments about 5 cc. By coating large glass tubes they can be made of 500 cc. capacity, the large bags being useful for chemic dialysis.

CHAPTER XI

THE IDENTIFICATION OF SPECIES

THE most difficult thing in bacteriology is the identification of the species of bacteria that come under observation.

A few micro-organisms are characteristic in morphology and in their chemic and other products, and present no difficulty. Thus, the tubercle bacillus is characteristic in its reaction to the anilin dyes, and can usually be recognized by this peculiarity. Some, as Bacillus mycoides, have characteristic agar-agar growths. The red color of Bacillus prodigiosus and the blue of Bacillus janthinus speak almost positively for them. The potato cultures of Bacillus mesentericus fuscus and vulgatus are usually sufficient to enable us to recognize them. Unfortunately, however, there are several hundreds of described species that lack any one distinct characteristic that may be used for differential purposes, and require that for their recognition we shall well-nigh exhaust the bacteriologic technic.

Tables for the purpose have been compiled by Eisenberg, Migula, Lehman and Neumann, Chester, and others, and are indispensable to the worker. The most useful are probably the "Atlas and Grundriss der Bakteriologie und Lehrbuch der speziellen bakteriologischen Diagnostik," by Lehmann and Neumann,* and the "Manual of Determinative Bacteriology," by F. D. Chester (1901), from which, through the courtesy of the author and publisher, the following synopsis of groups is taken. Unfortunately, in tabulating bacteria we constantly meet species described so insufficiently as to make it impossible to properly classify and tabulate them.

The only way to determine a species is to study it thoroughly, step by step, and compare it with the description and tables. In this regard the differentiation of bacteria resembles the determination of the higher plants with the aid of a botanic key, or the qualitative analysis for the detection of unknown chemic compounds. Such a key for specific bacterial differentiation is really indispensable, even though it be imperfect, and every student engaged in research work should have one. As Chester says: "probably nine-tenths of the forms of bacteria already described might as well be forgotten or given a respectful burial. This will then leave comparatively few well-defined species to form the nuclei of groups in one or another of which we shall be able to place all new and sufficiently described forms." "That typical forms or species of bacteria do exist, no one can deny. These typical forms furthermore

* J. F. Lehmann, München, 1907.

STANDARD CHART FOR BACTERIAL ANALYSIS.

NAME ..... SOURCE ..... HABITAT ..... DATE ..... REPORTED BY .....

Form and arrangement in bouillon, grown ..... hours at 18°–20° C.; ditto, grown ..... hours at 36°–38° C.

Micrococcus, single, pairs, chains, tetrads, or cubical packets; *Bacillus*, single, pairs, chains, or filaments; *Spirillum*, comma, spiral.

Size, length ..... μ, breadth ..... μ; extreme lengths from ..... μ to ..... μ.

Capsules, none observed, easily observed or demonstrated. Conditions under which they are present, agar, serum, milk, or .....

Spores, none observed within ..... hours at .....° C. on ..... When present are polar, central, cells ..... swollen.

Germinate within ..... hours at .....° C. Stain by ..... method. Are killed at 100° C. in ..... minutes.

Vacuoles, observed when grown on ..... at .....° C., or when stained with .....

Motility, sluggish or active, rotary or direct, more pronounced in ..... cultures grown at .....° C. for ..... hours.

Flagella stain by ..... method; are monotrichous, lophotrichous, amphitrichous, peritrichous.

Pleomorphism, observed in ..... cultures grown at .....° C. for ..... days.

Stain, easily or with difficulty with ....., uniformly or irregularly. Stained or decolorized by Gram's method.

GELATIN OR AGAR PLATES.	Surface colonies.		Deep colonies.	
	Gelatin.	Agar.	Gelatin.	Agar.
Size.				
Shape.				
Margin.				
Textures.				
Color.				
Under mica plate.				

GELATIN OR AGAR-TUBE.

Puncture.

Form.
Surface growth.
Size.
Shape.
Margin.
Texture.
Color.
Consistence.

BOUILLON.

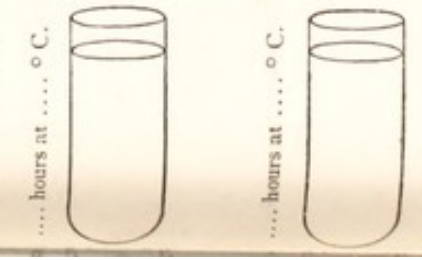
Opacity begins after .... hrs. at ....° C.
Pellicle forms in .... hrs. at ....° C.
Color .... appears in ... hrs. at ....° C.
Thickness.
Consistence.
Deposit forms in .... hrs. at ....° C.
Amount.
Color.
Character, compact, flocculent, granular, flaky, or viscid on agitation.
Color.
Odor.
Reaction, ... after ....hrs. at ....° C.

POTATO OR BLOOD-SERUM.

Size.
Shape.
Margin.

SKETCH OF GERM AND COLONY.

STAB CULTURES.



Surface relief.
Light transmission.
Color.
Luster.
Consistence.

Change in medium.

Color.
Consistence.
Odor.

Gas production.

NITRATE BROTH.

Reduction of nitrates.
Dextrose-free bouillon.
Indol production.

.... hours at ....

.... hours at ....

MILK.

Curd, curdles or does not curdle in .... days at 18°–20° C. or 36°–38° C.
" " " " " on boiling after .... days at ....° C.
hard or soft, in one mass or in fragments, gas-bubbles.
changed or unchanged by boiling.
Whey, separates from curd or not, amount, transparent or turbid.
Reaction, ... in ..... days at ....° C. *Color.* *Gas production.*
Digestion becomes gradually transparent without forming curds.
complete in .... days at ....° C.
effects of boiling, odor, clear or cloudy, watery or viscid.
reaction at end of digestion .....

SUGAR BOUILLON IN FERMENTATION-TUBES	Amount of gas in % formed at 18°–20° C.	Amount of gas in % formed at 36°–38° C.
	in 1 d., 2 ds., 3 ds., ... days.	in 1 d., 2 ds., 3 ds., ... days.
Dextrose	....	....
Lactose	....	....
Saccharose	....	....

Reaction of fluid in..... after....days at ....° C.	CO_2 %.	H %.	Pellicle.	Opacity.	Color.
.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	.....

PIGMENT,
developed in presence or absence of oxygen.
" in ..... cultures at .....° C. in ..... hours.
color ....., changed to ..... by acid or alkali.
soluble in ....., spectrum .....

PATHOGENESIS.

OPTIMUM TEMPERATURE ....° C. GROWTH LIMITS ....° C. to ....° C. THERMAL DEATH-POINT ....° C., time of exposure .... minutes.

PRODUCTION OF ACIDS OR ALKALIES.
Carbohydrates absent or present.

RELATION TO FREE OXYGEN.
Obligatory aërobe.
Facultative anaërobe.
Obligatory anaërobe.

RELATION OF GROWTH TO ACIDITY OR ALKALINITY OF MEDIUM.
.... % acid to .... % alkaline.

present certain definite morphologic, biologic, cultural, and perhaps pathogenic characters which establish the types independently of minor variations.

"The most marked of these types we select to become the centers of groups, around which are gathered all related species or varieties." "The division of the bacteria into groups, so far as grouping was possible, is outlined in the following tables:"

A PROPOSED SYNOPSIS OF GROUPS OF BACTERIA

Bacterium

I. Without endospores.
  A. Aërobic and facultative anaërobic.
    a. Gelatin not liquefied.
      * Decolorized by Gram's method.
        † Obligate aërobic. Acetic Ferment Group.
        †† Aërobic and facultative anaërobic.
          Gas generated in glucose bouillon.
            Gas generated in lactose bouillon. Bact. Aerogenes Group.
            Little or no gas generated in lactose bouillon. Friedländer Group.
          No gas generated in glucose bouillon.
            Milk coagulated. Fowl Cholera Group.
            Milk not coagulated. Swine Plague Group.
      ** Stained by Gram's method.
        † Gas generated in glucose bouillon. Lactic Ferment Group.
    b. Gelatin liquefied.
      * Colonies on gelatin ameboid or proteus-like. Bact. radiatum Group.
      ** Colonies on gelatin round, not ameboid. Bact. ambiguum Group.
II. Produce endospores.
  1. No growth at room temperature, or below 22°–25°C. Thermophilic Group.
  2. Grow at room temperatures.
    a. Gelatin liquefied. Anthrax Group.
    b. Gelatin not liquefied. Bact. fæcalis Group.

Bacillus

I. Without endospores.
  A. Aërobic and facultative anaërobic.
    a. Gelatin colonies roundish, not distinctly ameboid.
      * Gelatin not liquefied.
        † Decolorized by Gram's method.
          Gas generated in glucose bouillon.
            Milk coagulated. Colon Group.
            Milk not coagulated. Hog Cholera Group.
          No gas generated in glucose bouillon. Typhoid Group.
        †† Stained by Gram's method. B. muripestifer Group.
      ** Gelatin liquefied.
        † Gas generated in glucose bouillon. B. cloacæ Group.
        †† No gas generated in glucose bouillon. Include a large number of bacteria not sufficiently described to arrange in groups.
    b. Gelatin colonies ameboid, cochleate, or otherwise irregular.
      * Gelatin liquefied. Proteus vulgaris Group.
      ** Gelatin not liquefied. B. zopfi Group.
II. Produce endospores.
  A. Aërobic and facultative anaërobic.
    1. Rods not swollen at sporulation.
      a. Gelatin liquefied.

* Liquefaction of the gelatin takes place slowly. Ferment urea, with strong production of ammonia. URO-BACILLUS GROUP OF MIQUEL.

** Gelatin liquefied rather quickly.

† Potato cultures rugose. POTATO BACILLUS GROUP.

†† Potato cultures not distinctly rugose. B. SUBTILIS GROUP.

b. Gelatin not liquefied. B. SOLI GROUP.

2. Rods spindle-shaped at sporulation. B. LICHENIFORMIS GROUP.

3. Rods clavate at sporulation. B. SUBLANATUS GROUP.

B. Obligate anaërobic.

1. Rods not swollen at sporulation. MALIGNANT EDEMA GROUP.

2. Rods spindle-shaped at sporulation. CLOSTRIDIUM GROUP.

3. Rods clavate-capitate at sporulation. TETANUS GROUP.

PSEUDOMONAS (Migula)

I. Cells colorless, without a red-colored plasma and without sulphur granules.

A. Grow in ordinary culture-media.

1. Without endospores.

a. Aërobic and facultative anaërobic.

* Without pigment.

† Gelatin not liquefied.

Gas generated in glucose bouillon. PS. MONADIFORMIS GROUP.

No gas generated in glucose bouillon. PS. AMBIGUA GROUP.

†† Gelatin liquefied.

Gas generated in glucose bouillon. PS. COADUNATA GROUP.

No gas generated in glucose bouillon. PS. FAIRMONTENSIS GROUP.

* Produce pigment on gelatin or agar.

† Pigment yellowish.

Gelatin liquefied. PS. OCHRACEA GROUP.

Gelatin not liquefied. PS. TURCOSA GROUP.

†† Pigment blue-violet.

Gelatin liquefied. PS. JANTHINA GROUP.

Gelatin not liquefied. PS. BEROLINENSIS GROUP.

** Produce a greenish-bluish fluorescence in culture-media.

† Gelatin liquefied. PS. PYOCYANEA GROUP.

†† Gelatin not liquefied. PS. SYNCYANEA GROUP.

2. With endospores, aërobic and facultative anaërobic.

a. Non-chromogenic.

* Rods not swollen at sporulation. PS. ROSEA GROUP.

** Rods swollen at one end at sporulation. PS. TROMMELSCHLÄGER GROUP.

b. Produce a greenish-bluish fluorescence in culture-media.

* Gelatin liquefied. PS. VIRIDESCENS GROUP.

** Gelatin not liquefied. PS. UNDULATA GROUP.

B. Do not grow in nutrient gelatin or other organic media. NITRIMONAS GROUP.

II. Cell plasma with a reddish tint, also with sulphur granules. CHROMATIUM GROUP.

MICROSPIRA (Migula)

I. Cultures show a bluish-silvery phosphorescence. PHOSPHORESCENT GROUP.

II. Cultures not phosphorescent.

A. Gelatin liquefied.

1. Cultures show the nitro-indol reaction.

a. Very pathogenic to pigeons. MSP. METSCHNIKOVI GROUP.

b. Not distinctly pathogenic to pigeons. CHOLERA GROUP.

2. Nitro-indol reaction negative or very weak, at least after twenty-four hours. CHOLERA NOSTRAS GROUP.

B. Gelatin not liquefied or only slightly so. MSP. SAPROPHILA GROUP.

MYCOBACTERIUM (Lehmann-Neumann)

I. Stain with basic anilin dyes, and easily decolorized by mineral acids when stained with carbol-fuchsin.

A. Grow well on nutrient gelatin. Gelatin liquefied very slowly or merely softened.
1. Stain by Gram's method. SWINE ERYSIPELAS GROUP.
2. Not stained by Gram's method. GLANDERS GROUP.

B. Little or no growth in ordinary nutrient gelatin.
1. Grow well in nutrient bouillon at body temperatures.
a. Stained by Gram's method. Rods cuneate—clavate—irregularly swollen. DIPHTHERIA GROUP.
2. No growth in nutrient bouillon or on ordinary culture-media. Rods slender, tubercle-like.
a. Stain by Gram's method. LEPROSY GROUP.
b. Do not stain by Gram's method. INFLUENZA GROUP.
3. No growth in nutrient bouillon or on ordinary culture-media. Rods variable. ROOT-TUBERCLE GROUP.

II. Not stained with aqueous solutions of basic anilin dyes; not easily decolorized by acids. TUBERCLE GROUP.

COCCACEÆ

Cells in their free condition globular, becoming slightly elongated before division. Cell division in one, two, or three directions of space.

A. Cells without flagella.
1. Division in only one direction of space. *Streptococcus* (Billroth).
2. Division in two directions of space. *Micrococcus* (Hallier).
3. Division in three directions of space. *Sarcina* (Goodsir).

B. Cells with flagella.
1. Division in two directions of space. *Planococcus* (Migula).
2. Division in three directions of space. *Planosarcina* (Migula).

CHAPTER XII

THE BACTERIOLOGY OF THE AIR

MICRO-ORGANISMS are almost universally suspended in the dust of the air, their presence being a constant source of contamination in our bacteriologic researches and occasionally a menace to our health.

Such aërial organisms are neither ubiquitous nor uniformly disseminated, but are much more numerous where the air is polluted and dusty than where it is pure. The purity of the atmosphere bears a distinct relation to the purity of the surfaces over which its currents blow.

The micro-organisms of the air are for the most part harmless saprophytes taken up and carried about by the wind. They are almost always taken up from dry materials, experiment having shown that they arise from the surfaces of liquids with much difficulty. Not all the micro-organisms of the air are bacteria, and a plate of sterile gelatin exposed to the air for a brief time will generally grow molds and öidia as well.

In some cases the bacteria are pathogenic, especially where discharges from diseased animals have been allowed to collect and dry. On this account the atmosphere of hospital wards and of rooms in which infectious diseases are being treated is more apt to contain them than the air of the street. However, because of the expectoration from cases of tuberculosis, influenza, and pneumonia, which is often ejected upon the sidewalks and floors of public places, the presence of occasional pathogenic bacteria is far from uncommon in street-dust.

Günther points out that the greater number of the bacteria which occur in the air are cocci, sarcina being particularly abundant. Most of them are chromogenic and do not liquefy gelatin. It is unusual to find more than two or three varieties of bacteria at a time.

To determine whether bacteria are present in the air or not, all that is necessary is to expose a film of sterile gelatin on a plate or Petri dish to the air for a while, cover, and observe whether or not bacteria grow upon it.

To make a quantitative estimation is, however, more difficult. Several methods have been suggested, of which the most important may be briefly mentioned:

Hesse's method is simple and good. It consists in making a measured quantity of the air to be examined pass through a horizontal sterile glass tube about 70 cm. long and 3.5 cm. wide, the interior of which is coated with a film

of gelatin in the same manner as an Esmarch tube. The tube is closed at both ends with sterile corks carrying small glass tubes plugged with cotton. When ready for use the tube at one end is attached to a hand-pump, the cotton removed from the other end, and the air slowly passed through, the bacteria having time to sediment upon the gelatin as they pass. When the required amount has passed, the tubes are again plugged, the apparatus stood away for a time, and subsequently, when they have grown, the colonies are counted. The number of colonies in the tube will represent pretty accurately the number of bacteria in the volume of air that passed through the tube.

In such a tube, if the air pass through with proper slowness, the colonies will be much more numerous near the point of entrance than near that of exit. The first to fall will probably be those of heaviest specific gravity—*i. e.*, the molds.

Petri's Method.—A more exact method is that of Petri, who uses small filters of sand held in place in a wide glass tube by small wire nets. The sand used is made to pass through a sieve whose openings are of known size, is heated to incandescence, then arranged in the tube so that two of the little filters, held in place by their wire-gauze coverings, are superimposed. One or both ends

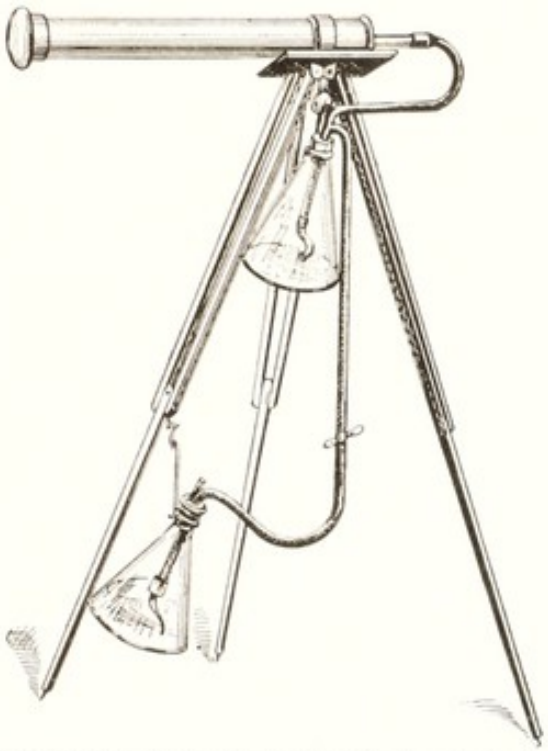


Fig. 80.—Hesse's apparatus for collecting bacteria from the air.

of the tube are closed with corks having a narrow glass tube. The apparatus is sterilized by hot air, and is then ready for use. The method of employment is very simple. By means of a hand-pump 100 liters of air are made to pass through the filter in from ten to twenty minutes, the contained micro-organisms being caught and retained by the sand. The sand from the upper filter is then carefully mixed with sterile melted gelatin and poured into sterile Petri dishes, where the colonies develop and can be counted. Petri points out in relation to his method that the filter catches a relatively greater number of bacteria in proportion to molds than the Hesse apparatus, which depends upon sedimentation. Sternberg points out that the chief objection to the method is the presence of the sand, which interferes with the recognition and counting of the colonies in the gelatin.

Sedgwick's Method.—Sedgwick and Miquel have recommended the use of a soluble material—granulated or pulverized sugar—instead of the sand. The apparatus used for the sugar experiments differs a little from the original of Petri, though the principle is the same, and can be modified to suit the experimenter.

A particularly useful form of apparatus, suggested by Sedgwick and Tucker, has an expansion above the filter, so that as soon as the sugar is dissolved in the

melted gelatin it can be rolled out into a film like that of an Esmarch tube. This cylindric expansion is divided into squares which make the counting of the colonies very easy.

Roughly, the number of germs in the atmosphere may be estimated at from 100 to 1000 per cubic meter.

The bacteriologic examination of air *is of very little importance* because of the numerous errors that must be met. Thus, when the air of a room is quiescent it may contain very few bacteria; let some

Fig. 81.—Petri's sand filter for air-examination.

Fig. 82.—Sedgwick and Tucker's expanded tube for air-examination.

one walk across the floor so that dust rises, and the number of bacteria becomes considerably increased; if the room be swept, the increase is enormous. From these and similar contingencies it becomes very difficult to know just when and how the air is to be examined, and the value of the results is correspondingly lessened.

The most sensible studies of the air aim rather at the discovery of some definite organism or organisms than at the determination of the total number per cubic meter.

CHAPTER XIII

BACTERIOLOGY OF WATER

Unless water has been specially sterilized, and received and kept in sterile vessels, it always contains some bacteria, the number usually bearing a distinct relationship to the quantity of organic matter present.

The majority of the water bacteria are bacilli, and are as a rule non-pathogenic. Wright,* in his examination of the bacteria of the water from the Schuylkill River, found two species of micrococci, two species of cladothrices, and forty-six species and two varieties of bacilli. Pathogenic bacteria, such as the spirillum of Asiatic cholera, the bacillus of typhoid fever, and the bacillus of dysentery may occur in polluted water, but are exceptional.

The method of determining the number of bacteria in water is very simple, and can be accomplished with very little apparatus. The method depends upon the equal distribution of a measured quantity

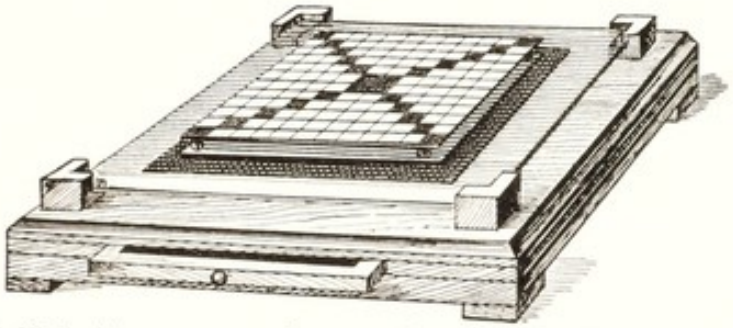


Fig. 83.—Wolfhügel's apparatus for counting colonies of bacteria upon plates.

of the water to be examined in some sterile liquefied medium, whose subsequent solidification in a thin layer permits the colonies to be counted.

The method originated with Koch, and may be performed with plates, Petri dishes, or Esmarch rolls. It is always best to make a number of cultures with different quantities of the water, using, for example, 0.01, 0.1, 0.5, and 1.0 cc., respectively, to a tube of liquefied gelatin, agar-agar, or glycerin agar-agar.

The details of the method depend upon the quality of the water to be examined. If the number of bacteria per cubic centimeter be small, large quantities may be used; but if there be millions of bacteria in every cubic centimeter, it may be necessary to dilute the water to be examined in the proportion of 1 : 10 or 1 : 100 with sterile water, mixing well, and making the plate cultures from the dilutions.

* "Memoirs of the National Academy of Sciences," vol. VII, Third Memoir.

It is best to count all the colonies developed upon the culture, if possible; but when hundreds of thousands are scattered over it, an estimate made by counting and averaging the number in each of the small squares of some counting apparatus, such as those devised by Wolfhügel, Esmarch, or Frost. In counting the colonies a lens is indispensable.

In some cases, where bacteria are exceedingly numerous, as badly contaminated waters, in the study of sewage, in inflammatory exudates, and in cultures intended for the preparation of bacterial vaccines, it is expedient to directly enumerate the bacteria without resorting to the cultivation method, where all of the organisms may not grow.

Fig. 84.—Esmarch's instrument for counting colonies of bacteria in Esmarch tubes.

Excellent methods for the computation of bacteria have been devised. That of Winslow and Willcomb* being as follows:

"The cover-slips should be boiled in a 10 per cent. solution of potassium bichromate in 50 per cent. sulphuric acid and allowed to lie in this cleansing mixture. Just before using they may be rinsed in 50 per cent. alcohol and dried on a silk cloth, not in the flame. One-twentieth of a cubic centimeter of water placed on such a cover-slip spreads evenly and should be allowed to dry in the air without sudden heating. After drying it is fixed by passing through the flame, covered with Ziehl-Neelson's carbol-fuchsin, warmed until steam just rises, washed, dried, and mounted. For counting the bacteria we use a Sedgwick-Rafter eye-piece micrometer, made for the study of the larger micro-organisms in drinking water." Very uniform results have followed.

The method of Wright† was devised for the computation of bacteria in suspensions used in making tests of the opsonic power of the blood and is given in the chapter upon "The Opsonic Index."

The majority of the water bacteria rapidly liquefy gelatin, on which account it is better to employ both gelatin and agar-agar in making the cultures.

In ordinary city hydrant-water the bacteria number from 2 to 50 per cubic centimeter; in good pump-water, 100 to 500; in filtered water from rivers, according to Günther, 50 to 200; in unfiltered river-water, 6000 to 20,000. According to the pollution of the water the number may reach as many as 50,000,000.

The waters of wells and springs are dependent for their purity upon the character of the earth or rock through which they filter, and the waters of deep wells are much more pure than those of shallow wells, unless contamination take place from the surface of the ground.

* "Jour. of Infectious Diseases," Supplement, May, 1905, No. 1, p. 273.
† "Lancet," July 5, 1902.

Ice always contains bacteria if the water contained them before it was frozen. In Hudson River ice Prudden found an average of 398 colonies in a cubic centimeter.

A sample of water when collected for examination should be placed in a clean sterile bottle or in a hermetically sealed pre-sterilized glass bulb, and must be examined as soon as possible, as the bacteria multiply rapidly in water which is allowed to stand for a short time. If the water to be examined must be transported any considerable distance before the manipulations are performed, it should be packed in ice. The greatest care must always be exercised that the unnatural conditions arising from the bottling of the water, the changes of temperature, and the altered relationship to light and the atmosphere, do not modify the number of contained bacteria.

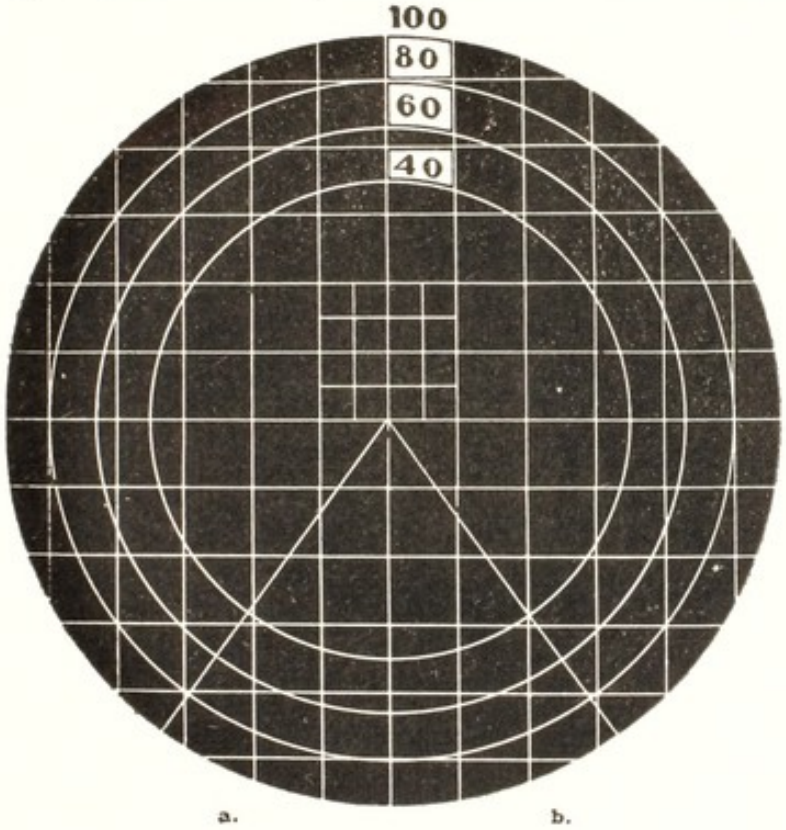


Fig. 85.—Frost's plate counter, for counting colonies of bacteria on Petri dish or plate cultures. The cross-lines divide the figure into square centimeters. The numbers at the top of the figure indicate the area in centimeters of the various discs. The area of each sector (a and b) is one-tenth of the whole area.

The detection of such important bacteria as the colon, typhoid and dysentery bacilli, and the cholera spirillum, will be considered in the chapters treating of those respective organisms.

Drinking-water, especially that furnished to large cities, is not infrequently contaminated with sewage, and contains intestinal bacteria—Bacillus coli. For the ready determination of this organism, which is an important indication that the water is polluted, Smith* has made use of the fermentation-tube in addition to the

* "Amer. Jour. Med. Sci.," 1895, cx, p. 301.

CLASSIFICATION OF BACILLI FOUND IN OHIO RIVER WATER AT CINCINNATI, OHIO.

Name of Organism.	First Investigator.	Morphology.				Biology.																								Pathogenesis
						Cultural Features.										Biochemical Features.														
		Bacillus.	Diameter Greater than 1 μ.	Motile.	Spores.	Scum.	Nutrient Broth Tube. Turbid.	Dull.	Nutrient Agar Tube. Wrinkled.	Character-istic Appearance.	Gelatin Plate. Visible.	Luxuriant.	Potato Tube. Growth in Closed Arm.	Fermentation Tube.	Grows at Body Temperature.	Facultative Anaerobe.	Affected by Range of Reaction.	Gelatin.	Liquefaction. Casein.	Blood-serum.	Gas Production. Dextrose Broth.	Nitrate Reduced.	Indol Produced.	Milk Coagulated.	Fecal Odor.	Nutrient Agar Tube. Chromogenesis.	Fluorescence.	White Mice. Intraperitoneal Inoculation Fatal.			
Group I. Fluorescent Type.																														
B. fluorescens liquefaciens	Flügge	+	—	+	—	+	+	—	—	+	+	—	—	—	—	—	+	+	+	+	—	+	—	—	—	+	—			
B. fluorescens non-liquefaciens	Eisenberg	+	—	—	—	—	+	—	—	—	+	—	—	—	+	—	+	—	—	—	—	—	—	—	—	+	—			
B. viridis	Lesage	+	—	+	—	+	+	—	—	+	+	+	+	+	+	+	+	+	+	+	—	+	—	+	—	+	+			
B. fluorescens ovalis	Ravenel	+	—	+	—	+	+	—	—	+	+	+	+	+	+	+	+	+	+	+	—	+	—	+	—	+	+			
B. pyocyaneus	Gessard	+	—	+	—	+	+	—	—	+	+	+	+	+	+	+	+	+	+	+	+	+	—	+	—	+	+			
B. fluorescens incognito	Wright	+	—	+	—	+	+	—	—	—	+	+	+	—	+	+	+	—	—	—	—	—	—	—	—	+	+			
Group II. Chromogenic Type—Red.																														
B. prodigiosus	Ehrenberg	+	—	+	—	+	+	—	—	+	+	+	+	+	+	+	+	+	+	+	+	+	—	+	—	Crimson	—			
B. rubidus	Eisenberg	+	—	+	—	+	+	—	—	+	+	—	+	—	—	+	+	+	—	—	—	—	—	—	—	Brick Red	—			
Group III. Chromogenic Type—Orange.																														
B. arborescens	Frankland	+	—	+	—	—	+	—	—	+	+	+	—	—	—	+	+	+	—	—	—	—	—	+	—	Orange	—			
B. aurescens	Ravenel	+	—	+	—	—	+	—	—	—	+	—	—	—	—	+	+	+	+	+	—	—	—	+	—	"	—			
B. fulvus	Zimmermann	+	—	—	—	—	+	+	—	+	+	—	+	—	—	+	+	+	—	—	—	—	—	+	—	"	—			
B. fuscus	Zimmermann	+	—	—	—	—	+	—	—	—	+	—	+	—	—	+	+	+	—	—	—	—	—	+	—	"	—			
B. aurantiacus	Frankland	+	—	+	—	—	+	—	—	+	+	—	—	—	—	—	+	+	—	—	—	+	—	—	—	"	—			
Group IV. Chromogenic Type—Yellow.																														
B. desidious	Wright	+	—	—	—	—	+	+	—	—	+	+	—	—	—	—	+	+	+	+	—	—	—	+	—	Yellow	—			
B. ochraceus	Zimmermann	+	+	+	—	+	—	—	—	+	+	—	—	—	+	—	+	+	+	+	—	+	—	+	—	"	—			

| | | |

plate. His method is to add to each of several fermentation-tubes containing 1 per cent. dextrose-bouillon a certain quantity of water. The evolution of 50–60 per cent. of gas by the third day is a strong indication that the colon bacillus is present. The presence of gas in a fermentation tube constitutes the "presumptive test" for the colon bacillus. It is not an infallible indication of their presence. A careful study of its usefulness has been made by Ruediger and Slyfield* who found that in making quantitative determination of B. coli in polluted waters by means of the fermentation tubes, the most accurate results were obtained by the use of neutral-red lactose bouillon. Gas appeared earlier in the neutral-red lactose tubes than in lactose bile broth tubes, and B. coli were more easily isolated, by plating, from the former than from the latter. The finding of B. coli in the fermentation tubes is greatly facilitated by making plates soon after the appearance of the gas. When the fermentation of the sugar and the appearance of gas in the tube occurs, some bacteriologists are satisfied that B. coli are present, and go no further, but a careful workman will always take pains to confirm the indications of their presence by plating and isolating the bacillus in pure culture.

It was at one time thought that the occurrence of the colon bacillus in water was sufficient to condemn its potability, but the evidence accumulated in recent years, showing that this organism may reach streams from manured soil, may enter it with the dejecta of domestic animals, wild animals, birds, and perhaps even of fishes, makes it doubtful whether anything but an exceptionally large number of the organisms should be looked upon as indicative of sewage pollution and proof that the water is not potable.

In determining the species of bacteria found in the water reference must be made to the numerous monographs upon the subject and to special tables. An excellent table of this kind, arranged by Fuller,† is given on pages 240 and 241.

Filtration with sand, etc., diminishes the number of bacteria for a time, but, as the organisms multiply in the filter, the benefit is not permanent and the filters must frequently be subjected to bacteriologic tests and the sand washed, spread out to dry and the filters renewed. Porcelain filters seem to be the only positive safeguard, and even these, the best of which seems to be the Pasteur-Chamberland, allow the bacteria to pass through if used too long without proper attention.

For those whose special line of work is the bacteriology of water, the report of the Committee on Standard Methods of Water Analysis to the Laboratory Section of the American Public Health Association, published in Supplement No. 1 of the "Journal of Infectious Diseases," May, 1905, will prove indispensable.

*Jour. of the American Public Health Association, 1911, 1, No. 11, p. 828.
†"Public Health and Journal of Experimental Medicine."

CHAPTER XIV

BACTERIOLOGY OF THE SOIL

The upper layers of the soil contain bacteria in proportion to their richness in organic matter. Near the habitations of men, where the soil is cultivated, the excrement of animals, largely made up of bacteria, is spread upon it to increase its fertility, this treatment not only adding new bacteria to those already present, but also enabling those present to grow much more luxuriantly because of the increased nourishment they receive.

Where, as in Japan, human excrement is used to fertilize the soil, or as in India, it is carelessly deposited upon the ground, bacteria of cholera, dystentery, and typhoid fever are apt to become disseminated by fresh vegetables, or through water into which the soil drains. In such localities fresh vegetables should not be eaten, and water for drinking should be boiled.

The researches of Flügge, C. Fränkel, and others show that the bacteria of the soil do not penetrate deeply, but gradually decrease in number until the depth of a meter is reached, then rapidly diminish until at a meter and a quarter they rather abruptly disappear.

The bacteria of soil are, for the most part, harmless saprophytes, though a few highly pathogenic organisms, such as the bacilli of tetanus and malignant edema, occur. Many of them are anaërobic, and it is interesting to speculate upon their biology. Whether they develop and multiply in the soil in intimate association with strongly aërobic organisms by which the free oxygen is aborbed, or whether they remain latent in the soil and develop only in the intestines of animals, is not known.

The estimation of the number of bacteria in the soil seems to be devoid of any practical importance. C. Fränkel has, however, originated an accurate method of determining it. By means of a special boring apparatus earth can be secured from any depth without digging and without danger of mixing with that of the superficial strata. A measured quantity of the secured soil is thoroughly mixed with liquefied sterile gelatin and poured into a Petri dish or solidified upon the walls of an Esmarch tube. The colonies are counted with the aid of a lens. Flügge found in virgin earth about 100,000 colonies in a cubic centimeter.

Samples of earth, like samples of water, should be examined as soon as possible after being secured, for, as Günther points out, the number of bacteria changes because of the unusual dryness, warmth, exposure to oxygen, etc.

The most important bacteria of the soil are those of tetanus and

malignant edema, in addition to which, however, there are a great variety of organisms pathogenic for rabbits, guinea-pigs, and mice.

In the "Bacteriological Examination of the Soil of Philadelphia," Ravenel* came to the conclusion that—

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

2. They furnish conditions more suited to the multiplication of bacteria than do virgin soils, unless the latter are contaminated by sewage or offal.

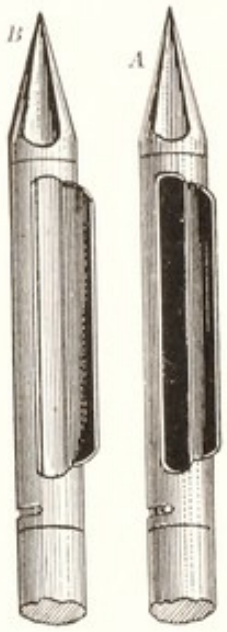


Fig. 86.—Tip of Fränkel's instrument for obtaining earth from various depths for bacteriologic study. *B* shows the instrument with its cavity closed, as it appears during boring; *A*, open, as it appears when twisted in the other direction to collect the earth.

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

In seventy-one cultures that were isolated and carefully studied by Ravenel, there were two cocci, one sarcina, and five cladothrices; all the others were bacilli.

* "Memoirs of the National Academy of Sciences," First Memoir, 1896.

CHAPTER XV

THE BACTERIOLOGY OF FOODS

The relation of bacteria to foods is an important one and should be as thoroughly understood as possible by both the profession and the laity. The relationship may be expressed thus:

I. Foods serve as vehicles by which infectious agents are conveyed to the body.

II. Foods are chemically changed and made unfit for use by the bacteria.

I. Foods as Fomites.—In animal food the first source of infection is the animal itself, danger of infection always accompanying the employment of foods derived from diseased animals. Thus, milk apparently normal in appearance has been found to contain dangerous pathogenic bacteria. The tubercle bacillus is one of the most important of these, and at the present time the consensus of opinion inclines toward the view that the great prevalence of tuberculosis among human beings depends partly upon the ingestion of tubercle bacilli in milk. It does not appear necessary that the udder of the cow be diseased in order that the organisms enter the milk, as they seem to have been found in milks derived from cows whose udders were entirely free from demonstrable tuberculosis. It is, therefore, imperative to retain only healthy cows in the dairy, and careful legislation should provide for the detection and destruction of all tuberculous animals. The detection of tubercle bacilli in milk can only be certainly accomplished by the injection of a few cubic centimeters of the fluid into guinea-pigs and noting the results.

In addition to the tubercle bacillus, pyogenic streptococci have been observed in enormous quantities and almost pure culture in milk drawn from cows suffering from mastitis. Stokes* has observed a remarkable case of this kind in which the milk contained so much pus that it floated upon the top like cream. Such seriously infected milk could not be used with safety to the consumer.

In market milk one occasionally finds pathogenic organisms, such as the diphtheria bacillus, typhoid bacillus, streptococcus, etc., derived from human sources. Such polluted milks have been known to spread epidemics of the respective diseases whose micro-organisms are present. Bacteria may enter milk from careless handling, from water used to wash the cans or to dilute the milk, or from dust; and as milk is an excellent medium for the growth of bacteria, it should

*"Maryland Medical Journal," Jan. 9, 1897.

always be treated with the greatest care to prevent such contamination, as saprophytic bacteria produce chemical changes in the milk, such as acidity and coagulation, which destroy its usefulness or render it dangerous as food for infants and invalids. Where the necessary precautions are not or cannot be taken, Pasteurization of the milk as soon after its reception as possible may act as a safeguard.

The student interested in the sanitary relations of milk cannot do better than refer to Bulletin No. 35 of the Hygienic Laboratory, Washington, D. C., 1907, "Upon the Origin and Prevalence of Typhoid Fever in the District of Columbia," and to Bulletin No. 41 of the same laboratory, upon "Milk and its Relation to the Public Health" (1908); also to the "Bacteriology of Milk," by Swithinbank and Neuman, New York, E. P. Dutton & Co., 1903.

Meat from tuberculous animals might cause disease if eaten raw or but partially cooked. As cooking suffices to kill the organisms, the danger under ordinary conditions is not great. Moreover, tuberculosis rarely affects the muscles, the parts usually eaten.

Butter made from cream derived from tuberculous milk may also contain tubercle bacilli, as has been shown by the researches of Rabinowitsch.*

Foods may become polluted with bacteria in a variety of ways that will suggest themselves to the reader. The common source is dust, which is more or less rich in bacteria according to the soil from which it arises. The readiness with which raw foods, such as meats, milk, etc., can be thus contaminated in the barnyard, dairy, slaughter-house, and shop, teaches but one lesson—that the greatest cleanliness should prevail for the sake of the dealer, whose goods may be spoiled by carelessness, and the consumer, who may be injured by the food.

Any food may carry infectious organisms upon its surface, such organisms being derived from the hands of the dealer, from dust, from water, as when green vegetables are sprinkled with impure water to keep them fresh, or from other sources.

The cleanliness of the merchant and the protection from contamination that he bestows upon his goods should be taken into consideration by his customers.

Shell-fish, especially oysters, seem to be common carriers of infection, especially of typhoid fever. The oysters seem to be contaminated with infected sewage carried to their beds. It is not yet satisfactorily determined whether typhoid bacilli multiply in the juices in the shells of the oysters or not, but a number of epidemics of typhoid fever have been very conclusively traced to the consumption of certain oysters at a definite time and place. As cooking the oysters will kill the contained bacilli, the prophylaxis of disease in this case is very simple.

* "Deutsche med. Wochenschrift," 1900, No. 26; abstract in the "Centralbl. f. Bakt.," etc., 1901, XXIX, p. 309.

II. Food Poisons.—The nomenclature, suggested by Vaughan and Novy,* contains the following terms:

Bromatotoxism—food-poisoning;
Galactotoxism—milk-poisoning;
Tyrotoxism—cheese-poisoning;
Kreotoxism—meat-poisoning;
Ichthyotoxism—fish-poisoning;
Mytilotoxism—mussel-poisoning;
Sitotoxism—cereal-poisoning.

The most important chemic alterations effected by bacteria occur in milk and meat.

1. **Milk-poisoning** (*Galactotoxism*).—Milk, even when freshly drawn from the cow, always contains some bacteria, whose numbers gradually diminish for a few hours, then rapidly increase until almost beyond belief. These organisms are for the most part harmless to the consumer, but ultimately ruin the milk. Although much attention has been paid to the subject, bacteriologists are not agreed whether the number of bacteria contained in milk is a satisfactory guide as to its harmfulness.

The poisonous change in milk, cream, ice-cream, etc., has been shown by Vaughan to depend in part upon the presence of a ptomain known as *tyrotoxicon*, formed by the growth of bacteria in the milk, but whether by any particular bacterium is not known. The milk may become poisonous during any time of the year, but chiefly in the summer, when, because of the higher temperature, bacteria develop most rapidly. The change takes place in stale milk, and it is supposed that many cases of what was formerly looked upon as "summer complaint" in infants were really poisoning by this toxic ptomain.

Ice-cream poisoning depends upon the growth of the bacteria in the milk before it is frozen. In some cases the error made has been to prepare the cream for freezing and then keep or transport it, the freezing operation being delayed until the development of the bacteria has led to the poisonous condition.

Cheese-poisoning (*Tyrotoxism*) is also thought to depend upon tyrotoxicon at times, though it has been shown that other cheese poisons exist. It is more or less a question whether cases of milk- and cheese-poisoning do not depend upon the toxic products of the colon bacillus growing in the foods.

2. **Meat-poisoning** (*Kreotoxism*).—Botulism or meat-poisoning depends upon the growth of certain bacteria, Bacillus botulinus of van Ermengem,† in the meat. The symptoms following infection by the organism sometimes closely resemble those of typhoid fever, and are characterized by acute gastro-intestinal irritation, nervous

* "Cellular Toxins," Phila., 1902.
† "Zeitschrift für Hygiene," Bd. XXVI, Heft 1.

disturbances, and, in case of death, by fatty degenerations in the organs and minute interstitial hemorrhages.

3. **Fish-poisoning** (*Ichthyotoxism*) sometimes follows the consumption of canned and presumably spoiled fish, sometimes the consumption of diseased fish. It is not known whether it depends upon ptomains or upon toxicogenic germs, though probably the latter, as Silber has isolated a Bacillus piscicidus that is highly toxicogenic.

4. **Mussel-poisoning** (*Mytilotoxism*) depends partly upon irritating and nervous poisons in the mussel substance, in part upon toxicogenic germs that they harbor.

5. **Canned Goods.**—Improperly preserved canned goods not infrequently spoil because of the growth of bacteria, but the occurrence of gas-formation, acidity, insipidity, etc., causes rejection of the product, and but few cases of supposed poisoning from canned goods can be authenticated.

CHAPTER XVI

THE DETERMINATION OF THE THERMAL DEATH-POINT OF BACTERIA

SEVERAL methods may be employed for this purpose. Roughly, it may be done by keeping a bouillon culture of the micro-organism to be investigated in a water-bath whose temperature is gradually increased, transplantations being made from time to time until the fatal temperature is reached.

It is economy to make the transplantations less frequently at first than later in the experiment, when the ascending temperature approaches a height dangerous to life. In ordinary determinations it is well to make a transfer at 40°C., another at 45°, another at 50°, still another at 55°, and then, beginning at 60°, make one for every additional degree. The day following the experiment it will be observed that all the cultures grow except those heated beyond a certain point, say 62°C., when it can properly be concluded that 62°C. is the thermal death-point. If all the transplantations grow, of course the maximum temperature was not reached, and the experiment must be repeated and the bacteria exposed to still higher temperatures.

When more accurate information is desired, and one wishes to know how long the micro-organism can endure some such temperature as 60°C. without losing its vitality, a dozen or more bouillon-tubes may be inoculated with the organism to be studied, and stood in a water-bath kept at the temperature to be investigated. The first can be removed as soon as it is heated through, another in five minutes, another in ten minutes, or at whatever intervals the thought and experience of the experimenter shall suggest, the subsequent growth in each culture showing that the endurance of the organism had not yet been exhausted. By using gelatin, pouring each culture into a Petri dish, and subsequently counting the colonies, it can be determined whether many or only a few of the organisms in a culture possess the maximum resisting power. To determine the percentage, it is necessary to know how many bacteria were present in the tubes before exposure to the destructive temperature. Approximately the same number can be placed in each tube by adding the same measured quantity of a fluid culture to each.

In both of the procedures one must be careful that the temperature of the fluid in the test-tube is identical with that of the water in the bath. A sterile thermometer introduced into an uninoculated tube

exposed under conditions similar to those of the experiment can be used as an index for the others.

Another method of accomplishing the same end is by the use of Sternberg's bulbs. These are small glass bulbs blown on one end of a glass tube, drawn out to a fine point at the opposite end. If such a bulb be heated so that the air is expanded and partly driven out, its open tube, dipped into inoculated bouillon, will in cooling draw the fluid in, so as to fill it one-third or one-half. A number of these tubes are filled in this manner with a freshly inoculated culture medium and then floated, tube upward, upon a water-bath whose temperature is gradually elevated, the bulbs being removed from time to time as the required temperatures are reached. As the bulbs are already inoculated, all that is necessary is to stand them aside for a day or two, and observe whether or not the bacteria grow, determining the death-point exactly as in the other case.

CHAPTER XVII

DETERMINATION OF THE VALUE OF ANTISEPTICS, GERMICIDES, AND DISINFECTANTS

THE student must bear in mind that an antiseptic is a substance capable of restraining the growth of bacteria; a germicide, one capable of killing them. All germicides are antiseptic in dilute solutions, but not all antiseptics are germicides. Disinfectants must be germicides.

Antiseptics are chiefly employed for purposes of preservation, and are largely used in the industries to protect organic substances from the micro-organisms of fermentation and decomposition. The problem is to secure a satisfactory effect with the addition of the least possible preservative in order that its presence shall not chemically destroy the good qualities of the substances preserved. In the case of foods it becomes necessary to use preservatives free from poisonous properties.

Disinfectants and germicides are employed for the purpose of destroying germs of all kinds, and the chief problem is to secure efficiency of action, rather than to endeavor to save on the reagent, which would be a false economy, in that the very object desired might be defeated.

The following methods of determining the antiseptic and germicidal values of various agents can be elaborated according to the extent and thoroughness of the investigation to be made.

I. The Antiseptic Value.—Remembering that an antiseptic is a substance that inhibits bacterial growth, the determination of its value can be made by adding varying quantities of the antiseptic to be investigated to culture-media in which bacteria are subsequently planted. It is always well to use a considerable number of tubes of bouillon containing varying strengths of the reagent to be investigated. If the antiseptic be non-volatile, it may be added before sterilization, which is to be preferred; but if volatile, it must be added by means of a sterile pipet, with the greatest precaution as regards asepsis, after sterilization and immediately before the test is made. Control experiments—*i.e.*, bouillon cultures without the addition of the antiseptic—should always be made.

The results of antiseptic action are two: *retardation* of growth and *complete inhibition* of growth. As the inoculated tubes containing the antiseptic are watched in their development, it will usually be observed that those containing very small quantities develop almost as rapidly as the control tubes; those containing more, a little

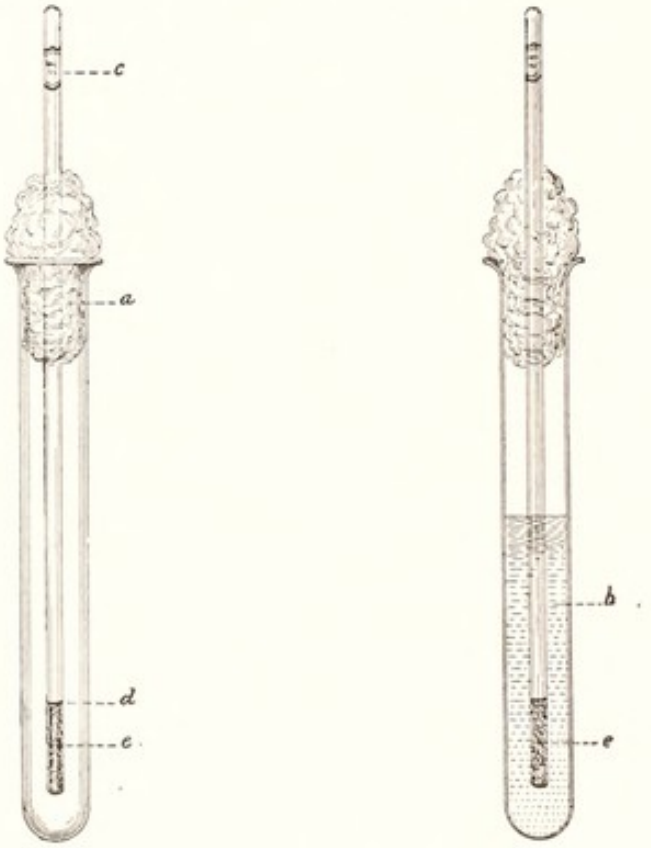
more slowly; those containing still more, very slowly, until at last there comes a time when the growth is entirely checked.

Sternberg points out that the following conditions, which must be avoided, may modify the results of experiment:

1. The composition of the nutrient media, with which the antiseptic may be incompatible (as bichloride of mercury and albumin).

2. The nature of the test-organism, no two organisms being exactly alike in their susceptibility.

3. The temperature at which the experiment is conducted, a



Same rod immersed in broth after exposure to disinfectant.

Fig. 87.—Glass rod in test-tube, for use in testing disinfectants. Tube 6 in. by ¾ in.; rod 9 in. by ¼ in. Ring marked with diamond 1 in. from lower end, to show upper limit of area on which the organisms are dried. After exposure the rod is placed in a similar tube containing broth, to test development. *a*, Cotton plug wrapped around glass rod; *b*, broth; *c*, gummed label on handle of rod, for identification; *d*, ring marked by diamond; *e*, dried organisms.

relatively greater amount of the antiseptic being necessary at temperatures favorable to the organism than at temperatures unfavorable.

4. The presence of spores which are always more resistant than the asporogenous forms.

II. The Germicidal Value.—Koch's original method of determining this was to dry the micro-organisms upon sterile threads of linen or silk, and then soak them for varying lengths of time in the germicidal solution. After the bath in the reagent the threads were washed in clean, sterile water, transferred to fresh culture-media, and their

growth or failure to grow observed. This method also determines the *time* in which a certain solution will kill micro-organisms, so is advantageous.

Sternberg suggested a method by which the *dilution* necessary to kill the bacteria could be determined, the time remaining constant (two hours' exposure) in all cases. "Instead of subjecting test-organisms to the action of the disinfecting agent attached to a silk thread, a certain quantity of a recent culture—usually 5 cc.—is mixed with an equal quantity of a standard solution of the germicidal agent, . . . and after two hours' contact one or two loopfuls are transferred to a suitable nutrient medium to test the question of disinfection."

A very simple and popular method of determining the germicidal value is to make a series of dilutions of the reagent to be tested; add to each a small quantity of a fresh liquid culture, and at varying intervals of time transfer a loopful to fresh culture-media. By a little ingenuity this method may be made to yield information as to both *time* and *strength*.

Hill* has suggested a convenient method of handling the cultures, which are dried upon the ends of sterile glass rods and can then be transferred from one solution to another or otherwise manipulated.

The Modern Method of Testing the Germicidal Value of Liquids.—The methods of testing germicidal strength given above are uncertain and inaccurate, and can only be looked upon as "rough and ready" methods, that should be willingly abandoned for anything better. Three methods are now offered that hold out the promise of scientific accuracy through an established standard of comparison. In the order of their appearance, which is also, probably, the order of their importance, these are the method of Rideal and Walker,† "The Lancet Method,"‡ and the method of Anderson and McClintic.§ The methods are similar in general principles, and have the same object in view, *i.e.*, the expression of the germicidal value of any substance as the carbolic acid or phenol "coefficient." Experience with the methods leads to the conviction that the Rideal and Walker method is the more easy to execute, but that the Anderson-McClintic method is the more accurate. As the latter in addition to its accuracy has now become the standard method of the United States Government, it is the method with which the student should be acquainted and which will be given in detail.

I. The Apparatus, Reagents, etc., Required for the Test.—1. *A Phenol Solution* that shall act as the standard of comparison. In the preparation of this solution, pure phenol—as free from cresols, etc., as possible—should be employed. Walker recommends that only

* "Public Health," vol. XXIV, p. 246.

† Journal of the Royal Sanitary Institute, London, 1903, p. 424.

‡ "The Standardization of Disinfectants" (unsigned article), Lancet, London, vol. CLXXVII, Nos. 4498, 4499 and 4500.

§ Bulletin No. 82 of the Hygienic Laboratory, Washington, D. C., 1912.

phenol with a melting point of 40.5°C., be used, as only such is entirely free from impurities. The Eighth Revision of the U. S. Pharmacopœia declares phenol with a melting point of 40°C. to be pure and that is the quality that may be accepted as the standard.

The phenol used at the Hygienic Laboratory is Merck's "Silver Label." The standard dilution, made by the U. S. P. method (Koppeschaar), contains exactly 5 per cent. of pure phenol by weight, in distilled water. From this stock solution, the higher dilutions are made fresh each day for that day's tests.

2. *The Solution to be Tested.*—A 5 per cent. solution is made by adding 5 cc. of the disinfectant to 95 cc. of sterile distilled water with a standardized 5 cc. capacity pipet. After filling the pipet, all excess of the disinfectant on its outside is wiped off with sterile gauze. The contents of the pipet are then delivered into a cylinder containing 95 cc. of sterile distilled water and the pipet washed out as clean as possible by aspiration and blowing out the contents into the cylinder. The contents of the cylinder are then thoroughly shaken.

3. *The Test Organism* selected is Bacillus typhosus. Before beginning the tests, the organisms in bouillon culture should be transplanted to fresh media every twenty-four hours for at least three successive days. In making the transfers one loopful of a 4-mm. platinum loop is carried over. In exposing the culture to the disinfectant, $\frac{1}{10}$ cc. of the culture is always added to 5 cc. of the diluted disinfectant, the amount being measured by pipets graduated in tenths of a cubic centimeter.

4. *The Inoculating Loops.*—These loops are made of No. 23 U. S. standard gauge platinum wire, each loop being 4 mm. in diameter. There should be four, and preferably six, such loops mounted in the usual glass handles, ready for use. In order to facilitate their sterilization, a special holder is used.

5. *The Water-bath.*—As variations in the temperature of the disinfecting solutions hasten or retard their destructive action, a temperature of 20°C. has been arbitrarily adopted as the standard. For its maintenance the following simply constructed water-bath has been devised. It consists of a wooden box 20 inches deep, 21 inches long and 21 inches wide. Inside this box a 14-quart agate-ware pail, 10 inches deep, is placed and saw-dust is well packed around, sufficient being placed in the bottom of the box to bring the rim of the pail on a level with the top of the box. A tightly fitting wooden cover, so made that the edges project slightly over the rim, is placed over the pail. In the cover are a sufficient number of holes for the seeding tubes, a thermometer, and the tube containing the culture. About 3 inches below the rim of the pail a false bottom of wire gauze is placed; this is for the seeding tubes, etc., to rest on. Water is placed in the pail to within half an inch of the top. When an experiment is about to be made the tempera-

ture of the water in the pail is taken, and if above or below 20°C., it is brought to the desired temperature by the addition of either

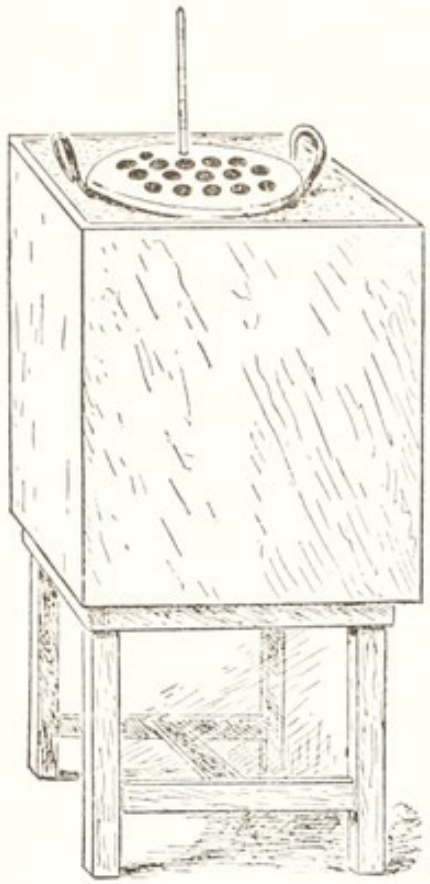


Fig. 88.—Water-bath showing position of holes for seeding tubes and thermometer in place (Anderson and McClintic, in Bulletin No. 82, Hygienic Laboratory).

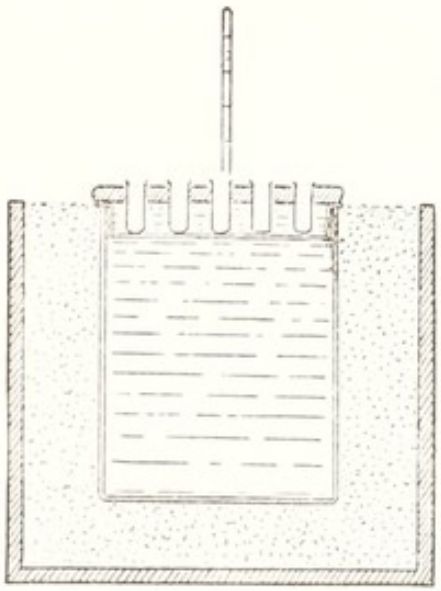


Fig. 89.—Cross section of water-bath showing seeding tubes in place (Anderson and McClintic, in Bulletin No. 82, Hygienic Laboratory).

hot or cold water. When the proper temperature has thus been adjusted, very little change takes place in an hour's time. The apparatus is shown in the cut.

6. *The Culture-media* used for the primary culture, and for the subcultures, made after exposure of the micro-organism to the disinfectant, is nutrient bouillon made with Leibig's beef extract in the usual manner and given a reaction of exactly + 1.5. Anderson and McClintic achieve this by so carrying out the titrating of the medium that a distinctly perceptible pink color marks the point at which the addition of the alkali stops (see directions for titrating culture-media).

7. *The Tubes* for the culture and subcultures are ordinary culture tubes, containing 5 cc. of the nutrient bouillon mentioned above. They are filled, plugged and sterilized in the usual manner.

The tubes for "seeding," *i.e.*, exposing the bacteria to the germicide, are more convenient when shorter. At the time of transfer, the platinum loop is to be introduced into the tube as it stands in the water-bath and as this is not easy with tubes of standard length, Anderson and McClintic recommend tubes 1 inch in diameter and 3 inches long. These are plugged and sterilized by dry heat, or as recommended by the authors quoted, are sterilized mouth down, without plugs in a paper-lined wire basket.

8. *The Dilution of the Phenol and Test Solutions.*—This is done in standardized graduates with standardized pipets, according to the requirements of the particular case. Anderson and McClintic give tables that are useful for making the dilutions, though with the aid of a little arithmetic it is easy to calculate the proportions of the 5 per cent. solutions already prepared, and sterile distilled water necessary to make the test solutions required. As it is certain that some of the dilutions will be below germicidal strength, and as "weeds" may be more difficult to kill than the test organism (B. typhosus) it is important to see that the distilled water used for dilution is sterile, and that the cylinders and bottles or pipets used for making the dilutions are all sterile and that the dilutions themselves are made with aseptic precautions.

Under the standard conditions recommended, the phenol solution that destroys all of the B. typhosus introduced, in 2½ minutes is 1 : 80, but it is always wise to make additional dilutions to control the strength, as shown in the table below. When the strength of the disinfectant or germicide to be tested is entirely unknown, it is well to begin by making a number of tests with widely separated dilutions, by one of the "rough and ready" methods, so as to arrive at an approximate strength, before commencing the more difficult technic required for the determination of the phenol coefficient, which should be looked upon as the final test for exact comparison.

9. *Racks for Holding the Tubes* are indispensable. The "seeding tubes," that is, the tubes in which the actual exposure of the culture to the germicidal solutions is to take place, have already been provided for in the construction of the water-bath.

For the "subculture" tubes, any test-tube rack will do, but it is more convenient to have a special rack or stand made. That recommended contains five rows of 14 holes each. Each tube of culture-medium is carefully marked with a blue pencil to show three things, 1, the germicide; 2, the dilution; 3, the time of exposure, and stood in its place in the rack as will be explained below.

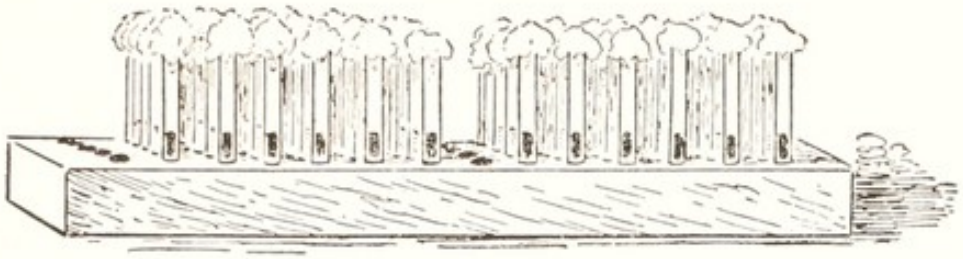


Fig. 90.—Block for subculture tubes (Anderson and McClintic, in Bulletin No. 82, Hygienic Laboratory).

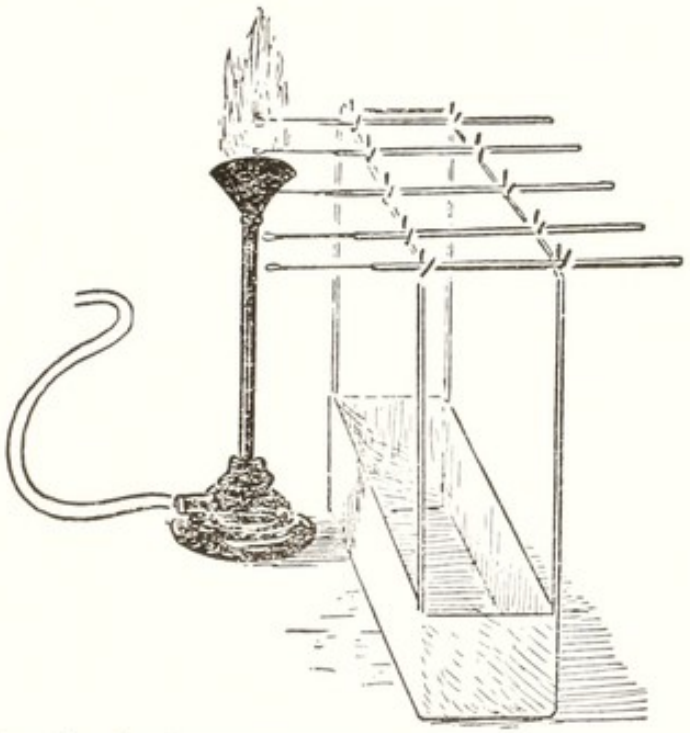


Fig. 91.—Device for flaming inoculating loops (Anderson and McClintic, in Bulletin No. 82, Hygienic Laboratory).

The transplantations from the seeding tubes to the culture tubes are to be made every 2½ minutes up to 15 minutes, so that for each strength of dilution to be tested, there will be six tubes. In addition to these test-tubes there will be four dilutions of phenol to act as controls so that every 2½ minutes ten transplantations must be made. As 2½ minutes contain 150 seconds, and as the picking up and opening of the subculture tube, the transfer of the seed-culture to the medium, the replacement of the stopper and the return of the tube to the rack require about 15 seconds at the hands of an

expert manipulator, the ten tubes in the series comprise the maximum number that can be handled.

The illustration shows one of the racks, and indicates how the tubes are placed in ten rows of six each, each row with an empty hole on the left. As the first tube of each series is inoculated, it is stood in the left-hand empty hole, the second stood in the hole from which the first was taken, the third in that from which the second was taken, and so on, so that there is always an empty hole to show the operator which tube to take up for the next inoculation.

The Technic of Determining the Phenol Coefficient.—Everything being ready as outlined above, one proceeds as follows: The 24-hour bouillon culture of B. typhosus is shaken, then poured through a sterile filter-paper in a sterile glass funnel and caught in a sterile tube. In this way clumps of bacteria are removed and uniformly distributed bacteria secured for addition to the "seeding tubes."

Exactly 5 cc. of each dilution to be tested is now measured into a seeding tube. To economize glassware the same pipet may be used for a whole series, by beginning at the lowest dilution, measuring out the necessary 5 cc. into the first seeding tube, with a 5-cc. delivery pipet. The contents of the pipet are then thoroughly blown out, and a pipetful of the next weaker dilution taken up to wash out the pipet. After this has been thoroughly blown out and thrown away, a pipetful of this second strength of diluted disinfectant is carefully measured into a second seeding tube, after which the same is done with each remaining dilution in turn. The tubes are so marked and so arranged in the rack of the water-bath that no mistake can be made in transplanting from them in regular order later. As each tube is filled, the stopper is replaced and when all have been filled and stood in the rack, it is placed in the water-bath and the temperature raised to 20°C. Anderson and McClintic do not use cotton plugs for the seeding tubes but sterilize them, open end down in a paper-lined wire basket. Some feel safer, however, in using tubes with plugs. The culture now being filtered, and the seeding tubes each with the required 5 cc. of each dilution of the disinfectant to be tested, all at 20°C. in the water-bath, the subculture tubes marked and stood in their respective places in the racks, sterilized pipets at hand, and four or six platinum loops on the block ready sterilized, with the burner in place ready to re-sterilize them, the technic is continued by the addition of the culture to the seeding tubes. At this point one should make a slight calculation: if the culture is to be added to each of ten of the seeding tubes, it must be done before the expiration of 150 seconds or 2½ minutes for at the conclusion of that time, the first transplantation from each seeding tube to a culture tube must take place. We have averaged 15 seconds for each operation. If each transfer takes an average of

15 seconds, the operator must have every detail of the technic so well in hand, and the materials so conveniently placed, etc., that he can complete the entire performance of the technic from the addition of the culture to the seeding tubes to the last transplantation from seeding tubes to subculture tube without a hesitation and without a distraction. It is on account of the necessity of this "continuous performance" that such care was taken to point out the exact details of apparatus and materials needed, before describing the technic.

To return to the seeding of the tubes, a sterile pipet graduated in $\frac{1}{10}$ cc. is used. The cotton stoppers are removed from the seeding tubes and thrown away as of no further use. One by one as the time arrives, tubes are taken in one hand, inclined to an angle of about 45 degrees, while the tips of the pipet are lightly touched to that side of the tube from which the fluid has run away on account of the slanting, and exactly 0.1 cc. of the culture delivered. This may under no circumstances take longer to perform than 15 seconds, and if one succeed in finishing it in a shorter time, he must wait until the calculated time arrives before delivering the culture into the next tube and so on until the end is reached. Each tube is given three gentle shakes after being straightened up, then returned to the water-bath.

With a ten-tube series, and a time allowance of 15 seconds for each tube, the entire series of tubes is no sooner completed than the time ($2\frac{1}{2}$ minutes) for making the first series of transplantations to the subculture tubes has arrived. The operator therefore seizes at once the first of the culture tubes in the $2\frac{1}{2}$-minute series with one hand, and a sterile platinum loop with the other. He cautiously removes the cotton plug from the culture tube, and at the proper moment introduces the platinum loop into the first seeding tube all the way to its bottom, withdraws it, and carries one drop of the contained fluid into the first subculture tube which he plugs and places in the empty hole to the left of the row in the block, at once taking up its neighbor on the right. As only 15 seconds are allowed for each such transfer, the operator must proceed without hesitation. There is no time to sterilize the platinum loop, so he lays it on the block, pushes the flame under it and takes up an already sterilized loop with which he performs the same act of transplantation for the second tube that was done for the first, doing it on the appropriate second of time, and so continuing through the whole series.

Every test of the phenol coefficient of disinfection must embrace two such series, one made with the dilutions of the phenol that is to act as the standard, the other made with the dilutions of the disinfectant to be determined. If, however, a variety of different germicides are to be tested the same day, one phenol test will answer the requirement of the whole group. The following

tabulation will make clear the details of a test (Table 17 from Anderson and McClintic's paper).

TABLE 17

Name "A."
Temperature of medication 20°C.
Culture used. B. typhosus, 24-hour extract broth-filtered.
Proportions of culture and disinfectant, 0.1 cc. × 5 cc.

Sample	Dilution	Time culture exposed to action of disinfectant for minutes						Phenol coefficient
		2½	5	7½	10	12½	15	
Phenol.....	1:80	−	−	−	.....	.....	.....	$\frac{375\times650}{80\ \ 110}$
	1:90	+	−	−	−	.....	.....	$\frac{}{2}$
	1:100	+	+	+	−	−	−	
	1:110	+	+	+	+	+	.....	
Disinfectant,	1:350	−	−	−	.....	.....	.....	$\frac{4.69\times5.91}{2}$
"A"	1:375	−	−	−	.....	.....	.....	
	1:400	+	−	−	−	.....	.....	5.30
	1:425	+	+	−	−	−	−	
	1:450	+	+	−	−	−	−	
	1:500	+	+	−	−	−	−	
	1:550	+	+	+	−	−	−	
	1:600	+	+	+	+	−	−	
	1:650	+	+	+	+	+	−	
	1:700	+	+	+	+	+	+	
	1:750	+	+	+	+	+	+	

To calculate the phenol coefficient, the figure representing the degree of dilution of the weakest strength of the disinfectant that kills within 2½ minutes is divided by the figure representing the degree of dilution of the weakest strength of the phenol control that kills in the same time. The same is done for the weakest strength that kills in 15 minutes. The mean of the two is the coefficient. The coefficient of any disinfectant may, for practical purposes, be defined as the figure that represents the ratio of the germicidal power of the disinfectant to the germicidal power of the phenol, both having been tested under the same conditions.

As many disinfectants and germicides are greatly modified through precipitation, combination or other transformation in the presence of organic matter, in all of those whose coefficient is considerably more than 1, it is wise to perform a second series of tests in which the disinfectant is tested, and the control tests made in the presence of organic matter and the coefficient calculated accordingly. It is usually found that under these conditions the coefficient falls. In a general way, those disinfectants are most valuable for general employment, whose coefficients are highest in the presence of organic matter in the test solutions.

The difference in the details of the test given and the new test to be made are as follows:

1. The test dilutions are made 20 per cent. stronger to allow for the dilution made by the addition of the solution of organic matter.

2. An organic matter solution is to be prepared. It consists of water containing 10 per cent. of peptone and 5 per cent. of gelatin. The solids are dissolved and the solution sterilized. Titration is not essential.

The variations in technic are simple. Of the dilutions made 20 per cent. stronger than for the other experiment, 4 cc. (not 5 cc.) are measured into each seeding tube. The culture after being filtered is added to the organic matter in the proportion of 0.1 cc. to each 1 cc. to be employed in seeding. The addition of 1.1 cc. of the organic solution culture mixture to each seeding tube, gives a total of 5 cc. of diluted disinfectant containing 0.1 cc. of culture and a total of 2 per cent. of peptone and 1 per cent. of gelatin. Except for the slight difference in the dilutions and the seeding with mixed culture and organic fluid the method is the same, and the method of calculating the results is the same.

Anderson and McClintic point out that it is manifestly cheaper to purchase a disinfectant for 60 cents a gallon than to purchase one for 30 cents a gallon, providing the former has four times the efficiency of the latter. The true cost of a disinfectant can only be determined by taking into consideration the phenol coefficient and the cost of the disinfectant per gallon. The cost of a disinfectant per 100 units of efficiency as compared with pure phenol is obtained by first dividing the cost per gallon of pure phenol; the efficiency ratio is of course obtained by dividing the coefficient of the disinfectant by the coefficient of phenol, but as the coefficient is always 1, the efficiency ratio is represented by the phenol coefficient of the disinfectant.

The cost ratio divided by the efficiency ratio (the coefficient of the disinfectant) gives the cost of the disinfectant per unit of efficiency as compared with the cost per unit of efficiency of pure phenol = 1. By multiplying by 100 the relative cost of 100 units is obtained thus:

$$\frac{\text{Cost of disinfectant per gallon.}}{\text{Cost of phenol per gallon.}} \ (=\text{Cost ratio}) \div \frac{\text{Coefficient of disinfectant.}}{\text{Coefficient of phenol. } (=1.)} \ (=\text{Efficiency ratio.})$$

= cost of the disinfectant per unit of efficiency as compared with phenol = 1, and by multiplying by 100 the cost of 100 units is obtained. For instance, the cost of disinfectant "Can" is $0.30 per gallon and it has a coefficient of 2.12; the cost of phenol is $2.67 and it has a coefficient of 1. Then,

$$\frac{0.30}{2.67} \div \frac{2.12}{1} = 0.052$$

Therefore, the comparative cost per unit of efficiency of "Can" and phenol respectively, is as 0.052 : 1; or, by multiplying by 100, the relative cost per 100 units—5.2 : 100 is obtained.

Gaseous Disinfection.—If the germicide to be studied be a gas, as in the case of sulphurous acid or formaldehyd, a different method must, of course, be adopted.

It may be sufficient to place a few test-tube cultures of various bacteria, some with plugs in, some with plugs out, in a closed chamber in which the gas is evolved. The germicidal action is shown by the failure of the cultures to grow upon transplantation to fresh culture-media. This crude method may be supplemented by an examination of the dust of the room. Pledgets of sterile cotton are rubbed upon the floor, washboard, or any dust-collecting surface present, and subsequently dropped into culture media. Failure of growth under such circumstances is very certain evidence of good disinfection. These tests are, however, very severe, for in the cultures there are immense numbers of bacteria in the deeper portions of the bacterial mass upon which the gas has no opportunity to act, and in the dust there are many sporogenous organisms of extreme resisting power. Failure to kill all the germs exposed in such manner is no indication that the vapor cannot destroy all ordinary pathogenic organisms.

A more refined method of making the tests consists in saturating strips of blotting-paper, absorbent cotton, various fabrics, etc., with cultures and exposing them, moist or dry, to the action of the gas. Such materials are best made ready in Petri dishes, which are opened immediately before and closed immediately after the experiment. If, when transferred to fresh culture media, the exposed objects fail to give any growth, the disinfection has been thorough so far as the particular test organism is concerned. If the penetrating power of a gas, such as formaldehyd, is to be tested, it can be done by inclosing the infected paper or fabrics in envelopes, boxes perforated with small holes, tightly closed pasteboard boxes, and by wrapping them in towels, blankets, mattresses, etc.

Easier of execution, but rather more severe, is a method in which cover-glasses are employed. A number of them are sterilized, spread with cultures of various bacteria, allowed to dry, and then exposed to the gas as long as required. They are subsequently dropped into culture media to permit the growth of the organisms not destroyed.

Animal experiments may also be employed to determine whether or not a germ that has survived exposure to the action of reagents has its pathogenic power destroyed. An excellent example of this is seen in the case of the anthrax bacillus, a virulent form of which will kill rabbits, but after being grown in media containing an insufficient amount of a germicide to kill it, will often lose its rabbit-killing power, though still able to fatally infect guinea-pigs, or may lose its virulence for both rabbits and guinea-pigs, though still able to kill white mice.

CHAPTER XVIII

BACTERIO-VACCINES

A BACTERIC-VACCINE is a culture of micro-organisms so modified as to be no longer a source of dangerous infection, and so administered as to stimulate the body defenses and thus assist either in preventing or overcoming more virulent infection.

The small amount of benefit that occurred from the employment of the Oriental method of "inoculating against small-pox" was based upon the theory that virus of low virulence, obtained from a sporadic case of small-pox if introduced into the healthy body, must result in a mild attack of the disease, by which the individual would be left immune against the more virulent viruses by which epidemics of the disease are brought about. The observation of Jenner, that the virus of cow-pox would protect against small-pox, led to the supposition that the essential causes of the two diseases had originally been the same, but had so diminished that the one became comparatively harmless for man after many generations of residence in the cow.

The success of Pasteur's preventive inoculation against chicken-cholera depended upon the fact that the bacilli of the disease rapidly lost their disease-producing power when grown artificially in culture-media, though they still retained the power of effecting a change in the fowls which thereafter remained immune. His vaccination against anthrax was based upon the observation that the spore-forming power and virulence of the anthrax bacillus could be destroyed by cultivation at temperatures beyond a certain point, and that animals infected with bacilli of this modified form subsequently resisted more virulent infections. His vaccination against rabies was based upon the supposed diminution in virulence that the unknown micro-organisms underwent when exposed to artificial inspissation of the nervous tissue in which they were contained. Such organisms of very low virulence protected against those of higher virulence, and so on.

From the periods during which these early observations were made, to the present time, when the term "bacterio-vaccine" is in daily use, studies in immunity have been conducted in so great a variety of ways by such a multitude of investigators, that it becomes tedious to endeavor to trace the logical and orderly steps that lead to present knowledge, theory and practice. Two names, however, stand out conspicuously in connection with the present topic, because of the importance of their contributions, those of Haffkine and Wright. The former used heated and killed cultures

of the cholera spirillum as a prophylactic against cholera, and later with equal success, heated and killed cultures of the plague bacillus as a prophylactic against plague. Wright somewhat modified the method, by using two or even three doses of modified cultures of the typhoid bacillus at intervals of ten or even twenty days, to secure complete prophylaxis against typhoid fever.

From prophylactic measures it was but a step to therapeutic measures, and the endeavor to facilitate the cure of disease by the administration of cultures of vaccine. The patient suffering from an infectious disease was already impressed by the toxic, enzymic or other disease-producing substances in his body, and the administration of cultures of micro-organisms seemed like adding so much fuel to an already widespread conflagration. Indeed, experience and experiment seemed to prove this to be the case, for when by any mischance a patient in the early stages of plague received an injection of the Haffkine plague prophylactic, he straightway became much injured by the added culture and might even die quickly.

But there are certain infections in which conditions are different both with regard to the bacteria and the disease. Thus, a certain micro-organism with limited power of invasion and with difficultly soluble toxic products (endo-toxins), whose injurious effects are local and limited in extent, particularly when their effects are prolonged and the disturbances chronic, are essentially different from actively invasive agents that quickly over-run the body, or those with considerable soluble products by which it is generally disturbed.

In the former group it is not unreasonable to hope that through a method of treatment by which the general body defences are stimulated, the local infections may be overcome. Such cases of disease were, therefore, selected, especially by Wright, for investigation and treatment. Success of varying degree has followed, and though it is difficult to calculate accurately the benefits obtained in cases that are not susceptible of numerical expression, the almost uniform opinion of clinical and laboratory men is to the effect that certain cases of sluggish infection, with little tendency to recover are benefited and sometimes rapidly cured by treatment with bacterio-vaccines.

From these preliminary considerations it should be clear to the reader that the theoretical conditions necessary to success are the following:

1. That the disease should be of subacute or chronic duration.
2. That it should be fairly well localized.
3. That it should be caused by a micro-organism incapable of ready invasion or much soluble toxin formation.
4. That the micro-organism be known and capable of cultivation so that the appropriate-specific vaccine can be made.

From these conditions certain lesions resulting from infection by pus cocci, colon bacilli, acne bacilli, typhoid bacilli (post-typhoidal

suppurations), tubercle bacilli, etc., etc., ought to be appropriate. And, indeed, for them the treatment is highly recommended, and in many cases remarkable success is claimed.

Remembering that the reactions of immunity are specific, it is imperative that the essential organism of the lesion be found and cultivated, and cultures of that organism used in the treatment. So important is this that Wright insists that only "*autogenous vaccines*"—that is, vaccines made of cultures of bacteria cultivated from the very lesion to be treated—be used. This somewhat limits the usefulness of the method for the rank and file of practitioners can scarcely be supposed to have the knowledge, apparatus, or time required for carrying out the technic, nor can all patients afford to patronize the laboratory man. Commercial manufacturers are therefore justified in the preparation and sale of what are known as "*stock vaccines*" that can be tried in lieu of autogenous vaccines, though in checking up the results note should always be taken of the fact that "autogenous" or "stock" vaccines were used.

In spite of the general principles laid down above, there are reports and observations to show that the theoretical considerations may be faulty and that in some cases the method of treating by vaccination may be beneficial in acute maladies, even when the condition to be treated is toxic. It will be necessary, however, to secure much more evidence with regard to the employment of the method in such cases before it can be recommended as sound practice.

Should a case of appropriate kind, when investigated, yield more than one species of micro-organism, of such kind as to make it uncertain which is responsible for the injury done, both should be cultivated, two vaccines made and mixed, and both infections simultaneously antagonized.

The Method of Making the Vaccine.—A pure culture of the necessary micro-organisms is obtained from the lesion to be treated, and cultivated in agar-agar.

One pint "Blake bottles," pint or quart white glass whisky flasks, or other good sized bottles with large flat sides, are selected and washed. Into each enough melted agar-agar is filled to spread out over one of the flat surfaces to a thickness of about 1 centimeter, after which a cotton plug is placed in the mouth of the bottle, and it and its contents are sterilized in the autoclave. Upon removal, after sterilization, the bottle is laid on its side so as to distribute the agar-agar and permit it to solidify over the greatest surface, without flowing into the neck and touching the cotton stopper. To the agar-agar culture of the micro-organism to be used, about 10 cc. of sterile 0.85 per cent. sodium chloride solution is added, the culture mass being detached with a platinum loop and thoroughly mixed with the fluid. When the agar-agar is firm, each bottle receives by means of a carefully sterilized pipet, about 1 cc. of the culture suspension which is thoroughly distributed over the entire flat surface of the agar-agar by tilting the bottle this way and that until it has been completely covered. The bottles are then placed in the incubating oven, lying upon the side so as to permit the bacteria to vegetate undisturbed upon the moist flat surface of the medium. After 24 hours, the growth having matured, the bottles are removed and about 10 cc. of sterile distilled water containing 0.85 per cent.

of sodium chloride and 0.5 per cent. of phenol is added to each, for the purpose of washing off the bacteria that have grown. This is done by tilting the bottle and permitting the solution to wash over and over the surface. If the culture does not detach, it may be necessary to remove it with a sterilized glass rod, or by means of a sterile swab made by fastening a small pledget of cotton batting upon the end of a wire.

When the growth is detached and thoroughly mixed with the salt solution, it is removed to a sterile receptacle by means of a sterile pipet.

What is next done will depend upon the theory upon which the treatment is based. The culture washings contain: (A) substances derived from the culture-medium that certainly cannot be regarded as useful or beneficial and may be harmful;

(B) bacterial products, of soluble quality, eliminated from the cells during the life activities, some of which may be useful;

(C) the bacteria themselves, which with their contained products—endo-toxins, etc.—are commonly regarded as the essential immunizing agents.

If one's theory is that the bacterial cells are essential, and there seems to be a growing tendency toward this view, further treatment is necessary before actually preparing the vaccine for administration; if, however, the collected products of their growth are thought to be of partial or equal value, and are to be preserved, this cannot be done without also retaining the less desirable matters from the culture-medium.

Let us suppose that only the bacterial cells are to be employed.

The suspension of bacteria, under these circumstances, is transferred to appropriate sterile tubes, plugged, and whirled in a powerful centrifuge until the bacteria are thrown down to the end of the tube, leaving the supernatant fluid fairly clear. The fluid is then removed by decantation or with a pipet, and replaced by an equal volume of 0.5 per cent. phenol in 0.85 per cent. sodium chloride solution in distilled water. In this the sediment is thoroughly mixed by stirring. As the bacteria are often in masses, groups or chains, it is now necessary to separate them. This is best done by adding a few small glass beads to the contents of the tubes, changing the cotton stopper for a sterile rubber cork, and shaking either in a shaking machine or by hand, until it can be supposed that the micro-organisms are all separated. This is easily accomplished by the aid of the shaking machine but is tedious to effect by hand. The tube is then returned to the centrifuge and again whirled until the bacteria are again sedimented, after which the fluid is again removed and again replaced and the bacteria again distributed. A few turns in the centrifuge now throw down particles of culture-media and contained flakes of the culture and leave a uniformly clouded fluid above.

If it be desired to conserve all of the bacterial products, the washings from the culture bottles are immediately transferred to the appropriate tube, shaken with the glass beads, given a few turns in the centrifuge to throw out flakes of culture and culture-media, and we again arrive at the point of having a uniformly cloudy fluid with which to continue the preparation of the vaccine.

If the vaccine is to be of scientific value, it should be made in such manner that its composition represents what is desired—bacterial cells only, or bacterial cells with their collected products—and some means should be provided by which a reasonably accurate

determination of its value can be estimated. This is done by calculating the number of contained bacteria per cubic centimeter of the fluid, and then either diluting or concentrating by means of centrifugation until an appropriate result is reached. As the concentration by centrifugation is more difficult than dilution it is best to take care at the very beginning of the process not to add too much fluid to the culture bottles for the purpose of washing off the culture. Whatever dilution of the final product may be necessary is made by the use of the 0 5 per cent. phenol solution.

The most ready method of calculating the number of bacteria in the fluid is that of A. E. Wright which will be found in the chapter upon the "Calculation of the Opsonic Index."

After having determined the number of bacterial cells per cubic centimeter of fluid, dilution with the phenol solution is made until single doses are contained in quantities easily injected into the patient. As the doses vary with the particular organism to be injected, the operator must calculate from the number of bacteria in the fluid, how much solution must be added to constitute a dose. Several doses of each desired size should be prepared. Quantities of the dilutions containing single doses or a number of doses as may be preferred are now transferred, by means of a sterile pipet, into previously sterilized, appropriate sized "ampules" or glass bulbs made for the purpose, and the necks sealed in a flame.

The bacteria are, however, still alive, and though many of them no doubt undergo autolysis in the phenol salt solution, it is necessary to make certain that none remains alive to infect the patient.

The destruction of the vitality of the micro-organisms which is the final step in the process of vaccine preparation is effected by exposure to the lowest temperature that is known to be positively destructive. As spore-producing micro-organisms may maintain this vitality at temperatures beyond 100°C., at which the micro-organismal substance as well as their products are altered by coagulation and other destructive transformation, they are inappropriate organisms to employ for purposes of vaccines, unless, through some such ingenious means as was devised by Pasteur for the anthrax bacillus, the production of spores can be prevented.

With very few exceptions non-sporogenous bacteria are destroyed by exposure for 60 minutes to a temperature of 60°C. Should any escape destruction, they are probably so injured as to be incapable of further injurious effect upon the human body.

The destruction of the bacteria is, then, effected by heat:

The ampules of vaccine are placed in some sufficiently commodious receptacle filled with water, the heat being supplied by a flame below, and the temperature determined by a thermometer whose bulb is at the center of the bath. When small quantities of the vaccine are to be made for special cases, a large beaker supported upon an asbestos plate upon a chemical tripod and heated by a Bunsen's flame answers very well. The burner is allowed to heat the bath until the proper temperature is reached, when it is removed. As soon as the tem-

perature begins to fall, it is replaced. Thus by alternately heating and removing the source of heat for 60 minutes, the destruction is affected.

If there are many of the small ampules, containing different doses or different cultures, each separate lot may be done up in a piece of gauze, and labelled.

J. H. Small uses orange-colored "string tags" for this purpose, writing upon them with either pen or pencil, and fastening them to the gauze packages. In the water of the water-bath, the writing does not wash off of the tag, but the color comes out and gives the water an orange tinge. This is found to be of the greatest use, for as one or more of the factory-made ampules commonly cracks in the water-bath, the color penetrates the contained fluid. Upon removal from the water-bath, to glance at each ampule will inform the observer whether it is cracked or not, through the change in the color of the contents. The tags, therefore, subserve a double purpose.

After heating, one of the ampules can be opened and a drop of the contents transferred to a tube of culture to make sure that the bacteria are no longer alive.

The vaccine is now ready for use, but in what dose shall it be administered? There is no other information upon this subject than that which is derived from the experience that certain doses seem to accomplish good without producing ill effects. Thus experience with doses at first selected arbitrarily has led to a fairly accurate standard dosage. As the beginning dose for most vaccines 50–250 millions may be recommended, to be increased to 1000 millions or more, the injections being given every 4–6 days or as controlled by the opsonic index.

The benefit of the vaccine is commonly supposed to depend upon the stimulation of the phagocytic cells of the body. This is very probably the case, but when the bacterial bodies are administered, their dissolution results in the liberation of the contained endo-toxin, and when the entire culture is given, endo-toxins and perhaps exo-toxins and other substances are also given so that the increased phagocytosis is not likely to be the only effect of the treatment.

A. E. Wright who is a firm believer in the stimulating influence upon the cells seeks to control the dosage and estimate the value of the injections by such study of phagocytic activity, as is shown in the next chapter. If after an injection of vaccine, the phagocytic activity of the leukocytes is diminished (negative phase) harm is supposed to have been done and the inference is drawn that the dose was too large; if, on the other hand, the phagocytic activity is increased for the respective organism, good is supposed to have been done, and at the next injection the same or a larger dose may be given.

Besredka and Metschnikoff* have modified the vaccines by what

*Ann. d. l'Inst. Pasteur, 1913, XXVII, 597.

is called *sensitization*. This they accomplish by treating the bacteria to be used with an antiserum, prepared by injecting animals with such organisms as form the vaccine. In this manner the specific bacteriolytic amboceptors are supposed to anchor themselves to the bacterial cells, and so pave the way for immediate destructive treatment in the body. To achieve such sensitization, some of the appropriate serum is added to the bacterial suspension which need not be subsequently killed, as the sensitized bacteria meet with prompt destruction through the normal complement of the body juices. However, if the bacteria are first killed by heat and then sensitized, a similar result may be brought about, and one is relieved of all anxiety as to the possibility of infection accidentally resulting from the injections.

CHAPTER XIX

THE PHAGOCYTIC POWER OF THE BLOOD AND THE OPSONIC INDEX

FROM the time that Metschnikoff connected the phenomena of phagocytosis with those of immunity, there was no recognized technic for the observation and comparison of the bacteria-consuming and bacteria-destroying power of the cells until 1902, when Leishman* suggested the following simple method:

A thin suspension of bacteria in normal salt solution is mixed with an equal volume of blood by drawing in and out of a capillary tube, then dropped upon a clean slide, covered carefully, placed in a moist chamber, and incubated at 37°C. for a half hour. The cover is then slipped off carefully, as in making blood-spreads, dried, stained, and the number of bacteria in each of 20 leukocytes counted and averaged. For comparison with the normal, the patient's blood and normal blood are simultaneously examined.

This was greatly improved by Wright and Douglas,† the accuracy of whose methods enabled them to discover the "opsonins," work out the "opsonic index," and formulate methods by which sufficiently accurate observations could be made for controlling the specific treatment of infectious diseases.

The opsonic theory teaches that the leukocytes are disinclined to take up bacteria unless they are prepared for consumption or phagocytosis by contact with certain substances in the serum that in some manner modify them. This modifying substance is the opsonin (*opsono*, I cater to, I prepare for).

To make a test of the opsonic value of the blood it is necessary to prepare the following:

A uniform suspension of bacteria.
A suspension of washed leukocytes in physiological salt solution.
The serum to be tested.
A normal serum for comparison.

The Bacterial Suspension.—This is prepared like the similar suspensions used for determining agglutination, but with greater care, since the bacteria taken up by the corpuscles are to be counted, and any variation in the number of bacteria with which they come into contact may modify the count. It is also necessary to avoid all clumps of bacteria for the same reason.

The culture is best grown upon agar-agar for twelve to twenty-four hours, the bacteria in young cultures being more easy to sepa-

* "British Medical Journal," Jan. 11, 1902, I, p. 73.
† "Proc. Royal Soc. of London," 1904, LXXXII, p. 357.

rate than those in old cultures. Such a culture may be taken up in a platinum loop, transferred to a test-tube containing some 0.85 per cent. sodium chloride solution, and gently rubbed upon the glass just above the fluid, allowing the moistened and mixed bacterial mass to enter the fluid little by little.

If the culture be older or of a nature that will not separate in

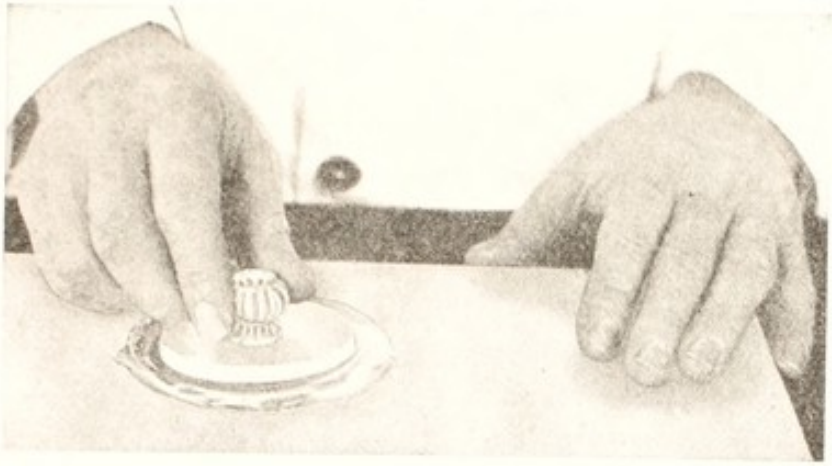


Fig. 02.—Grinding bacteria (Miller).

this manner (tubercle bacillus), it may be necessary to rub it between two glass plates, or in a small agate mortar with a drop or two of salt solution, other drops being added one at a time, until a homogeneous suspension is secured. Such clumps of bacteria as may remain in the suspension are easily removed by whirling for a few seconds in a centrifuge.

The next step is the standardization of the suspension. Wright recommends for this purpose and for the standardization of the bacterio-vaccines that the number of bacteria shall actually be counted. This he does by mixing one part of the bacterial suspension with an equal volume of normal blood and three volumes of physiological salt solution. After thorough mixing a smear is made upon a slide, the smear stained, and the number of bacteria and corpuscles in successive fields of the microscope counted until at least 200 red blood-corpuscles have been enumerated. As the number of red corpuscles per cubic millimeter of blood is 5,000,000, the number of bacteria per cubic centimeter can be determined from the results of the counting by a simple arithmetical process. To facilitate the counting the eyepiece of the microscope is prepared by the introduction of a diaphragm. The prepared suspension must usually be greatly diluted before using, but the reduction of bacteria is, of course, easily cal-

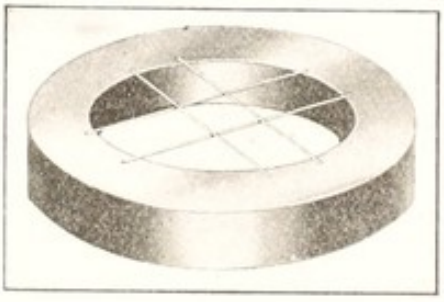


Fig. 03.—Diaphragm of eyepiece showing hairs in position (Miller).

culated. It requires experience to determine the appropriate number of bacteria to be employed. When this is once determined, future manipulations are made easy, because one first makes his suspension,

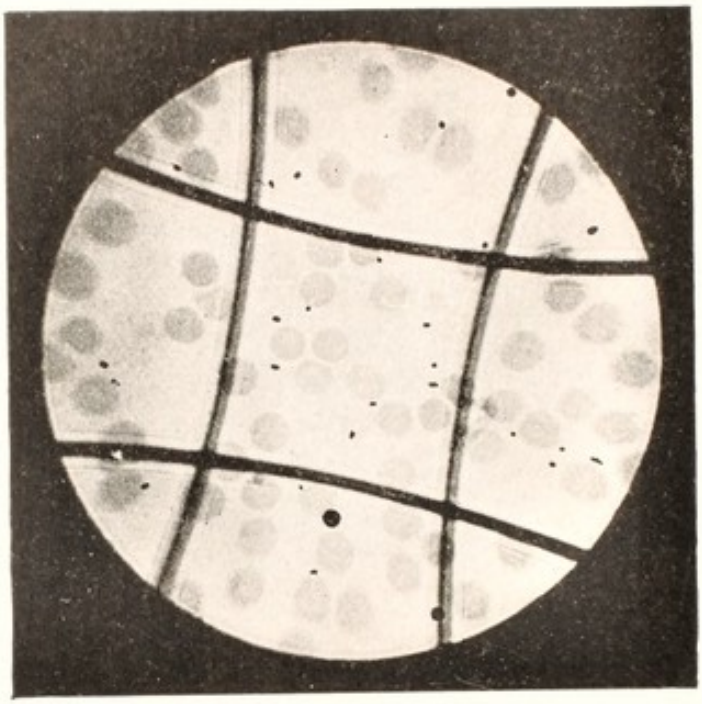


Fig. 94.—Photomicrograph showing cross-hairs, bacteria, and red blood-corpuscles (Miller).

then enumerates the bacteria, and having determined their number, immediately arrives at the appropriate concentration by dilution.

Fig. 95.—Collecting blood for corpuscles (Miller).

The Washed Leukocytes.—It is not necessary to have the leukocytes free from admixture with the erythrocytes, but it is necessary to have large numbers of them. They are collected by citrating the blood so as to prevent coagulation, and then separating the citrated plasma from the corpuscles by centrifugalization.

The hands of the patient are washed, and a piece of elastic rubber tubing or some other convenient fillet wound about the thumb or a finger to produce venous congestion. With a convenient lancet (Wright uses a pricker made by drawing a bit of glass tubing or a glass rod to a fine point in the flame) a prick is made about a quarter inch from the root of the nail. From this the blood is permitted to flow into small test-tubes previously filled about three-fourths with 1.5 per cent. sodium citrate solution. The blood and citrate solution are mixed, and the tubes placed in a centrifuge, balanced, and centrifugalized until the corpuscles are collected at the bottom of the tube. The citrated plasma is now withdrawn and replaced with 0.85 per cent. sodium chloride solution, through which the corpuscles are distributed by shaking. The tubes are now again centrifugalized until the corpuscles are collected, when the saline is removed carefully, the last drop from the back of the meniscus. In the corpuscular mass that remains the leukocytes form a thin creamy layer on the top.

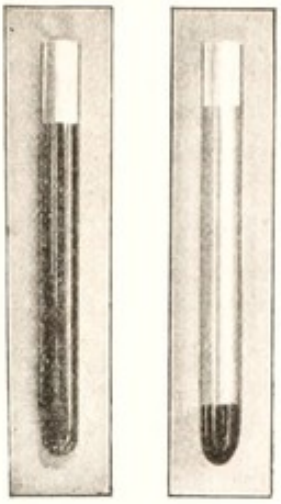


Fig. 96.—Tube of blood and citrate solution before and after centrifugalizing (Miller).

The serum to be tested and the normal serum for comparison are secured in the same manner, the former from the patient, the latter from the operator. As it is advisable to wound the patient

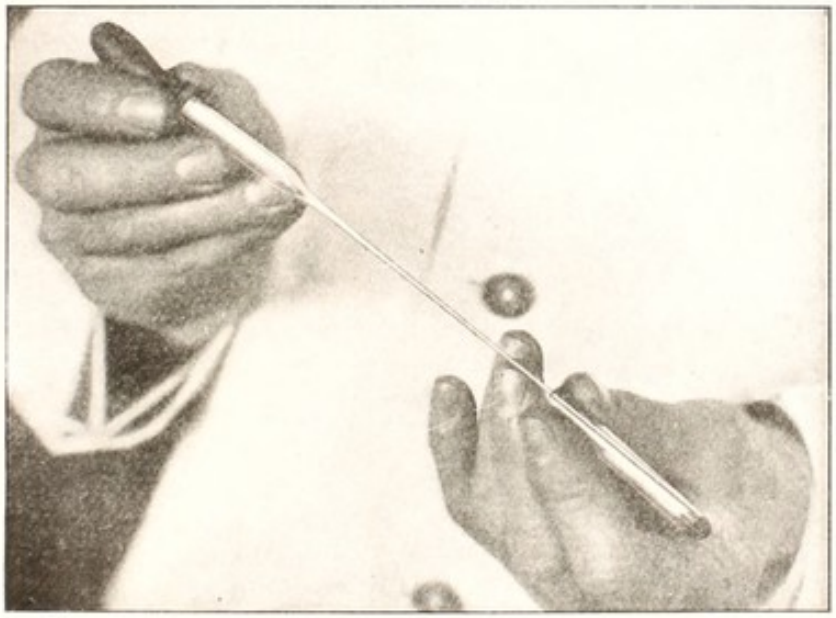


Fig. 97.—Removing last drops of saline solution (Miller).

but once, the tube for obtaining the serum should be filled at the same time that the citrated blood is taken.

The blood to furnish the serum is taken in a small bent tube shown in the illustration.

The blood from the puncture is allowed to flow into the bent end of the tube, into which it enters by capillary attraction and from which it descends to the body of the tube by gravity. At least 1 cc. of the blood is required to furnish the serum. The ends of the tube are closed in the flame and the tube stood in the thermostat for fifteen to thirty minutes. Coagulation takes place almost immediately, and the serum usually separates quickly. If it does not do so, Wright recommends hanging the curved arm of the tube over the centrifuge tube and whirling it for a moment or two, when the clot is driven into the straight arm of the tube and the clear serum appears above. The tube is then cut with a file so that the serum can be removed when needed. Mixing the factors concerned in the test is a matter that requires practice and a steady hand. It is best done, as recommended by Wright, in a capillary tube controlled by a rubber bulb. The object of the experimenter is to take up into this pipette equal quantities of the creamy layer of blood-corpuscles, of

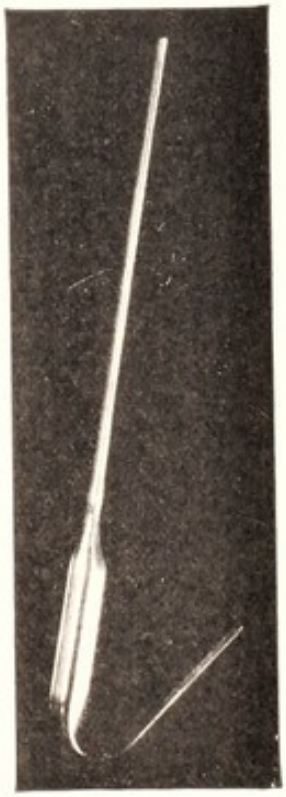


Fig. 98.—Special blood pipetet (Miller).

Fig. 99.—Opsonizing pipette containing blood-corpuscles, bacterial emulsion, and blood-serum (Miller).

the blood-serum, and of the bacterial suspension. Wright first makes a mark with a wax pencil about 1 centimeter from the end of the capillary tube. He first draws up the leukocytic layer of blood-corpuscles to this mark, then removing the tube, permits the column to ascend a short distance. Next he draws up the bacterial suspension to the same point, withdraws the tube, and permits the column to ascend; then draws up the serum to be taken to the same point; thus in the same capillary tube he has three equal volumes of three different fluids, separated by bubbles of air. It is next necessary to mix these, which is done by repeatedly expelling them upon a clean glass slide, and redrawing them into the tube. After thus being thoroughly mixed, the fluid is once more permitted to enter the capillary tube and come to rest

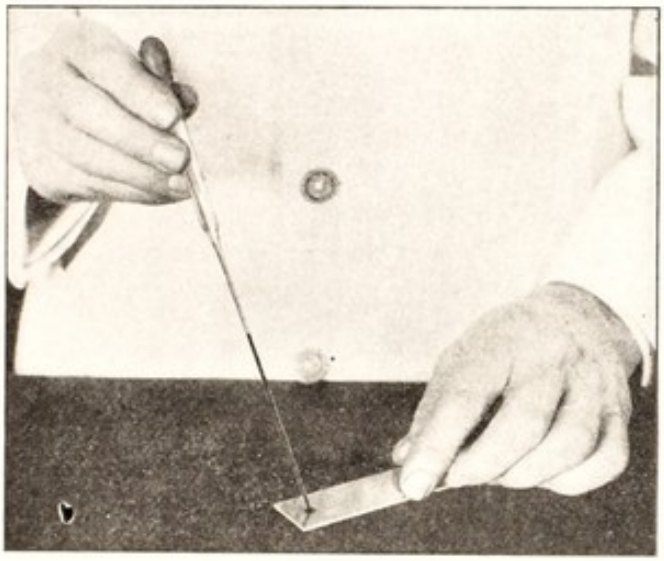


Fig. 100.—Mixing liquids by repeatedly expelling on to slide and redrawing into pipette (Miller).

there. The end is now sealed in a flame, the rubber bulb removed and the tube placed in a thermostat, or in case much work of the kind is being done, to an opsonizing incubator in which the temperature is not modified by opening and closing the doors. The tube remains in the incubating apparatus at 37°C. for fifteen minutes (some use twenty, some thirty, minutes as their standard), is then removed, whirled about its long axis between the thumbs and fingers a few times to mix the contents from which the corpuscles have sedimented, its end is broken off, and a good-sized drop is allowed to escape upon a perfectly clean glass slide and spread over its surface.

The spreading is a matter of some importance, as an even distribution of the leukocytes is desirable. The capillary tube from which the drop has escaped will form a good spreader if laid flat upon the glass and drawn along, but the edge of another slide is better, and in distributing the fluid, it is better to push than to pull it with the end of the slide, rather than its side.

Miller* says that "a good smear should be uniform in consistency and most of the leukocytes should be found along the edges and at the end. For convenience in counting, it is well to have the smear terminate abruptly and not be drawn out into threads or irregular forms."

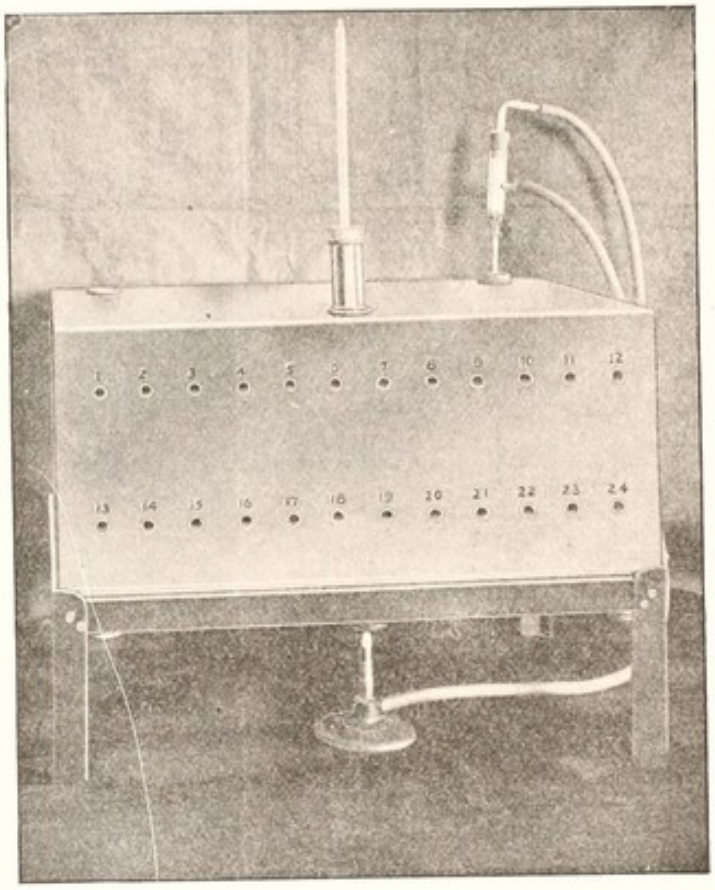


Fig. 101.—A small incubator of special design for opsonic work (Miller).

This mixing, incubating, and spreading is done twice—once with the serum of the patient, and once with the normal serum of the operator. The technic is the same each time. In order that the enumeration of the bacteria taken up by the leukocytes can be accomplished, it is next necessary to stain the blood smears. This can be done by any method that will demonstrate both the bacteria and the cells. For staphylococci and similar organisms, Leishman's stain, Jenner's stain, or J. H. Wright's stains are appropriate. Marino's stain, recommended by Levaditi,† gives beautiful results. For the tubercle bacillus the spreads may be stained with carbol-fuchsin

Fig. 102.—The smear (Miller).

* "Therapeutic Gazette," March 15, 1907.
† "Ann. de l'Inst. Pasteur," 1904, XVIII, p. 761.

and counterstained with methylene-blue, or perhaps better with gentian violet and counterstained with Bismarck brown or vesuvin.

The final step in the process is the enumeration of the bacteria in the corpuscles by averaging the number taken up by the cells. Only typical polymorphonuclear cells should be selected for staphylococcic cases, and separate averages made for polymorphonuclear and mononuclear cells in tubercle bacillus cases. It is best to follow certain routine methods of enumeration. Some who content themselves with a count of the number of bacteria in 20 cells, secure less accurate results than those who count 50 cells. It is usually best to count one-third of the cells in the central portion of the spread, one-third at the edge, and one-third at the end. In each portion no other selection of cells should be made than the elimination of other than polymorphonuclear cells and the elimination of all crushed or injured cells; the others should be taken one after the other, as they are brought into the field with the mechanical stage. After the bacteria included in each of the accepted number of cells selected as the standard has been enumerated, an average is struck.

The "opsonic index" is determined by dividing the average number in the patient's serum preparation by the average in the normal serum preparation.

Leishman's* studies of the phagocytic power of the blood show that in cases of furunculosis, etc., with each recrudescence of boils, there is a marked diminution of the phagocytic power of the blood, and with each improvement, a marked increase.

McFarland and l'Engle† found by an examination of the blood of 24 supposedly healthy students and laboratory workers that it was possible to prejudge, by the phagocytic activity of the cells, the past occurrence of suppuration and present liability to it.

Wright and Douglas use the opsonic index as a guide to the specific therapy of the infectious diseases. If the opsonic index is low they believe bacterio-vaccination is indicated. In its administration, however, care must be taken to administer a counted number of bacteria, and to make frequent opsonic estimations to determine the good or ill effects accomplished. Thus, the administration is always followed by a temporary diminution (negative phase) of the opsonic index, soon followed, if the dose be not too large, by a marked increase (positive phase). It is supposed, upon theoretic grounds, and proved by practical experience, that the increase of phagocytic activity brings about improvement. The care of the operator should be to avoid giving so large a dose of the vaccine that the negative phase will be so long continued that harm instead of good may be achieved.

Although Wright is said to cling to the study of the opsonic

* "Lancet," 1902, I, p. 73.
† "Medicine," April, 1906.

index as a guide to bacterio-vaccination and the resulting degree of immunity, the greater number of workers have abandoned it upon grounds which the writer long ago expressed—"that the estimation of the value of bacterio-vaccination by means of the opsonic index was a very complicated way of finding out very little."

CHAPTER XX

THE WASSERMANN REACTION FOR THE DIAGNOSIS OF SYPHILIS

This now popular and fairly reliable method for assisting in the diagnosis of atypical syphilitic infections was devised by Wassermann, Neisser, and Bruck.* It is a method of making the diagnosis of syphilis by demonstrating in the blood (cerebrospinal fluid, milk, or urine) of the patient a complement-fixing substance (antibody?) not present in normal blood.

The test is twofold: (1) A combination of syphilitic antigen, complement, and suspected serum. (2) A subsequent addition to the mixture of blood-corpuscles and hemolytic amboceptor. If the suspected serum contain the syphilitic antibody the antigen and complement unite with it, and the complement being thus "fixed," no hemolysis can take place upon the subsequent addition of the blood-corpuscles and hemolytic serum. If, on the other hand, the suspected serum contain no antibody, the complement cannot be fixed, and is, therefore, free to act upon the subsequently added blood-corpuscles in the presence of the hemolytic serum, and hemolysis results.

It is thus seen that the first test is made for the purpose of fixing the complement, and the second for the purpose of finding out whether it has been fixed or not.

It is quite clear that such a test is very delicate, and can only be successful when executed with great precision and with reagents or factors titrated, so that their exact value may be known.

CONSIDERATION OF THE REAGENTS EMPLOYED

I. For the first, or fixation, test it is necessary to bring together—
Syphilitic antigen.
Serum to be tested.
Complement.

(1) **The Syphilitic Antigen.**—It was supposed by Wassermann, Neisser, and Bruck, who first devised the test, that the syphilitic antigen must contain the essential micro-organisms of syphilis. No method for the cultivation of Treponema pallidum having at that time been devised, cultures of the specific micro-organism could not be employed. Histologists had, however, shown that greater numbers of the organisms were to be found in the livers of the congenitally syphilitic stillborn infants than anywhere else. With the

* "Deutsch. Med. Wochenschr.," 1906, No. 19.

purpose, therefore, of securing the greatest possible number of micro-organisms for the antigenic function, such livers were used. The tissue, having been cut into small fragments, was spread out in Petri or other appropriate dishes and dried, and the fragments rubbed to a fine powder with a mortar and pestle. Such a powder can be kept indefinitely in an exsiccator over calcium chlorid if placed where it is cool and dark. When the powder is to be used, 0.5 gm. is extracted either at room temperature or in the ice-box with 25 cc. of 95 per cent. alcohol for twenty-four hours, filtered through paper, and the filtrate used in quantities later to be mentioned.

Instead of drying the liver tissue, pulverizing, and then extracting it, many investigators now prefer to cut it up, rub it into a uniform paste with a mortar and pestle, and add 5 volumes of 95 per cent. or absolute alcohol, with which the paste is thoroughly macerated and shaken many times or in a shaking machine. The alcohol may then be filtered off, or may be permitted to remain upon the sedimented liver tissue remnants, and the clear supernatant fluid pipeted off and diluted, at the time of employment, with the isotonic sodium chlorid solution. When this alcoholic extract is added to the salt solution a turbidity occurs, but this must not be filtered out, as it consists of the lipoids or other substances in the extract that are essential to the test, and the quantity of the cloudy fluid in the final mixtures is so small as not in any way to interfere with the results. The small amount of alcohol in the diluted extract is negligible and has no influence upon the reagents used for the test.

The mention of the lipoids now brings us to the point where it seems advisable to state that one of the most interesting facts about the Wassermann reaction is that its theoretic basis was founded upon the erroneous assumption that the essential antigenic substance consisted of the whole or fragmented treponemata in the liver extract. The method scarcely began to meet with practical application, however, before it was discovered that the active antigenic substance was soluble in alcohol, was present in other than syphilitic livers, and could be extracted not only from human tissues, but also from dogs' livers and from guinea-pigs' hearts. Porges and Meier, indeed, found that lecithin could play the rôle of syphilitic antigen, and Leviditi and Yamanouchi place sodium glycocholate, sodium taurocholate, protogon, and cholin among those bodies capable of acting as syphilitic antigens, and Noguchi goes so far from the original that he regularly employs an extract of the normal guinea-pig's heart as the antigen to be employed in his modification of the test.

These discoveries now make it clear that the complement fixation that takes place in syphilis is not identical with that of the Bordet-Gengou reaction, in which it had its beginning. Happily, however, the error does not destroy the usefulness of the method for diagnosis.

The probable nature of the reaction will be described below. For

the present we must be content to follow the beaten path, and for this purpose will use the congenitally syphilitic liver extract as the antigen, preparing it as described above.

(2) **The Serum to be Tested.**—Wassermann, Neisser, and Bruck

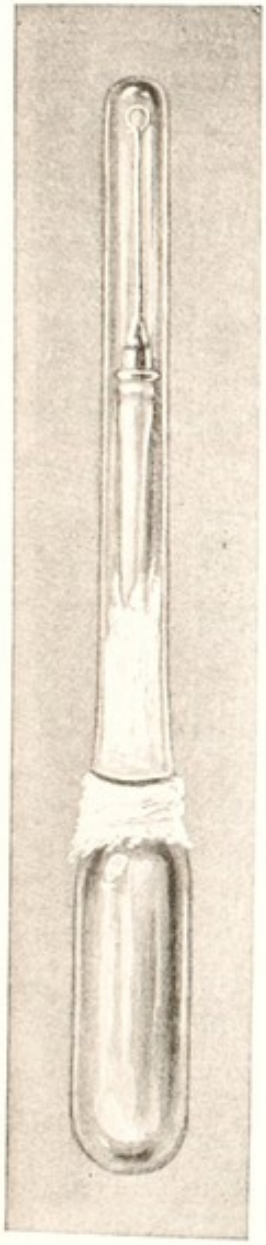


Fig. 103.—The Keidel tube for collecting blood. (Manufactured by the Steele Glass Co., of Philadelphia.)

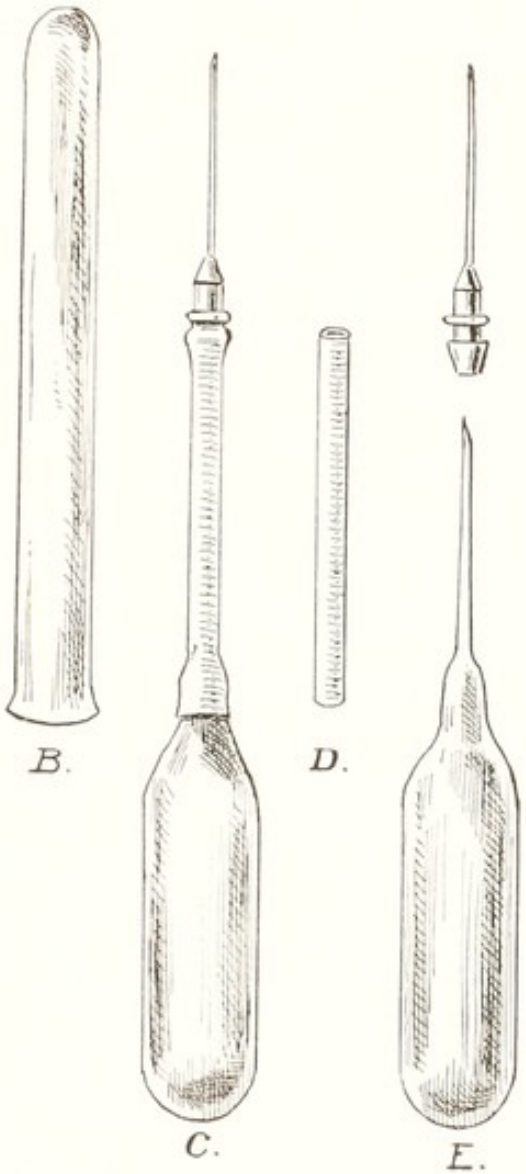


Fig. 104.—Parts of the Keidel tube. *E* is the vacuum bulb which is attached to the needle by a piece of rubber tubing (*D*); the glass tube (*B*) covers the needle and the whole is sterilized. (Kolmer.)

at first employed the cerebrospinal fluid, but now the blood-serum of the suspected patient is almost universally used. As is usual with antibodies, the substances engaging in the complement-fixation test are widely distributed throughout the body, and reach the

cerebrospinal fluid, the milk, the urine, and the other body fluids through the blood, in which it exists in greatest concentration. The blood is, moreover, readily obtainable for study, which is another reason it is at present used for making the test under all ordinary circumstances. Noguchi, who works with very small quantities of the reagents, secures the blood by obstructing the venous circulation of the thumb or of a finger by means of a rubber band (see directions for obtaining the blood for making the opsonic index) but the greater number prefer to obtain it by introducing a large hypodermic needle into one of the veins near the bend of the elbow. The arm above the elbow is compressed by a fillet, as though for the purpose of performing phlebotomy, and a conspicuous vein selected for the purpose. The skin is first carefully washed, then treated with tincture of iodin. If the patient is nervous, a momentary spraying with chlorid of ethyl will make the operation entirely painless. Some prefer to use the iodin without the preliminary washing, believing that soap makes it difficult for the iodin to effect satisfactory disinfection of the skin. The sterilized needle is thrust into the vein, care being taken that the vein is not too compressed and the point of the needle thrust entirely through instead of into it. From 15 to 25 cc. of blood may be withdrawn in a Keidel tube, or into a large syringe or may be allowed to flow into a sterile test-tube. The blood, however secured, is permitted to coagulate and the clear serum removed by a pipette, or the clotted blood is placed in a centrifuge tube and whirled, so that clear serum is secured in a few minutes.

As normal human blood-serum, when fresh, contains a certain amount of complement which would interfere with the success of the experiment, the serum is next placed in a test-tube and kept in a water-bath between 55° to 58°C. for a half-hour. This degree of heat destroys the complement and leaves the complement-fixing substance uninjured. The serum is now ready for use.

(3) **The Complement.**—The complement generally employed is contained in the blood of a healthy adult guinea-pig. To obtain it a piece of cotton moistened with ether or chloroform is held to the guinea-pig's nose until it becomes unconscious, when the head is forcibly extended and a longitudinal incision made through the skin of the neck. The skin is then drawn back between the finger, on the one side, and the thumb, on the other side, of the operator's left hand, while, with a sharp knife held in the right hand, he cuts through all the tissues of the neck down to the spinal column and thus opens both carotid arteries. The spurting blood is caught in a sterile Petri dish and the animal permitted to bleed to death. The blood soon coagulates when undisturbed, and in a short time clear serum exudes from the clot. As, however, the complement seems to be at least in part derived from the corpuscles, the serum should not be removed as soon as it forms, but permitted to remain in contact with the clot for three hours. If it is desired to save

time, the clot, as soon as formed, may be cut into strips and placed in the tubes of a centrifuge and whirled for a half-hour. This secures a greater quantity of the serum and at the same time gives it its full value, probably by injuring the leukocytes.

Such serum containing the complement is useful for twenty-four hours. Longer it should not be kept or used, as it begins to deteriorate almost at once, and the deterioration increases in rapidity in proportion to the length of time it is kept. The quantity of the complement in the serum of the guinea-pig is fairly constant, when the animal is regularly fed, and furnishes a fairly uniform reagent that requires no titration.

II. For the second, or hemolytic, test two additional reagents are required:

Blood-corpuscles to be dissolved.

Hemolytic amboceptors by which complement may be united to them.

(4) **The Blood-corpuscles.**—It makes no difference what kind of blood-corpuscles are employed. Ehrlich and Morgenroth, in their pioneer experiments into the mechanism of hemolysis, used goat corpuscles. Bordet used rabbit corpuscles; Wassermann, Neisser, and Bruck, sheep corpuscles; Detre, horse corpuscles; Noguchi, human corpuscles.

As those who do many tests require a considerable quantity of blood, it seems wisest to make use of some kind that is readily obtainable in any quantity, hence most investigators now follow Wassermann and his collaborators and use sheep blood, which is easily obtained at a slaughter-house or from sheep kept for the purpose.

The flowing blood is caught in some open receptacle, stirred until it is defibrinated (it must not be permitted to coagulate), and then taken to the laboratory.

The corpuscles must next be washed with care, so as to free them from all traces of amboceptors and complement belonging to the serum in which they are contained. For this purpose a centrifuge is indispensable. The tubes of the apparatus are filled with the defibrinated blood and then whirled for fifteen minutes until the corpuscles form a compact mass below a fairly clear serum. The serum is then cautiously removed and replaced by 0.85 per cent. sodium chlorid solution, the top of each tube closed by the thumb, and vigorously shaken so as to distribute the corpuscles throughout the newly added fluid. The tubes are next returned to the centrifuge and again whirled until the corpuscles are sedimented, when the fluid resulting from this first washing is removed and replaced by fresh salt solution, in which the corpuscles are again thoroughly shaken up. They are now again whirled until again sedimented, when the second washing is removed, leaving the corpuscular mass undisturbed. Some prefer to give the corpuscles a third washing,

but it does not seem to be necessary. Of the remaining corpuscular mass, 5 cc. are added to 95 cc. of salt solution to make a 5 per cent. volume suspension, in which form they are ready for use. As the corpuscles of healthy sheep thus treated form a practically invariable unit, no titration or other preliminary is needed before they are used. They must, however, be used within seventy-two hours to secure satisfactory results, as they tend to soften when kept and so to lose their standard value. If kept longer than twenty-four hours they should be washed before using.

(5) **The Hemolytic Amboceptor.**—As the validity of the test depends upon the ability or inability of the complement to dissolve the corpuscles, and as this can only be achieved when appropriate amboceptors are added, the hemolytic amboceptors must correspond to the kind of blood-corpuscles employed in the experiment. As has been shown, the greater number of investigators now employ sheep corpuscles, hence must use such corpuscles as the antigen through whose stimulation the amboceptors or antibodies are excited.

The usual method of obtaining the amboceptor is in the blood-serum of an experimentally manipulated rabbit. A large healthy rabbit is employed for the purpose, and is given a series of intraperitoneal injections of the 5 per cent. suspension of washed and sedimented sheep corpuscles prepared as above described. These injections are usually given about five days apart, and the dosage is usually 5, 10, 15, 20 and 25 cc. respectively.

A serum of higher amboceptor content may be prepared by using a greater number of corpuscles, and for this purpose the solid corpuscular mass thrown down by centrifugalization after the second washing is employed. Of this, 2, 4, 8, and 12 cc., diluted with just enough salt solution to make it pass readily through the hypodermic needle, may be regarded as appropriate doses, the intervals being the same, viz., five days. The amboceptor content of the rabbit serum seems to be greatest about the ninth or tenth day after the last injection. Much care must be taken to see that the injected fluid is sterile and the operations performed under aseptic precautions, as the rabbits are easily infected and not infrequently die. They also seem prone to die after the last injection, so that it is best to have more than one rabbit under treatment at a time.

When the appropriate time has arrived, the rabbit is bled from the carotid artery, according to the directions given in the chapter upon Experiments upon Animals.

The blood thus obtained is permitted to coagulate, and the serum, which should be clear, removed with a pipette. More serum may be obtained from the clot by cutting it into strips, placing these in a centrifuge tube, and whirling them for fifteen minutes.

Having thus described the preparation of the reagents to be employed in making the Wassermann test, the next step, that of titrating them, becomes essential. One of the first questions that pre-

sents itself is how successful titration of reagents that may all be more or less variable can be effected. To achieve this it is necessary to begin with those that can be assumed to be least variable and work up to those that are most so.

(1) *The Sheep Corpuscles.*—As these come from a healthy animal, are always treated in precisely the same manner and used under standard conditions of freshness, they can be looked upon as an invariable factor. 1 cc. of the 5 per cent. suspension forms a good working quantity and constitutes the *unit.*

(2) *The Normal Guinea-pig Serum Containing the Complement.*—As this also comes from a normal animal, is always treated in precisely the same manner, and is also used under standard conditions of freshness, etc., it may also be looked upon as a factor subject to very slight variation. Of this serum, 0.1 cc. (1 cc. of a 1:10 dilution, made with physiological salt solution) forms the *unit*, or working quantity.

These two reagents, therefore, may be regarded as the standards of measurement through which the titer of a third is made possible.

(3) The *hemolytic serum* from the rabbit treated with the sheep corpuscles.

This is subject to very great variation, according to the treatment of the rabbit, and apparently, also, according to the ability of the individual rabbit to respond to the treatment by the formation of hemolytic amboceptors. It is, therefore, imperative to make a careful titration of it.

To do this we proceed as follows, the quantities recommended being such as experience has proved most satisfactory:

Into each of a series of common test-tubes or culture-tubes 1 cc. of the 5 per cent. suspension of sheep corpuscles and 1 cc. of the 1:10 dilution of the normal guinea-pig serum (complement) are measured with graduated pipettes, and then to each of these tubes the rabbit serum (amboceptor), diluted with physiological salt solution so as to make the correct measurement of the minute quantities necessarily employed a matter of ease and convenience, is added in diminishing quantities for the purpose of determining the least quantity that will bring about complete hemolysis in two hours at the temperature of 37°C. The occurrence of the hemolysis is shown by a very striking change in the appearance of the fluids. The mixture is at first opaque and pale red, but after hemolysis, or solution of the red corpuscles, becomes a beautiful transparent Burgundy wine red.

The actual "set-up" or working scheme for determining the *unit* or least hemolyzing addition of the amboceptor serum may be represented as follows, the tubes being placed in a thermostat and observed every fifteen minutes:

Five per cent. suspension of corpuscles.	Normal guinea-pig serum.	Hemolytic rabbit serum.	Result (final readings after two hours).
1 cc.	0.1 cc.	0.01 cc.	Complete hemolysis.
1 "	0.1 "	0.005 "	" "
1 "	0.1 "	0.002 "	" "
1 "	0.1 "	0.001 "	" "
1 "	0.1 "	0.0005 "	" "
1 "	0.1 "	0.0003 "	Partial "
1 "	0.1 "	0.0002 "	No "
1 "	0.1 "	0.0001 "	" "

After the reagents are added, enough 0.85 per cent. salt solution is added to each tube to bring the total bulk of the mixture up to 5 cc.

From the results shown in the tubes it is evident that the hemolyzing quantity of the rabbit serum lies between 0.0005 and 0.0003 cc., and is probably 0.0004 cc. To be as accurate as possible, a second series of experiments should be made with 0.0005, 0.00045, and 0.0004 cc., so that the proportion of amboceptor serum necessary to effect hemolysis be known within small limits. This least quantity, that will certainly cause hemolysis in two hours at 37° C., is known as the *unit*. The combination of the unit of corpuscular suspension (1 cc.), the unit of complement (0.1 cc.), and the unit of hemolytic amboceptor is known as the *hemolytic system*.

As soon as this unit is known accurately, we are in position to reverse the conditions of the test. Thus, if we should desire to know how much variation there may be in the complements from different animals under different conditions of age, feeding, health, etc., we can now do so by determining whether, when 1 cc. of the corpuscles, 1 unit of amboceptor and varying quantities of complementary serums are combined, any variation in the final results will obtain.

Or, if we desire to know to what extent the sheep corpuscles may change through prolonged keeping or other manipulation, it can be done by maintaining the unit of amboceptor and the unit of complement and adding larger or smaller quantities of the corpuscles.

The conditions under which the unit of amboceptor is titrated constitute the standard conditions of the Wassermann reaction. In it are always employed 1 unit of sheep corpuscle suspension, 1 unit of complement, and 1 unit of amboceptor. Here, however, a slight difference of opinion is reached, it being argued by many experimenters that such exact proportions may make the test uncertain, because, should there be the slightest tendency on the part of the remaining reagents to inhibit hemolysis by means other than complement fixation, it would result in positive readings where the final result should be negative. To overcome this possibility, they differentiate between the amboceptor *unit* and the amboceptor *dose*, the latter being commonly twice and sometimes four times the unit.

Now, though the amboceptor unit is determined by the method given, it by no means follows that those proportions are the only

ones that will lead to hemolysis. By increasing the amboceptor we can diminish the complement with the same end-result, a matter that has been graphically shown by Noguchi,* who says "that hemolysis is merely the relative expression of the combined action of amboceptor and complement, and is not the absolute indication of the amount of the hemolytic components present in the fluid. The same amount of hemolysis can be produced by 1 unit of complement and by 1 unit of amboceptor as by 20 units of amboceptor and 0.1 unit of complement or any other appropriate combination of these two components."

As in the performance of the test we work always with 1 unit of complement, we do not want to unduly disturb its proper proportional action by any excessive addition of amboceptor, but simply to increase the latter sufficiently to provide for the accidental presence, in the serum to be tested, of substances affecting hemolysis. Fortunately, means are provided for controlling this action, as will be shown below.

The amboceptor serum keeps indefinitely. When it is to be kept and used from time to time, many experimenters prefer to seal it in a number of small tubes, one of which is opened when the serum is needed, the remainder being kept in an ice-box. Others prefer a stoppered bottle that can be opened and a measured quantity removed as needed. The most convenient way of treating it seems to be Noguchi's method of drying it upon filter-paper.

For this purpose a good quality of filter-paper is cut into strips 10 to 20 cm. in length and 6 to 8 cm. in breadth, and saturated with the serum, which is permitted to dry. It is well to make a preliminary titration of the serum, for if it be very active it may have to be diluted in order that the piece of dry paper containing the dose be of a size convenient to handle; 1 drop of serum usually covers about ½ sq. cm., which is about as small a piece as can be measured, cut, and used with satisfaction if sufficient allowances are to be made for variations in distribution and other conditions that may modify the accuracy of the method. If the unit-strength of a serum be, say, 0.00005 and the dose 0.0001, *water* should be added to the extent of about 9 volumes and the mixture gently agitated, so that diffusion may occur without frothing. The diluted serum is poured into a large flat dish, and the strips of paper passed lengthwise and slowly to and fro until not only wet, but thoroughly saturated. Each strip, when the dipping is finished, is held first by one end, then by the other, to drain off the free drops, and then laid flat upon a clean glass plate and permitted to dry. The use of an electric fan is recommended to hasten drying. Paper so prepared contains everywhere about the same quantity of serum.

The real titration of the serum now begins. With a ruler, one piece of paper is divided into squares of, say, ½ cm., and a series of tubes prepared with cor-

* "Serum Diagnosis and Syphilis," 1910, p. 13 et seq.

puscle suspension and complement and the paper added 1 square, 2 squares, 2½ squares, and so on until the unit is determined. When that is achieved, the exact size of the paper containing the unit being known, one sheet of the paper can be ruled into squares of that size or into squares of twice that size—since the "dose" is two units—at the option of the investigator.

The sheets of paper are kept in a clean envelope, the quantity for each test being cut off as needed. The dry serum changes so little that the dose once determined, the size of the square of paper needed for the test remains about the same.

The method has the advantage that the amboceptor serum cannot be spoiled or spilled. It has the disadvantage of being slightly less accurate, though it must be admitted that the chances of error in measuring and diluting the fluid serum are probably as great as those arising from inequalities in the distribution of the serum throughout the paper.

(4) *The Antigen.*—It has already been shown that complement is labile, and it may have occurred to the reader that its activity is similar to that of ferments. It is now necessary to point out the many conditions (some of which may arise in the performance of a test so delicate as the Wassermann reaction) by which the complementary action may be affected or set aside. Thus, temperature affects it, and temperatures of 0°C. suspend it. It is on this account that the test is always made at 37°C. Like most of the ferments of the living organism, salts affect it, and in salt-free media its action ceases, to return when a small quantity of an alkaline salt is added. Not only inorganic salts, but salts of the fatty acids and the bile-salts may inhibit it. Certain lipoids, such as lecithin, cholesterin, protogon and tristearin, and neutral fats inhibit the complementary action. Some of these substances are always present in the serum containing the complement itself or in the other serums to be tested by its use, and, as Wassermann and Citron have pointed out, we really know nothing about complementary action. Aleuronat, inulin, peptone, albumose, tuberculin, natural and artificial aggressins, gelatin, casein, sitosterin, coagulated serum-albumin, and albuminous precipitates all act as inhibitives to complementary action.

Now, in all combinations of several serums and antigens it is always possible that some of these complement-binding or complement-inhibiting substances may be present, hence the first thing that has to be done in the way of titrating the antigen—which is a tissue extract, rich in lipoids which inhibit complementary action—is to determine how much of it can be added to the "hemolytic system" without disturbing hemolysis.

As, however, the antigen is not used by itself, but always in combination with a serum to be tested, we must always combine it with serum when making the titration, so that the requirements of the test may be conformed with. In order that the essential difference between the normal serum and the syphilitic serum can be reduced

to precise calculation it is imperative that, in all the tests, the same quantity of added serum be employed. Experience has shown this quantity to be 0.2 cc., and this we regard as the *unit* of serum to be tested.

To titrate the antigen we require (1) a normal human serum and (2) a known syphilitic serum, obtained from blood drawn from the arm veins of cases known to be well and cases known to be syphilitic respectively. These serums should be kept on hand in the laboratory in considerable quantity, as they are constantly needed for making the controls that must accompany each test, as well as for making the preliminary titration of the antigen.

TABLE I.—*Series with the Normal Serum*

Tubes									
1.	1 unit of complement	+	1 unit of normal serum	+	antigen	0.01	(These being mixed, are stood in the thermostat at 37°C. for one hour, then to each tube are added 1 unit of sheep corpuscles and 1 dose of amboceptor serum, after which the tubes are returned to the thermostat for another hour, when the following readings are obtained.)	=	Complete hemolysis.
2.	"	+	"	+	"	0.03		=	"
3.	"	+	"	+	"	0.05		=	"
4.	"	+	"	+	"	0.07		=	"
5.	"	+	"	+	"	0.08		=	"
6.	"	+	"	+	"	0.09		=	"
7.	"	+	"	+	"	0.01		=	"
8.	"	+	"	+	"	0.12		=	"
9.	"	+	"	+	"	0.15		=	"
10.	"	+	"	+	"	0.18		=	"
11.	"	+	"	+	"	0.2		=	No hemolysis.

TABLE II.—*Series with the Syphilitic Serum*

Tubes									
1.	1 unit of complement	+	1 unit of syphilitic serum	+	antigen	0.01	(These being mixed, are stood in the thermostat at 37°C. for one hour, then to each tube are added 1 unit of sheep corpuscles and 1 dose of amboceptor serum, after which the tubes are returned to the thermostat for another hour, when the following readings are obtained.)	=	Complete hemolysis.
2.	"	+	"	+	"	0.03		=	"
3.	"	+	"	+	"	0.05		=	Suggestion of hemolysis.
4.	"	+	"	+	"	0.07		=	Slight hemolysis.
5.	"	+	"	+	"	0.08		=	Partial hemolysis.
6.	"	+	"	+	"	0.09		=	No hemolysis.
7.	"	+	"	+	"	0.1		=	"
8.	"	+	"	+	"	0.12		=	"
9.	"	+	"	+	"	0.15		=	"
10.	"	+	"	+	"	0.18		=	"

The "set-up" for the titration of antigen is fairly simple. A series of tubes is prepared and divided into two groups. Into each tube in each group is placed 1 unit of complement. Each tube of one group receives the addition of 0.2 cc. of the normal serum; each tube of the other group, 0.2 cc. of the known syphilitic serum. All the tubes now receive additions of antigen, so that one tube of each group contains the same quantity. The quantity of antigen not being known, it is only through the experience of others that we can guess where to start. An idea can be formed through study of the tabulation on page 289.

From this we find that the unit of antigen is 0.09 cc., the largest quantity of the antigen that can be added without preventing hemolysis when the normal serum is used is probably 0.18 cc. At the same time 0.09 cc. is the smallest quantity that can be added, when the syphilitic serum is used, to prevent it. In this case the dose exactly fulfils Kaplan's requirement that "The unit dose of antigen must completely inhibit hemolysis . . . of a known luetic serum, provided double the dose does not interfere with the complete hemolysis of cells using a known normal serum and complement."

We have now accomplished the titration of all five of the factors involved in making the Wassermann reaction, but we have done more, we have really done the test, and have seen positive and negative results, for in titrating the antigen we have developed the reaction by which we can confirm the diagnosis of syphilis in the case from whom the syphilitic serum was obtained, and have failed to develop it with the known normal serum.

However, in order that those who perform the test may be able to escape the numerous errors into which one may fall, it will be necessary to point out the controls by which they can be avoided.

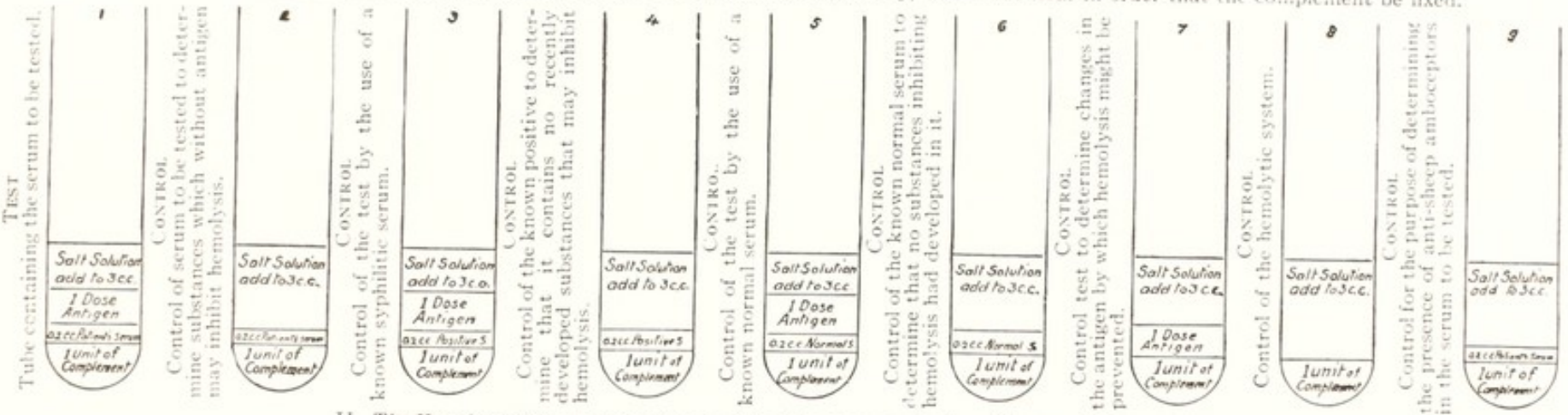
A Wassermann reaction at the present time comprises not only the test of the patient's serum, but simultaneously includes a long series of other tests by which the validity of every part of the test and the correct titer of all the reagents employed can be simultaneously ascertained. Every one who makes the test should practice some such systematic method as is suggested by the following scheme for the "set-up." Nine tubes are employed for the usual test. These are stood in a rack in the same order for every test, and in the course of time it becomes a matter of habit to know the tubes by number, and to recall for what each stands.

If many tests are to be made at one time, it is, of course, unnecessary to make more than one series of controls.

Of the complementary serum we add 1 cc. to 9 cc. of 0.85 per cent. (physiologic) salt solution, making each cubic centimeter of the dilution of the fluid equal 0.1 cc. This quantity, carefully measured by the same volumetric pipette, is dropped into each tube, and this pipette laid aside.

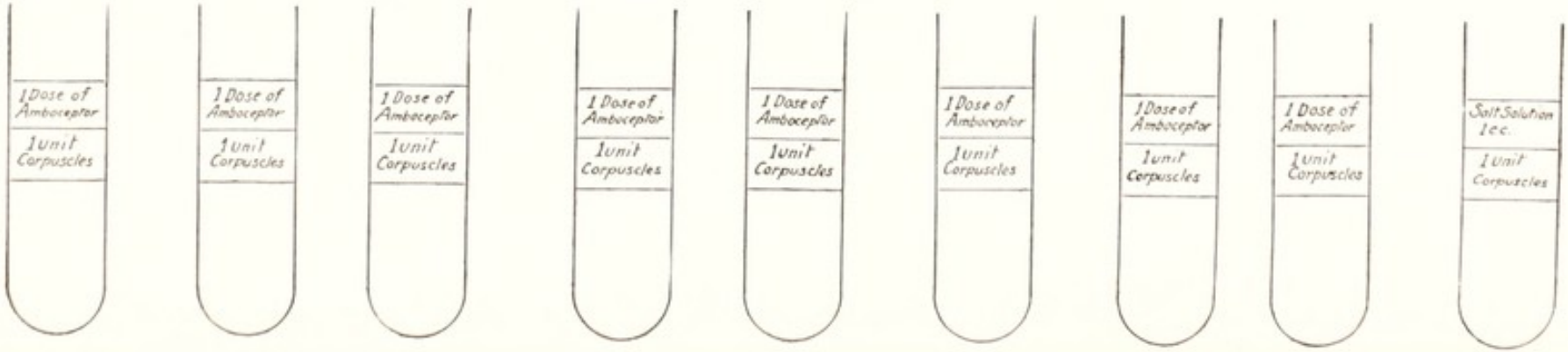
I. *The Fixation Test*, the first part of the Wassermann Test.

Each tube receives the reagents indicated, and is then stood in the thermostat at 37°C. for one hour in order that the complement be fixed.



II. *The Hemolytic Test*, the second part of the Wassermann Test.—*The same tubes as above.*

Each tube receives the additions indicated below, and is then again stood in the thermostat at 37°C. for two hours in order that the occurrence or non-occurrence of hemolysis may show whether the complement had previously been fixed or not. If the complement be fixed there is no hemolysis in tubes 1 and 3, and the patient has syphilis (within such limitations as the validity of the test necessitates).



The serum to be tested is drawn into a second finely graduated pipette, and 0.2 cc. added to tubes 1, 2, and 9, and that pipette laid aside.

The positive syphilitic serum used to control the test is similarly drawn up in a fresh pipette and 0.2 cc. of it measured into tubes 3 and 4, and the pipette laid aside.

The normal serum used as a control is similarly drawn into still another pipette and 0.2 cc. measured into tubes 5 and 6, and the pipette laid aside.

The alcoholic extract composing the antigen is next added, either by diluting it so that 1 cc. contains the unit, or measuring the unit quantity directly into the tubes. The antigen is added to tubes 1, 3, 5, and 7, and the pipette laid aside.

Lastly, each tube receives a correctly measured quantity of 0.85 per cent. sodium chlorid solution to bring the total bulk of fluid up to exactly 3 cc.

Each tube is now shaken carefully, so as not to cause frothing of the fluid, and the rack is stood in a thermostat kept at 37°C.

At the end of an hour the rack is removed, and every tube receives the addition of 1 unit of the sheep corpuscle suspension and, with the exception of tube 9, receives one dose of amboceptor, either the serum measured by diluting so that 1 cc. equals the dose, or the necessary square of paper. This, in the former case, brings the total bulk of fluid to 5 cc., in the latter makes it necessary to add 1 more cubic centimeter of salt solution to each tube. We aim to have exactly 5 cc. of fluid in each tube.

The tubes are again stood in the thermostat, where they are permitted to remain for an hour, when the readings are taken and carefully noted. After this the rack and all the tubes are placed in the ice-box until twenty-four hours old, when the final readings are taken and the conclusions are reached.

As a rule, the readings taken after the second hour of incubation and those taken after twenty-four hours correspond.

A valid test should show the following:

	Tubes	
Test Controls.	1. *No hemolysis in syphilis.*	*Hemolysis in health.*
	2. Complete hemolysis.	
	3. *No hemolysis* (this is the standard of comparison).	
	4. Complete hemolysis.	
	5. "	"
	6. "	"
	7. "	"
	8. "	"
	9. No hemolysis, as a rule.	

In the tubes in which hemolysis takes place the change is very marked. The hemoglobin dissolves out of the corpuscular stroma and saturates the fluid, transforming it from the opaque pale red to a transparent Burgundy red. Sometimes the corpuscular

stroma dissolves, sometimes it sediments as a colorless mass to the bottom of the tube.

In the tubes containing the positive or syphilitic serum, and in which there is complete complement fixation, the unaltered corpuscles sediment to the bottom of the tube, leaving a colorless fluid above.

When the complement fixation is complete there is no solution of the hemoglobin. Such a result has been described by Citron as + + + +. When the sedimented corpuscles lie at the bottom of a slightly reddened fluid, the result is said to be + + +; when at the bottom of a distinctly red fluid, + +, etc. Confusion will be avoided by making reports as *positive* in all cases in which there is

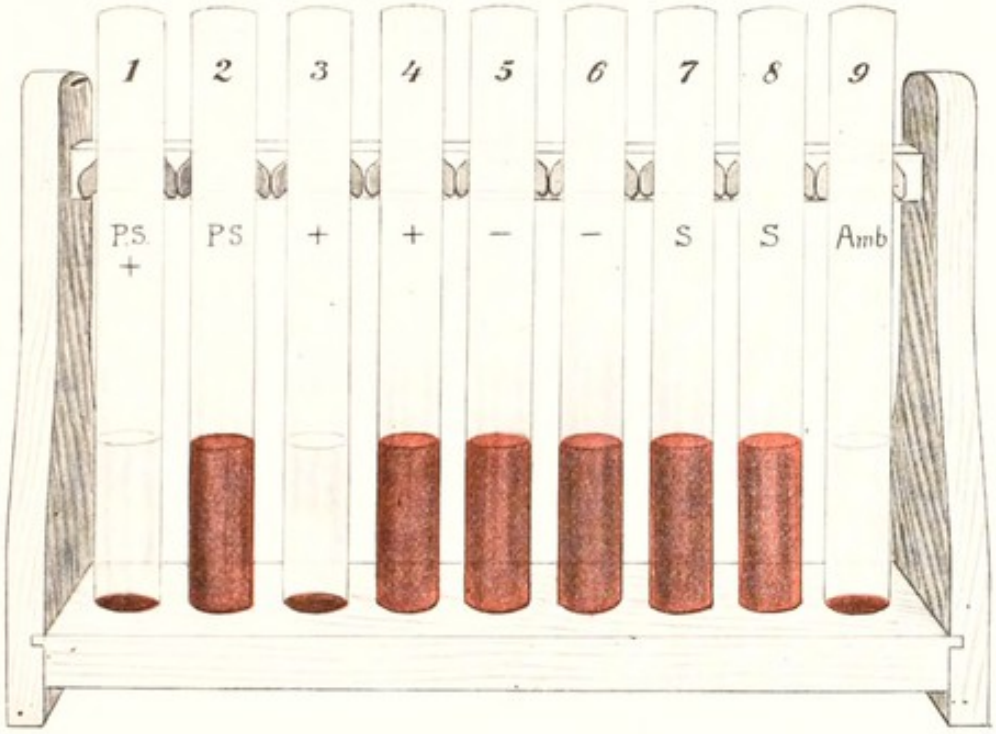


Fig. 105.—A typical positive Wassermann reaction with the recommended controls as it appears after standing twelve hours. Corpuscular sedimentation without hemolysis is seen in tubes 1, 3, and 9; complete hemolysis in the others.

a distinct red corpuscular deposit, regardless of the state of the supernatant fluid, and *negative* when there is no such deposit.

When we come to inquire why the supernatant fluid should be red, we reach a question that is not quickly answered. In order to be in a position to explain it in certain cases we introduced in our series tube 9, by which to discover whether the serum under examination contain, as is sometimes the case in health as well as in syphilis, sheep corpuscle amboceptors. If tube 9 shows such amboceptors to be in the serum, it explains the redness of the fluid bathing the corpuscles, and does not invalidate the test. If no such amboceptors are present and the fluid is still red, it may indicate that a little of

the complement remained unfixed and acted upon a few of the corpuscles.

The Validity of the Test.—The Wassermann reaction is not a certain test for syphilis. It is an aid in making the diagnosis, especially in cases in which there are no symptoms.

Of thousands of bloods of normal persons examined, the results are almost 100 per cent. negative. Basset-Smith has had a positive reaction in a case of scarlet fever and one in a case of malignant disease of the liver with jaundice; Oppenheim, one in a case of tumor of the cerebellopontine angle; Marburg, one in a similar case; Newmark reports 2 cases of brain tumors with positive reactions; Cohn, a positive in a patient with a cerebral tumor. The Wassermann reaction is of no value for the differential diagnosis of syphilis and frambœsia or yaws. All cases of the latter give a positive reaction. Positive reactions have been found in some cases of nodular leprosy, in a few cases of malaria, in some cases of pellagra, and in a good many cases of sleeping sickness. These seem to form the greater part of positive reactions in non-syphilitics thus far recorded.

In active syphilis Wassermann had 90 per cent. of positive reactions in 2990 cases; and most others report about the same. Basset-Smith in 458 such cases found 94 per cent. positive reactions.

In latent syphilis Wassermann found 50 per cent. positive reactions; Basset-Smith, 46 per cent.

In chronic, presumably syphilitic, disease of the nervous system, general paresis, and tabes dorsalis the positive reactions vary. In the former disease some have found as high as 90 per cent. positive; in the latter the usual figures vary about 50 per cent.

It is thus seen that the occurrence of the reaction is much more conclusive evidence of the presence of syphilitic infection than the failure of the reaction is of its absence.

Treatment greatly influences the test. When under active treatment, either with mercury and iodids or with salvarsan, the reaction of the serums is usually negative.

Nature of the Reaction.—We now reach the point of considering the nature of the reaction. It is certainly not a variation of the Bordet-Gengou phenomenon. It does not occur because of the presence in the blood of syphilitics of antibodies which combine with the antigen and fix the complement. It is probably not complement fixation so much as complementary inhibition, through the presence in the blood of syphilitics of certain metabolic products, whose action interferes with the complement in some entirely different manner.

NOGUCHI'S MODIFICATION OF THE WASSERMANN REACTION

Noguchi* has modified the Wassermann reaction, first by employing as an antigen an extract of the heart of a normal guinea-pig,

* "Serum Diagnosis of Syphilis," Philadelphia, 1910, J. B. Lippincott Co.

and, second, by making use of human instead of sheep corpuscles for the hemolytic test. The advantage of the latter depends upon the fact, carefully determined by Noguchi, that human blood-serum contains no amboceptors active in effecting hemolysis of human blood-corpuscles, though it not infrequently contains hemolytic amboceptors for sheep corpuscles. In the directions for making the Wassermann test a control test for determining their presence or absence was found expedient. It will also be remembered that the presence of these amboceptors causes no invalidity of the test, provided it be recognized.

Noguchi also varies the technic in such a manner that very small quantities of the various reagents are employed—a necessity that arises from the relatively small quantity of the patient's blood obtainable according to the method he employs. The reagents employed are as follows:

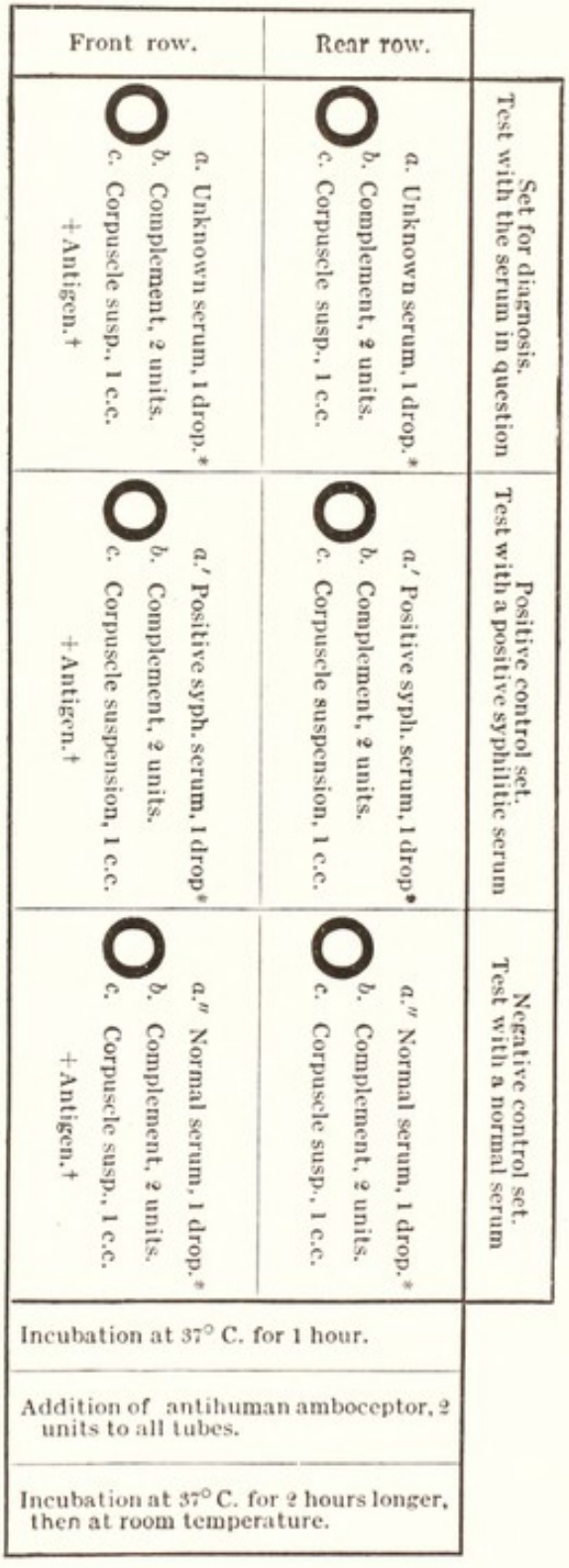
(1) *The Serum to be Tested.*—To obtain this, Noguchi binds the finger of the patient with a rubber band, makes a good-sized puncture near the root of the nail with a Hagedorn needle, and collects about 2 c c. of the blood in a Wright tube (see directions for making the opsonic index). The blood soon coagulates in the tube, which is then scratched with a diamond or file, broken, and the serum removed with a capillary pipet. The serum may or may not be inactivated by heat, according to the option of the experimenter. The dose of the unheated serum is 1 drop; of the inactivated serum, 4 drops. The same doses of the normal and syphilitic control serums are used.

(2) *The Complement.*—This consists of fresh guinea-pig serum. Of it he makes a 40 per cent. dilution in physiologic salt solution by adding one part of the serum to 1½ parts of the salt solution; 0.1 cc. is the unit. Two units constitute the "dose."

(3) *The Antigen.*—The antigen is made, according to the directions given in the description of the Wassermann test, out of normal guinea-pig heart. The extract is dried upon filter-paper, as has been recommended for the hemolytic amboceptor, and titrated according to the size of the square of paper needed, instead of the quantity of fluid to be added.

(4) *The Corpuscle Suspension.*—For this purpose either normal human corpuscles or the corpuscles of the patient whose blood is to be examined may be employed. Instead of a 5 per cent. suspension a 1 per cent. suspension is recommended. If normal corpuscles are employed, it is necessary to wash them free of the normal serum or plasma, which Noguchi accomplishes as follows: 8 cc. of normal salt solution are placed in a large test-tube, and the blood flowing from a puncture (in the operator's own finger, for example) permitted to drop in, the proportion being 1 drop each 4 cc. The fluid is then shaken and stood on ice over night, when the corpuscle sediment and the supernatant fluid containing the fibrin factors and ferment

is decanted and replaced by fresh salt solution, and the suspension made by shaking. Or, in a laboratory, the corpuscles can be



* When working with inactivated serum, 4 drops (0.8 cc.) should be employed; with cerebrospinal fluid, 0.2 cc. (not inactivated) is used.

† When using unheated serum, pure lipoids prepared by Noguchi's method should be used; with inactivated serum aqueous, alcoholic, or artificial antigen (Sachs and Roudoni) may also be used.

(This diagram and reading matter are reproduced from "Serum Diagnosis of Syphilis," by Hideyo Noguchi, M. D., with the kind permission of the publishers, J. B. Lippincott Co.)

washed as usual with the aid of the centrifuge. If the patient's own corpuscles are to be employed, some of them may be distributed, through the serum without any washing, by simply shaking

it up a little with the clot. It is not essential exactly to measure the corpuscles, as after a few trials with the suspension of normal corpuscles the eye becomes accustomed to the color, intensity, and density corresponding to the requirement.

(5) *The Antihuman Hemolytic Amboceptor.*—This is prepared by injecting rabbits, according to the method already described, with washed *human* corpuscles obtained from fresh human placentæ or from the heart of a fresh cadaver come to autopsy. The serum of the rabbit, when obtained, is dried upon blotting-paper and titrated as already described.

The "set-up" for the test, as given by Noguchi, is less cumbersome than that recommended for the Wassermann test and includes six tubes. It can best be understood by reference to the diagram.

The method recommends itself through its simplicity and convenience, no sheep corpuscles being used, and through the smaller quantity of blood required, it seeming to the patient that less damage is done by pricking the finger than by introducing a syringe needle into a vein. It is, moreover, a very sensitive test, and gives very accurate results as far as regards positive cases. Unfortunately, it seems to have the demerit of occasionally finding the reaction in negative cases, which is a serious defect.

Diagnosticians are still divided in opinion, some preferring the Wassermann test, some the Noguchi test, and some always doing both, permitting the one to control the other. In the long run the Wassermann test seems to meet with most favor, and in the hands of the majority leads to most satisfactory results.

PART II

THE INFECTIOUS DISEASES AND THE SPECIFIC MICRO-ORGANISMS

CHAPTER I

SUPPURATION

SUPPURATION was at one time looked upon as a normal and inevitable outcome of the majority of wounds, and although bacteria were early observed in the purulent discharges, the insufficiency of information then at hand led to the belief that they were spontaneously developed there.

From what has already been said about the evolution of bacteriology and the biology and distribution of bacteria, the relationship existing between bacteria and suppuration, and, indeed, between bacteria and disease in general, is found to be reversed. Instead of being the products of disease, the micro-organisms are the cause.

Suppuration, while nearly always the result of micro-organismal activity, is not a specific infectious process.

Being but the expression of tissue irritation arising through strong chemotactic influences, as many bacteria may be associated with it as can bring about the essential conditions. Bacteria with which these qualities are exceptionally marked appear as the common cause of the process; those with which it is less marked, as exceptional causes.

The relative frequency with which certain varieties of bacteria are associated with suppuration is shown in the following table from Karlinski:*

Suppuration in man—	Streptococci,	45	cases.
	Staphylococci,	144	"
	Other bacteria,	15	"
Suppuration in the lower animals—	Streptococci,	23	"
	Staphylococci,	45	"
	Other bacteria,	15	"
Suppuration in birds—	Streptococci,	11	"
	Staphylococci,	40	"
	Other bacteria,	20	"

Andrewes and Gordon,† after the examination of large numbers

* "Centralbl. f. Bakt.," etc., 1890, VII, S. 113.

† "Report of the Local Government Board of Great Britain," Supplement; "Report of the Medical Officers," 1905–06, vol. XXXV, p. 543.

of staphylococci from lesions of the human skin and mucous membranes, came to the conclusion that four varieties are differentiable. Of these, the Staphylococcus pyogenes is the most common and most important. When typical, it produces an orange-colored pigment; when atypical, it may be lemon yellow or white. Staphylococcus epidermidis albus is a distinct species. The differences between these cocci are shown in the table.

Staphylococcus Epidermidis Albus (Welch)

General Characteristics.—A non-motile, non-flagellate, non-sporogenous, slowly liquefying, non-chromogenic, aërobic and optionally anaërobic, doubtfully pathogenic coccus, staining by the usual methods and by Gram's method, and having its natural habitat upon the skin.

Under the name *Staphylococcus epidermidis albus*, Welch* has described a micrococcus which seems to be habitually present upon the skin, not only upon the surface, but also deep down in the Malpighian layer. He believes it to be Staphylococcus pyogenes albus in an attenuated condition, and if this opinion be correct, and there is seated deeply in the derm a coccus which may at times cause suppuration, the conclusions of Robb and Ghriskey, that sutures of cat-gut when tightly drawn may be a cause of skin-abscesses by predisposing to the development of this organism, are certainly justifiable. As the morphologic and cultural characteristics of the organism correspond fairly well to those of the following species, no separate description of them seems necessary.

Staphylococcus Pyogenes Albus (Rosenbach)†

General Characteristics.—A non-motile, non-flagellate, non-sporogenous, liquefying, non-chromogenic, aërobic and optionally anaërobic, mildly pathogenic coccus, staining by the ordinary methods and by Gram's method.

Although, as stated, Staphylococcus pyogenes albus is a common cause of suppuration, it rarely occurs alone, Passet so finding it in but 4 out of 33 cases investigated. When pure cultures of the coccus are subcutaneously injected into rabbits and guinea-pigs, abscesses occasionally result. Injected into the circulation, the staphylococci occasionally cause septicemia, and after death can be found in the capillaries, especially in the kidneys. From this it will be seen that the organism is feebly and variably pathogenic.

In its morphologic and vegetative characteristics Staphylococcus albus is almost identical with the species next to be described, differing from it only in the absence of its characteristic golden pigment.

* "Amer. Jour. Med. Sci.," 1891, p. 439.
† "Wundinfektionskrankheiten des Menschen," Wiesbaden, 1884.

TABLE OF THE CHIEF TYPES OF STAPHYLOCOCCI FOUND IN MAN.

	Character in broth cultures.	Pigment on agar-agar.	Clot formed in milk in a week.	Gelatin liquefied in a week.	Neutral red reduced.	Nitrites reduced in three days at 37° C.	Maltose fermented (acid).	Lactose fermented (acid).	Glycerin (acid).	Mannite (acid).	Pathogenic.
	Type.										
Staphylococcus aureus . . .	A.	Orange yellow.	+	+	—	+	+	+	+	+	Highly.
Staphylococcus epidermidis albus . . .	A.	White.	+	+	+	+	+	+	+	+	Feebly.
Staphylococcus salivarius . .	B.	White.	—	—	—	+	+	—	+	—	Not.
Scurf staphylococcus . . .	A and B.	White.	—	—	—	+	—	—	—	+	Not.

Staphylococcus Pyogenes Aureus (Rosenbach*)

General Characteristics.—A non-motile, non-flagellate, non-sporogenous' liquefying, chromogenic, pathogenic, aërobic and optionally anaërobic coccus' staining by the ordinary methods and by Gram's method.

Commonly present upon the skin, though in smaller numbers than the organisms already described, is the more virulent and sometimes dangerous *Staphylococcus pyogenes aureus*, or "golden staphylococcus," first observed by Ogston and cultivated by Rosenbach. As the morphology and cultural characteristics of this organism are identical with those of the preceding species, it seems convenient to describe them together, pointing out such minor differences as occur. In doing this, however, it must not be forgotten that, although Staphylococcus albus was first mentioned, Staphylococcus aureus is the more common organism of suppuration.

Staphylococci Pyogenes Aureus et Albus

Distribution.—The cocci are not widely distributed in nature, seeming not to find a purely saprophytic existence satisfactory. They

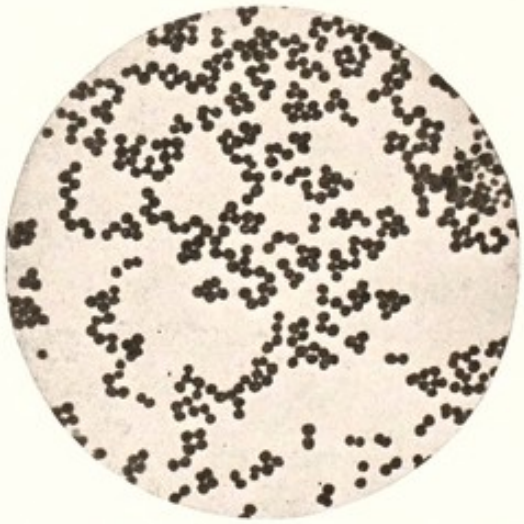


Fig. 106.—Staphylococcus pyogenes aureus (Günther).

occur, however, upon man and the lower animals, and can occasionally be found in the dusts of houses and hospitals—especially in the surgical wards—if proper precautions are not exercised. They are common upon the skin, in the nose, mouth, eyes, and ears of man; they are nearly always present beneath the finger-nails, and sometimes occur in the feces, especially of children.

Staphylococci are the most common micro-organisms in some acne pustules, in furuncles, in carbuncles, in superficial and deep abscesses, and in the ordinary run of surgical injections. So common are they that one should never be satisfied that he has exhausted

* "Mikroorganismen bei Wundinfektionskrankheiten des Menschen," Wiesbaden, 1884.

the etiological possibilities of the case through their demonstration. He should always seek for less evident though sometimes far more important organisms. In the absence of such, and in their absence only, should the case be referred to staphylococci.

Morphology.—The cocci are small spheres measuring about 0.7–1.0 μ in diameter. There is no definite grouping in either liquid or solid cultures. It is only in pus or in the organs or tissues of diseased animals that one can say that a true staphylococcus (bunch of grapes) grouping occurs.

The organisms are not motile and have no flagella. They do not form spores.

Staining.—They stain easily and brilliantly with aqueous solutions of the anilin dyes and by Gram's method.

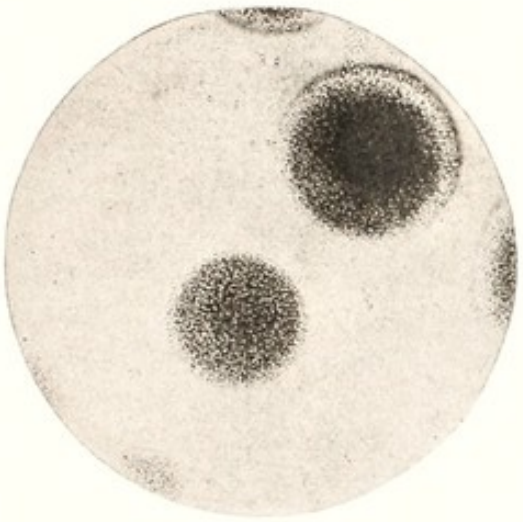


Fig. 107.—Staphylococcus pyogenes aureus. Colony two days old, seen upon an agar-agar plate. × 40 (Heim).

Isolation.—Staphylococci are easy organisms to isolate, and can be secured by plating out a drop of pus in gelatin or in agar-agar.

The colonies of Staphylococcus aureus differ considerably in color, some being much paler than others.

Cultivation.—The staphylococci grow well upon all the standard culture-media either in the presence or in the absence of oxygen at temperatures above 18°C., the most rapid development being at about 37°C.

Colonies.—Upon the surface of gelatin plates the colonies appear as small whitish points, after from twenty-four to forty-eight hours, rapidly extending to the surface and causing extensive liquefaction of the medium. The formation of the yellow pigment can be best observed near the center of the colonies. Under the microscope the colonies appear as round disks with circumscribed, smooth edges. They are distinctly granular and dark brown. When the colonies are grown upon agar-agar plates, the formation of the pigment is more distinct.

Gelatin Punctures.—In gelatin the growth occurs along the whole length of the puncture, causing an extensive liquefaction of the medium in the form of a long, narrow, blunt-pointed, inverted cone, sometimes described as being like a stocking, full of clouded liquid, at the apex of which a collection of golden or orange-yellow precipitate is always present in Staphylococcus aureus. It is this precipitate in particular that gives the organism its name, "golden staphylococcus."

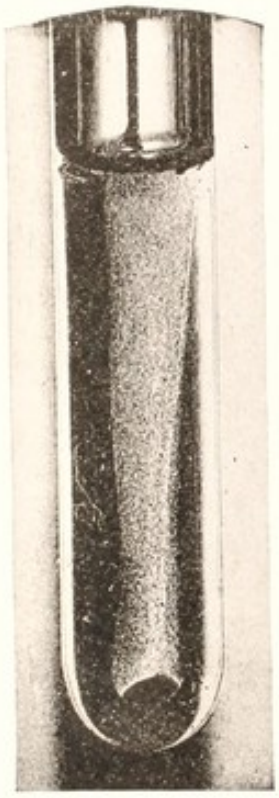


Fig. 108.—Staphylococcus pyogenes aureus. Puncture culture three days old in gelatin (Fränkel and Pfeiffer).

Agar-Agar.—The growth of the golden staphylococcus upon agar-agar is subject to considerable variation in the quantity of pigment produced. Sometimes, perhaps rarely, it is golden; more commonly it is yellow, often cream color. Along the whole line of inoculation a moist, shining, usually well-circumscribed growth occurs. When the development occurs rapidly, as in the incubator, it exceeds the rapidity of color production, so that the center of the growth is distinctly colored, the edges remaining white.

Potato.—Upon potato the growth is luxuriant, Staphylococcus aureus producing an orange-yellow coating over a large part of the surface. The potato cultures may give off a sour odor.

Bouillon.—When grown in bouillon the organism causes a diffuse cloudiness, with a small quantity of slightly yellowish sediment. The reaction of the medium becomes increasingly acid. Nitrates are reduced to nitrites.

Milk.—In milk, coagulation takes place in about eight days, and is followed by gradual digestion of the casein. In litmus milk slow acid production is observed.

Thermal Death Point.—Staphylococci are usually quite susceptible to the effect of heat, though their resistance is not uniform. Sternberg found them destroyed by an exposure to 62°C. for ten minutes, and to 80°C. for one and a half minutes, but three cultures studied by von Lingelsheim were not killed by an exposure to 60°C. for an hour, and one culture studied by him endured an exposure to 80°C. for ten minutes.

Metabolic Products.—Staphylococci can make use of free or combined oxygen, hence are aërobic or anaërobic. In liberating combined oxygen, no gas is generated in any culture medium. They produce ferments by which gelatin is liquefied, milk coagulated and digested, blood-serum digested and slowly liquefied. A yellow pigment is produced. Nitrates are reduced to nitrites in cultures

kept for three days at 37°C. Staphylococci are capable of producing fatty acids from sugars, hence acidity develops in media containing lactose, maltose, mannite and glycerin. The acids most commonly produced are acetic, valerianic, butyric and propionic.

Toxic Products.—Leber seems to have first conceived of suppuration as a toxic process depending upon the soluble products of parasitic fungi, and in 1888, through the action of alcohol upon staphylococci, prepared an acicular crystalline body soluble in alcohol and ether, but slightly soluble in water, to which he gave the name *phlogosin*.

Mannatti found that pus has substantially the same toxic properties as sterilized cultures of the staphylococcus; that repeated injections of sterilized pus induce chronic intoxication and marasmus; that injection of sterilized pus under the skin causes a grave form of poisoning; and that the symptoms and pathologic lesions caused by these injections correspond with those observed in men suffering from chronic suppuration.

Van de Velde* found that the staphylococcus has some metabolic products destructive to the leukocytes, which he has called *leukocidin*. This poison causes the cells to cease ameboid movement, become spheric, and gradually to lose their granules, until they finally appear like empty sacs containing shadow nuclei, which eventually disappear. The leukolysis occurs in about two minutes. These observations have been abundantly confirmed. Krauss† first observed that certain products of the staphylococcus were hemolytic and destroyed red blood-corpuscles. This hemolysin has been carefully studied by Neisser and Wechsberg,‡ by whom it was called *staphylolysin*.

Durme§ found staphylolysin produced most abundantly by virulent staphylococci.

Ribbert‖ found that both sterilized and unsterilized cultures when intravenously injected into animals produced definite changes in the heart, kidneys, lungs, spleen, and bone-marrow, and attributed the action to the toxin.

Morse** found that the toxic products of Staphylococcus aureus were capable of occasioning interstitial nephritis.

The staphylococci form very little extracellular toxin, as filtered cultures provoke little local or general reaction in animals, even when the staphylococcus is highly virulent.

To secure the endo-toxin, masses of culture, prepared as described in the section upon "Bacterio-vaccines," are ground in a mortar, or

* "La Cellule," 1896, XI, p. 349.
† "Wiener. klin. Wochenschrift," 1900.
‡ "Zeitschrift für Hygiene," 1911, XXXVI, p. 330.
§ "Hyg. Rundschau," 1903, Heft 2, p. 66.
‖ "Die pathologische Anatomie und die Heilung der durch den Staphylococcus pyogenes aureus hervorgerufenen Erkrankungen."
** "Journal of Experimental Medicine," 1896, vol. I, p. 613.

frozen by liquid air and then ground, or the culture masses are treated by dilute acids and alkalies according to Vaughan, or the culture masses are permitted to undergo autolysis in physiological salt solution or in diluted serum containing amboceptor and complement (see Bacteriolysis).

Pathogenesis.—The virulent Staphylococcus aureus is a dangerous and sometimes a deadly organism. Its virulence is, however, very variable both for the lower animals and for man. The most susceptible laboratory animal is the rabbit. Guinea-pigs, rats, mice, dogs and cats are much less susceptible.

The classical test for virulence is to inject $\frac{1}{10}$ cc. of a twenty-four hour old bouillon culture into the ear vein of a middle-sized rabbit. If of the ordinary virulence, the organism should kill the rabbit in from four to eight days, during which time the animal suffers from fever and wasting. Highly virulent cultures kill the animal in from one to two days.

The effects produced by different methods of inoculation are marked. Thus, if a few drops of a virulent culture be injected *beneath the skin* of a rabbit, there is a local reaction, an abscess forms, the temperature rises and the animal is ill. In a few days the abscess points and empties, the temperature returns to the normal and the animal recovers. In exceptional cases a generalized injection occurs and the rabbit dies.

If the injection be made into the *peritoneal cavity*, *pleural cavity* or into a joint, there is primarily a localized suppuration, peritonitis, pleuritis or arthritis, which is usually followed in a day or two by generalized infection and death.

Intravenous injections are immediately followed by rise of temperature, and the occurrence of multiple widespread foci of colonization with minute abscesses in many of the organs. The heart is sometimes the seat of purulent myocarditis, less frequently of septic endocarditis. The kidneys show minute abscesses, with aggregations of cocci in the glomeruli and in the tubules.

When the cocci enter human beings subcutaneously, furuncles, carbuncles and abscesses commonly result, according to the virulence of the organism and the resisting power of the individual. Garre* applied the organism in pure culture to the uninjured skin of his arm, and in four days developed a large carbuncle, with a surrounding zone of furuncles. Bockhart† suspended a small portion of an agar-agar culture in salt solution, and scratched it gently into the deeper layer of the skin with his finger-nail; a furuncle developed. Bumm injected the coccus suspended in salt solution beneath his skin and that of several other persons, and produced an abscess in every case. When conditions of invasion are most favorable, fatal generalization of the organisms may occur. In such cases they may be cultivated

* "Fortschritte der Med.," 1885, No. 6.
† "Monatschrift für prakt. Dermatologie," 1887, IV, No. 10.

from the streaming blood, though the greater number collect in, and frequently obstruct, the capillaries. In the lungs and spleen, and still more frequently in the kidneys, infarcts are formed by the bacterial emboli. The Malpighian tufts of the kidneys are sometimes full of cocci, and become the centers of small abscesses.

It enters the human system through scratches, punctures, or abrasions, and when virulent usually occasions an abscess.

Staphylococcus aureus is not only found in the great majority of furuncles, carbuncles, abscesses, and other inflammatory diseases of the surface of the body, but also plays an important rôle in a number of deeply seated diseases. Becker and others obtained it from the pus of osteomyelitis, demonstrating that if, after fracturing or crushing a bone, the staphylococcus be injected into the circulation, osteomyelitis may occur. Numerous observers have demonstrated its presence in ulcerative endocarditis. Rodet has been able to produce osteomyelitis without previous injury to the bones; Rosenbach was able to produce ulcerative endocarditis by injecting some of the staphylococci into the circulation in animals whose cardiac valves had been injured by a sound passed into the carotid artery; and Ribbert has shown that the injection of cultures of the organism may cause valvular lesions without preceding injury.

Virulence.—Experiments have shown that both Staphylococci aureus and albus exist in attenuated and virulent forms, and there is every reason to believe that in the majority of instances they inhabit the surface of the body in a feebly virulent condition.

Agglutination.—Kolle and Otto* have found that immune antistaphylococcic serums agglutinate the staphylococci. The reaction is not specific and is peculiar. All pathogenic staphylococci are agglutinated; non-pathogenic cocci are not agglutinated. The reaction cannot, therefore, be used for specific differentiation.

Specific Therapy.—The treatment of staphylococcus infections with immune serum has not met with encouraging success. Viquerat,† Denys and van de Velde,‡ and Neisser and Wechsberg§ and others have experimented in this direction, but the literature contains very little evidence that beneficial results have followed the employment of antistaphylococcus serums.

Bacterio-vaccination.—Although specific serums have failed, a promising form of specific treatment for subacute and chronic staphylococcic infections has been introduced by A. E. Wright,‖ who first isolates from the lesion the particular strain of staphylococci by which it is caused, cultivates this artificially, suspends the organisms in an indifferent fluid, of which a given quantity contains a known (counted) number, kills the organisms by heating them

* "Zeitschrift für Hygiene," etc., 1902, XLI.
† Ibid., XVIII, 1894, p. 483.
‡ "La Cellule," 1895, XI.
§ "Zeitschrift für Hygiene," 1901, XXXII.
‖ "Lancet," March 29, 1902, p. 874; "Brit. Med. Jour.," May 9, 1903, p. 1069.

for an hour at 60°C., and then uses them by subcutaneous injection for producing increased resistance on the part of the patient. (See "Bacterio-vaccination.")

The treatment is controlled by studying the "*opsonic index*" (*q.v.*), the objects being the avoidance of the "negative phase" or condition of diminished resistance, and the progressive establishment of the positive phase or stage of increased resistance. As the resistance increases the patient rapidly improves, and many cases of obstinate acne, furunculosis, and other pyogenic infections have quickly recovered under this treatment.

Staphylococcus Citreus (Passet)

An organism similar in many respects to the preceding, except that its growth on agar-agar and potato is of a brilliant lemon-yellow color and its pathogenicity for animals doubtful, is *Staphylococcus citreus* of Passet.* As it is not common and is doubtfully pathogenic, it is of much less importance than the previously described organisms.

Streptococcus Pyogenes (Rosenbach)

General Characteristics.—The streptococcus is a non-motile, non-flagellate, non-sporogenous, non-liquefying, non-chromogenic, aërobic and optionally anaërobic, spheric organism, infectious for man and the lower animals. It stains by ordinary methods and by Gram's method.

Streptococci were probably first seen by Pasteur and Doleris in the blood of women suffering from puerperal infection, and by Koch† in 1878. In 1881 Ogston‡ called attention to the fact that two distinct kinds of cocci were to be found in pus, mentioning both staphylococci and streptococci. The beginning of real knowledge of the streptococci, however, dates from the time of their isolation and cultivation by Fehleisen§ and of Rosenbach,‖ from 18 of 33 suppurative lesions, fifteen times alone and five times in association with Staphylococcus aureus.

Distribution.—Streptococci are parasitic pathogenic organisms, not known apart from human and animal hosts. They seem to occur not infrequently, in health, upon the surface of the body, in its various openings and in the alimentary canal. Such organisms are to be regarded as potentially virulent and pathogenic in all cases.

Streptococci have been the subject of extensive systematic

* "Untersuchungen über die Aetiologie der eitrigen Phlegmone des Menschen," Berlin, 1885, p. 9.

† "Untersuchungen über die Aetiologie der Wundinfektionskrankheiten," Leipzig, Vogel, 1878.

‡ "British Med. Jour.," March, 1881, p. 369.

§ "Aetiologie des Erysipels," Berlin, Fischer, 1883.

‖ "Mikroorganismen bei Wundinfektionskrankheiten des Menschen," 1884, p. 22.

study because of still existing uncertainty as to whether there is a single species or whether there are various species, but opinion, at present, seems in favor of the opinion that there is but one streptococcus whose various manifestations depend upon its virulence, upon the resistance of the host, upon its avenue of entrance, and the associated micro-organisms with which it happens to engage.

Streptococci may be primary pathogenic agents, or they may be secondary agents whose activities complicate, modify and sometimes outweigh in importance those of the primary agents.

They are the primary infecting agents in many inflammatory, purulent and septicemic disturbances—erysipelas, cellulitis, phlegmons, osteomyelitis, puerperal infection, pseudo-membranous angina, phlebitis, salpingitis, meningitis, endocarditis, etc.

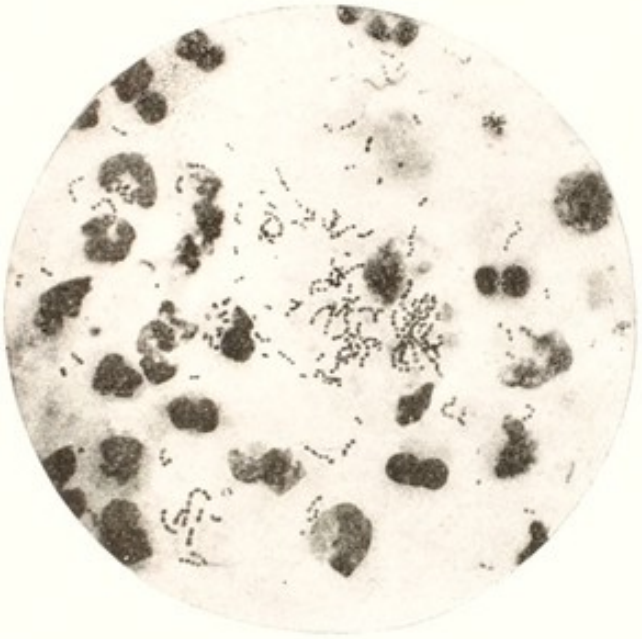


Fig. 109.—Streptococcus pyogenes, from the pus taken from an abscess. × 1000 (Fränkel and Pfeiffer).

Berson points out that they are secondary agents of importance in all pathological conditions of the throat of whatever nature.

Hektoen found them to be the most frequent complicating organism in scarlatina and Councilman the most frequent complicating organism in variola.

The suppurative conditions for which streptococci are held to be responsible, differ from those caused by staphylococci in being more rapidly spreading, more locally destructive, and more prone to generalized infection or septicemia.

Morphology.—The organisms are spheric, of variable size (0.4–1 μ in diameter), and are constantly associated in pairs or in chains of from four to twenty or more individuals. Special varieties, known as Streptococcus longus (chains of more than one hundred members)

and Streptococcus brevis (chains of from four to ten), have been described by v. Lingelsheim,* but do not hold as separate species.

The streptococcus is not motile and does not form spores.

Staining.—The organisms stain well with ordinary aqueous solutions of anilin dyes and by Gram's method.

Isolation.—The streptococcus can be isolated from pus containing it by plating or by the inoculation of a mouse or rabbit, from whose blood it may easily be secured after death.

Cultivation.—The organism grows at both the room temperature and that of incubation, its best and most rapid development being at about 37°C.

Colonies.—Upon gelatin plates very small, colorless, translucent colonies appear in from twenty-four to forty-eight hours. When superficial, they spread out to form flat disks about 0.5 mm. in diameter. The microscope shows them to be irregular and granular, to have a slightly yellowish color by transmitted light, and to have a frayed-out appearance around the edges, due to projecting chains of the cocci. No liquefaction of the gelatin occurs.

Gelatin Punctures.—In gelatin puncture cultures no liquefaction is observed. The minute spheric colonies grow along the whole length of the puncture and form a slightly opaque granular line.

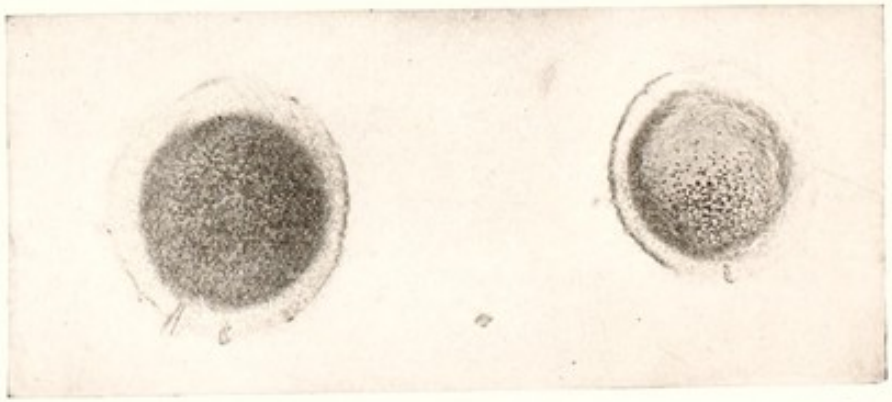


Fig. 110.—Streptococcus colonies on serum agar (From Hiss and Zinsser, "Text-Book of Bacteriology," D. Appleton & Co., Publishers).

Agar-agar.—Upon agar-agar a delicate transparent growth develops slowly along the line of inoculation. It consists of small, colorless, or slightly grayish transparent colonies which do not readily coalesce.

Blood-serum.—The growth upon blood-serum resembles that upon agar-agar. The colonies are small, white, discrete, and do not affect the medium.

Potato.—The streptococcus does not seem to grow well upon potato, the colonies being invisible.

Bouillon.—In bouillon the cocci develop slowly, seeming to prefer a neutral or feebly alkaline reaction. The medium remains clear,

* "Zeitschrift für Hygiene," 1891, Bd. x, p. 331; 1892, xii, p. 308.

while numerous small flocculi are suspended in it, sometimes adhering to the sides of the tube, sometimes forming a sediment. When the flocculi formation is distinct, the name *Streptococcus conglomeratus* (Kurth) is sometimes given to the organism; when the medium is diffusely clouded, it is called *Streptococcus diffusus*.

In mixtures of bouillon and blood-serum or ascitic fluid the streptococcus grows more luxuriantly, especially at incubation temperatures, distinctly clouding the liquid. As the lactic acid which is rapidly formed inhibits the growth of the cocci, Hiss recommends* that instead of eliminating the sugars in the broth, upon which the streptococci are nourished, 1 per cent. of sterile powdered $CaCO_3$ be added to the culture-media. This neutralizes the acid as rapidly as it is formed. It also maintains the life of the culture for a long time.

Milk.—The organism seems to grow well in milk, which is coagulated and digested.

Reaction.—The streptococcus is sensitive to acids, and can only grow well in media with a slightly alkaline reaction. All streptococci produce acids and eventually acidulate the media, thus checking their further development.

Vital Resistance.—The optimum temperature appears to be in the neighborhood of 37°C. It grows well between 25° and 40°C., above 40.5°C. the growth is slowed. The thermal death point is low. Sternberg found that the streptococci succumb at temperatures of 52° to 54°C. if maintained for ten minutes. Their vitality in culture is slight, and unless frequently transplanted they die. Bouillon cultures usually die in from five to ten days. On solid media they seem to retain their vegetative and pathogenic powers much longer, especially if kept cool and cultivated beneath the surface of the medium in a deep puncture. They resist drying fairly well.

Differential Features.—It is not always easy to differentiate Streptococcus pyogenes from other less important forms of streptococci and from the pneumococcus. One of the best methods is by the employment of *blood-agar plates*, suggested by Schottmüller.† Such plates are easily prepared by melting ordinary culture agar-agar, cooling to about 45°C., and then adding about 0.5 cc. of defibrinated human or rabbit's blood to the tube. The blood is first thoroughly mixed with the agar, then the tube inoculated, and poured into a Petri dish. As the Streptococcus pyogenes grows, it produces a hemolytic substance that destroys the blood-corpuscles in the vicinity of the colony, thus surrounding each by a clear, pale halo that contrasts with the red agar. The colonies themselves appear gray.

The test is not specific, and Ruediger‡ points out that the diph-

* "Text-book of Bacteriology," p. 338.
† "Münch. med. Wochenschrift," 1903, L, p. 909.
‡ "Jour. Amer. Med. Assoc.," 1906, XLVII, p. 1171.

theria and pseudodiphtheria bacilli also produce hemolyzing substance, so that the test cannot be used for the immediate separation of streptococci from other bacteria in cultures from the throat. Colonies of the pneumococcus usually appear green and without hemolysis, but Ruediger finds that they also sometimes cause solution of the hemoglobin. The streptococci whose colonies are green and without hemolysis are called *Streptococcus viridans* by Schottmüller. They were at first regarded as practically non-pathogenic, but it is now known that they cause endocarditis in rabbits and it is thought that they may do so in man.

Pathogenesis.—The streptococcus has been found in erysipelas, malignant endocarditis, periostitis, otitis, meningitis, empyema, pneumonia, lymphangitis, phlegmons, sepsis, puerperal endometritis, and many other forms of inflammation and septic infection. In man it is usually associated with active suppuration and sepsis.

The relation of the streptococcus to diphtheria is of interest, for, though in all probability the great majority of cases of pseudomembranous angina are caused by the Klebs-Löffler bacillus, yet a number are met with in which, as in Prudden's 24 cases, no diphtheria bacilli can be found, but which seem to be caused by the streptococcus alone.

There are few clinical differences between the throat lesions produced by the two organisms, and the only positive method of differentiating the one from the other is by means of a careful bacteriologic examination. Such an examination should always be made, as it has much weight in connection with the treatment; in streptococcus angina no benefit can be expected from the administration of diphtheria antitoxic serum.

Hirsh* has shown that streptococci are by no means rare in the intestines of infants, where they may occasion enteritis. In such cases the organisms are found in large numbers in the stomach and in the stools, and late in the course of the disease in the blood and urine of the child. They also occur in all of the internal organs of the cadaver.

The intestinal streptococci are often Gram-negative, when they are usually non-virulent.

Libman† has reported 2 carefully studied cases of streptococcic enteritis.

Flexner,‡ in a larger series of autopsies, found the bodies invaded by numerous micro-organisms, causing what he has called "terminal infections," and hastening the fatal issue. Of 793 autopsies at the Johns Hopkins Hospital, 255 upon cases dying of chronic heart or kidney diseases, or both, were sufficiently well studied bacteriologically, to meet the requirements of a statistical inquiry.

* "Centralbl. f. Bakt. u. Parasit.," Bd. XXII, Nos. 14 and 15, p. 369.
† "Centralbl. f. Bakt. u. Parasit.," Bd. XXII, Nos. 14 and 15, p. 376.
‡ "Journal of Experimental Medicine," 1896, vol. 1, No. 3.

Tuberculous infections were not included. Of the 255 cases, 213 gave positive bacteriologic results. "The micro-organisms causing the infections, 38 in all, were Streptococcus pyogenes, 16 cases; Staphylococcus pyogenes aureus, 4 cases; Micrococcus lanceolatus, 6 cases; gas bacillus (Bacillus aërogenes capsulatus), three times alone and twice combined with B. coli communis; the gonococcus, anthrax bacillus; B. proteus, the last combined with B. coli; B. coli alone; a peculiar capsulated bacillus, and an unidentified coccus."

It is interesting to observe in how many cases the streptococcus was present. All the streptococci found may not have been Streptococcus pyogenes, but for convenience in his statistics they were regarded as such.

The presence of streptococci in the blood in scarlatina has been observed in 30 cases by Crooke, by Fränkel and Trendenburg, Raskin, Leubarth, Kurth, and Babes. In 11 cases of scarlatina studied by Wright* a general streptococcus infection occurred in 4, a penumococcus infection in 1, and a mixed infection of pyogenic cocci in 1.

Lemoine† found streptococci in the blood during life in 2 out of 33 cases of scarlet fever studied. Pearce‡ studied 17 cases of scarlatina and found streptococci in the heart's blood and liver in 4, in the spleen in 2, in the kidney in 5 cases. In 2 of the cases Staphylococcus pyogenes aureus was associated with the streptococcus.

The streptococcus is the most common organism found in the suppurative sequelæ of scarlatina, frequently occurring alone; sometimes with the staphylococci; sometimes with the pneumococci.

Virulence.—Streptococci isolated from human beings vary greatly in pathogenic action upon the laboratory experiment animals. In many cases, although they have induced a fatal illness in human beings, they are without effect upon the lower animals; in other cases, although from a more simple lesion that recovered, they are extremely fatal for the most susceptible animals, rabbits and mice. Rats sometimes become ill when injected with virulent cultures in large doses, but usually recover. Guinea-pigs, cats, and dogs are but slightly susceptible even when the cultures are virulent. Large animals, like sheep, goats, cattle, and horses, react very slightly to large doses, but sometimes suffer from abscesses at the seat of injection. Mice die in from one to four days from general infection. If the organisms are less virulent, they die in from four to six days with edema and abscess formation at the site of inoculation, and subsequent invasion of the body. All streptococci seem to be most pathogenic for that species of animal from which they have been isolated.

* "Boston Med. and Surg. Jour.," March 21, 1895.
† "Bull. et Mém. Soc. d'Hôp. de Paris," 1896, 3 s., XIII.
‡ "Jour. Boston Soc. of Med. Sci.," March, 1898.

If the ear of a rabbit be carefully scarified, and cutaneously inoculated with a small quantity of a pure culture, local erysipelas usually results, the disturbance passing away in a few days and the animal recovering. If, however, the streptococcus be highly virulent, the rabbit may die of general septicemia in from twenty-four hours to six days. The cocci may then be found in large numbers in the heart's blood and in the organs. In less virulent cases minute disseminated *pyemic* abscesses are sometimes found.

When mildly virulent cultures of the variety called Streptococcus viridans are intravenously injected into rabbits, some time elapses before much disturbance is noted, then the animal becomes ill and eventually dies of cardiac disease. Verrucose endocarditis with marked calcification of the mitral valve, with secondary metastatic subacute glomerulonephritis was observed in those cases which were carefully studied by Libman.*

According to Marmorek,† the virulence of the streptococcus can be increased to a remarkable degree by rapid passage through rabbits, and maintained by the use of a culture-medium consisting of 3 parts of human blood-serum and 1 of bouillon. The blood of the ass or ascitic or pleuritic exudates may be used instead of the human blood-serum if the latter be unobtainable. By these means he succeeded in intensifying the virulence of a culture to such a degree that one hundred-thousand millionth (*un cent milliardième*) of a cubic centimeter injected into the ear vein was fatal.

Petruschky‡ found the virulence of the culture to be well retained when the organisms were planted in gelatin, transplanted every five days, and when grown, kept on ice.

Holst§ observed a virulent Streptococcus brevis that remained unchanged upon artificial culture-media for eight years without any particular precautions having been taken to maintain the virulence.

Dried streptococci are said by Frosch and Kolle‖ to retain their virulence longer than those growing on culture-media.

Metabolic Products.—The streptococcus produces a ferment by which milk is coagulated. A few streptococci (S. fæcalis of Andrewes and Horder) are said to produce gelatine softening ferments, but this Streptococcus pyogenes never does.

The organisms derive O from the atmosphere or from compounds, but no gas is ever evolved in the process, though acids are always produced in the presence of saccharose, lactose, rhamnose (isodulcite) raffinose, inulin, amygdalin, arbutin, coniferin, digitalin, helicin, populin, salicin, glycerin, sorbite and mannite (Gordon).

* Amer. Jour. Med. Sci., 1910, cxl, 516; 1912, clxiv, 313; Trans. Asso. Amer. Phys., 1912, xxvii, 157.

† "Ann. de l'Inst. Pasteur," July 25, 1895, p. ix, No. 7, 593.

‡ "Centralbl. f. Bakt. u. Parasitenk.," May 4, 1895, Bd. xviii, No. 16, p. 551.

§ Ibid., March 21, 1896, Bd. xix, No. 11.

‖ Flügge's "Die Mikroorganismen."

No acids are formed from starch, glycogen, arabin, convolvulin, huperidin, jalapin, methyl glucoside, saponin, glycol, erythrite or dulcite (Gordon).

Marmorek* and Lubenau† found that cultures of the streptococcus when grown in bouillon containing glucose, produced a hemolytic substance—*streptokolysin*—not seemingly present in cultures grown in ordinary bouillon. Besredka‡ found that streptokolysin was produced only by highly virulent cultures of the streptococcus and not by saprophytic organisms that have been for some time under cultivation in the laboratory.

Levin§ investigated the subject thoroughly and found that different strains of streptococci produced streptokolysin in varying quantities, that its production is entirely independent of virulence, that it is destroyed by heat (37°C. in some days; 55°C. in one-half hour); that acidity of the nutrient media hinders its formation, and that it is intimately associated with the bodies of the streptococci by which it is produced, so that in the sediment obtained by filtration or by centrifugation there is nearly one thousand times as much as in the filtered fluid culture. The streptokolysin is not destroyed by the death of the bacteria. *Antistreptokolysin* is present in antistreptococcus serum.

Toxic Products.—The toxic products of the streptococcus are not well known. Cultures from different sources vary greatly in the effects produced by hypodermic or intravenous injection after filtration through porcelain. Killed cultures produce a much more marked effect than filtered ones, so that the important product must be an endotoxin.

Simon‖ found that the toxic quality of the bodies of streptococci of different stocks had nothing to do with their virulence. Simon** also found that the toxic products of the streptococcus were diverse and peculiar. The bodies of the cocci contained an intracellular toxin the activity of which was independent of virulence. This poison is liberated only when the bactericidal activities of the body act upon the cocci. The cocci also excrete a toxic substance whose activity is greater than that of the intracellular toxin, but whose production is subject to great variation and is entirely independent of the intracellular toxin. The toxins and hemolysins are entirely different bodies.

In general, the effects of streptococcus intoxication are vague. The animals appear weak and ill, and have a slight fever; but unless the virulence of the culture be exceptional or the dose very large, they usually recover in a short time.

* "Annales de l'Inst. Pasteur," 1895, 593.
† "Centralbl. f. Bakt.," etc., 1901, Bd. XXX, Nos. 9 and 10.
‡ "Ann. de l'Inst. Pasteur," 1901, p. 880.
§ "Nord. Med. Ark.," 1903, II, No. 15, p. 20.
‖ "Centralbl. f. Bakt.," Dec. 18, 1903, XXXV, No. 3, p. 308.
** Ibid., Jan. 16, 1904, XXXV, No. 4, p. 350.

Coley's Mixture.—The clinical observation that occasional accidental erysipelatous infection of malignant tumors is followed by sloughing and the subsequent disappearance of the tumor, suggested the experimental inoculation of such tumors with Streptococcus erysipelatis as a therapeutic measure. The danger of the remedy, however, caused many to refrain from its use, for when one inoculates the living erysipelas virus into the tissues it is impossible to estimate the exact amount of disturbance that will follow.

To overcome this difficulty Coley* has recommended that the toxin instead of the living coccus be used for injection.

A virulent culture of the streptococcus is obtained, by preference from a fatal case of erysipelas, inoculated into small flasks of bouillon, and allowed to grow for three weeks. The flask is then reinoculated with Bacillus prodigiosus, allowed to grow for ten or twelve days at the room temperature, well shaken up, poured into bottle of about f℥ss capacity, and rendered perfectly sterile by an exposure to a temperature of 50° to 60°C. for an hour. It is claimed that the combined products of the streptococcus of erysipelas and Bacillus prodigiosus are much more active than a simple streptococcus culture. The best effects follow the treatment of cases of inoperable spindle-cell sarcoma, where the toxin sometimes causes a rapid necrosis of the tumor tissue, which can be scraped out with an appropriate instrument. Numerous cases are on record in which this treatment had been most efficacious; but, although Coley still recommends it and Czerny upholds it, the majority of surgeons have failed to secure the desired results.

Antistreptococcus Serum.—Since 1895 considerable attention has been bestowed upon the antistreptococcus serum of Marmorek† and Gromakowsky,‡ which is said to act specifically upon streptococcus infections, both general and local. Numerous cases of suppuration, septic infection, puerperal fever, and scarlatina are upon record in which the serum seems to have exerted a beneficial action.

The serum is prepared by the injection of cultures of living virulent streptococci into horses, until a high degree of immunity is attained. The serum is probably both antitoxic and bactericidal in action.

The success following the serums of some experimenters upon certain cases, and their occasional or constant failure in other cases, have suggested that there is considerable difference between different "strains" or families of streptococci. To obviate this inequality Van de Velde§ has made a polyvalent antistreptococcus serum by using a number of different cultures secured from the most diverse clinical cases of streptococcus infection. Another serum, of Tavel‖ and Moser,** is made by using cultures from different cases of scarlatina. The use of these serums, however, has not given the satisfaction expected, and at the present moment the whole subject of antistreptococcus serums is debatable both

* "Amer. Jour. Med. Sci.," July, 1894.
† "Ann. de l'Inst. Pasteur," July 25, 1895, IX, No. 7, p. 593.
‡ Ibid.
§ "Archiv. de. méd. Expér.," 1897.
‖ "Deutsche med. Wochenschrift," 1903, No. 50.
** "Berliner klin. Wochenschrift," 1902, 13.

from the standpoint of its theoretic scientific basis and its therapeutic application.

Streptococcus Vaccine.—Vaccines made by the method given in the chapter on "Bacterio-vaccines" are now used in all streptococcus infections with varying success. As, however, there is no knowledge by which one can foretell exactly what course a streptococcus infection will pursue, it is impossible to determine with accuracy what advantage results from the treatment. Judged upon its clinical merits, streptococcus vaccine does good, especially when the vaccine is homologous. When homologous vaccine cannot be prepared, preference might next be given the so-called "polyvalent" vaccines made by combining cultures from many sources. Such, especially when "sensitized" by admixture with antistreptococcus serum, according to the method of Besredka, give promise of benefit upon theoretical grounds.

Streptococcus Mucosus (Howard and Perkins)

This organism, described by Howard and Perkins,* was isolated from a case of tubo-ovarian abscess with generalized infection, and

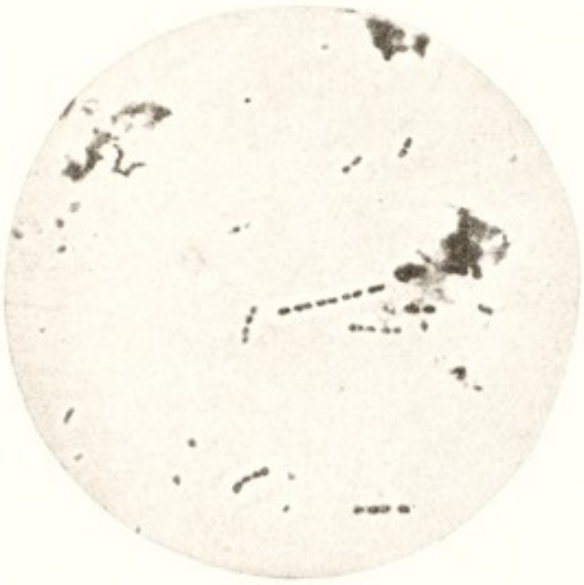


Fig. 111.—Streptococcus mucosus, from peritoneal exudate. × 1200 (Howard and Perkins, in "Journal of Medical Research").

again later by Schottmüller† from a case of parametritis, peritonitis, meningitis, and phlebitis.

It occurs as a rounded coccus in pairs and in short chains, though sometimes long chains of a hundred have been observed. The pairs resemble gonococci. They measure 1.25 to 1.75 μ in length and 0.5 to 0.75 μ in breadth. Each is surrounded by a halo that varies in width from 1.5 to 3.0 μ, which shows best in cultures grown on

* "Journal of Medical Research," 1901, N. S. 1, 163.
† "Münch. med. Wochenschrift," 1903, XXI.

human blood-serum. The usual capsule stains fail to color this halo when the organisms are from artificial cultures, though they show it well when they are in pus. The organisms stain with ordinary dyes and by Gram's method.

The cultures resemble those of Streptococcus pyogenes, but are rather more luxuriant, the colonies having a bluish cast. The organism ferments inulin, which makes Hiss think it related to the pneumococcus.

The organism taken at autopsy and inoculated into the peritoneum of a guinea-pig caused the animal to die, comatose, in thirty-six hours, with peritonitis. There were 15 to 20 cc. of peculiar viscid fluid in the peritoneal cavity. It had a grayish purulent character and contained numerous flakes of fibrin. There was no generalized infection. Mice and rabbits were susceptible and died of generalized infection.

The organism is not infrequently found as an apparently harmless tenant of the human mouth, where it may be confused with the pneumococcus. It has also turned up unexpectedly in a variety of inflammatory diseases.

Streptococcus Erysipelatis (Fehleisen)

The streptococcus of Rosenbach is generally thought to be identical with a streptococcus described by Fehleisen* as *Streptococcus erysipelatis.*

The streptococcus of erysipelas can be obtained in almost pure culture from the serum which oozes from a puncture made in the margin of an erysipelatous patch. They are small cocci, usually forming chains of from six to ten individuals, but sometimes reaching a hundred or more in number. Occasionally the chains occur in tangled masses.

They can be cultivated at the room temperature, but grow much better at 30° to 37°C. They are not particularly sensitive to the presence or absence of oxygen, but perhaps develop a little more rapidly in its presence. The cultural appearances are identical with those of Streptococcus pyogenes.

When injected into animals Fehleisen's coccus behaves exactly like Streptococcus pyogenes.

Micrococcus Tetragenus (Gaffky)

General Characteristics.—Large, round, encapsulated cocci, regularly associated in groups of four, forming tetrads. They are non-motile, non-flagellated, non-sporogenous, non-liquefying, non-chromogenic, non-aërogenic, aërobic and optionally aërobic, pathogenic for mice and other small animals, and stain well by all methods, including that of Gram.

A large micrococcus grouped in fours and known as Micro-

* "Verhandlungen der Würzburger med. Gesellschaft," 1881.

coccus tetragenus can sometimes be found in normal saliva, tuberculous sputum, and more commonly in the contents of the cavities of tuberculosis pulmonalis. It sometimes occurs in the pus of acute abscesses, and may be of importance in connection with the pulmonary abscesses which complicate tuberculosis. It was discovered by Gaffky.*

Morphology.—The cocci are rather large, measuring about 1 μ in diameter. In cultures they do not show the regular arrangement in tetrads as constantly as in the blood and tissues of animals, where they occur in groups of four surrounded by a transparent gelatinous capsule.

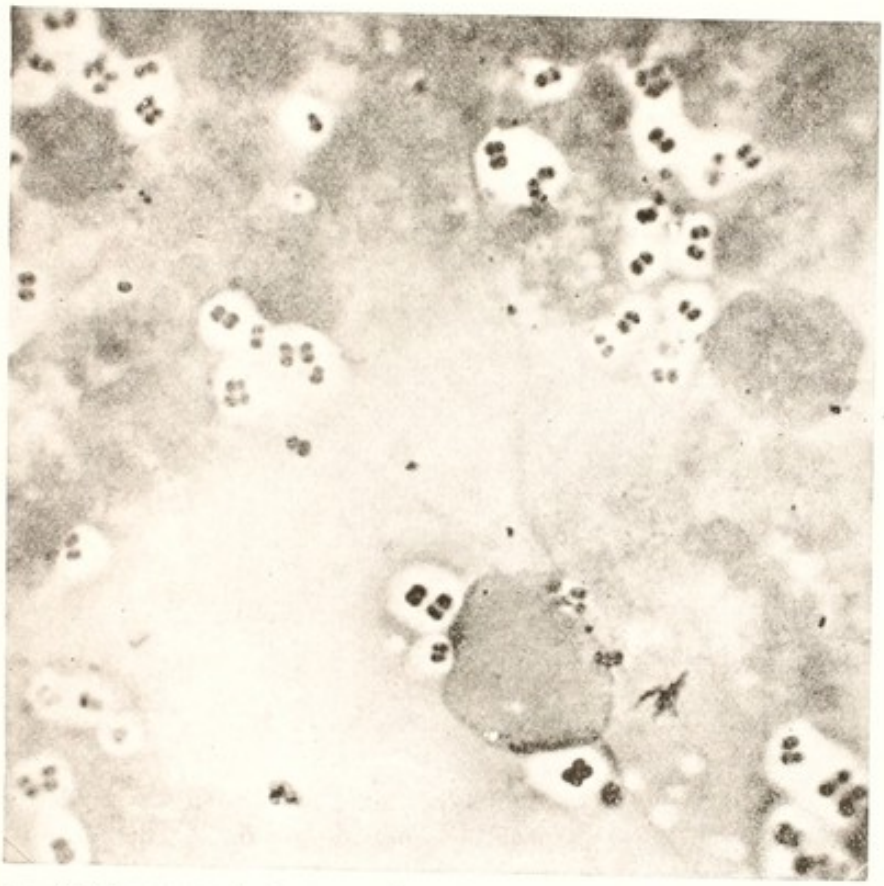


Fig. 112.—Micrococcus tetragenus in spleen of infected mouse. (From Hiss and Zinsser, "Text-Book of Bacteriology," D. Appleton & Co., Publishers.)

Staining.—The organisms stain well by ordinary methods and beautifully by Gram's method, by which they can best be demonstrated in tissues.

Isolation.—The organism can be isolated by inoculating a white mouse with sputum or pus containing it, and after death recovering it from the blood.

Cultivation.—It grows readily upon artificial media. Upon gelatin plates small white colonies are produced in from twenty-four to forty-eight hours. Under the microscope they appear

* "Archiv. f. Chirurgie," XXVIII, 3.

spheric or elongate (lemon shaped), finely granular, and lobulated like a raspberry or mulberry. When superficial they are white and elevated, 1 to 2 mm. in diameter.

Gelatin.—In gelatin punctures a large white surface growth takes place, but development in the puncture is very scant, the small spheric colonies usually remaining isolated. The gelatin is not liquefied.

Agar-agar.—Upon agar-agar spheric white colonies are produced. They may remain discrete or become confluent.

Potato.—Upon potato a luxuriant, thick, white growth is formed.

Blood-serum.—The growth upon blood-serum is also abundant, especially at the temperature of the incubator. It has no distinctive peculiarities.

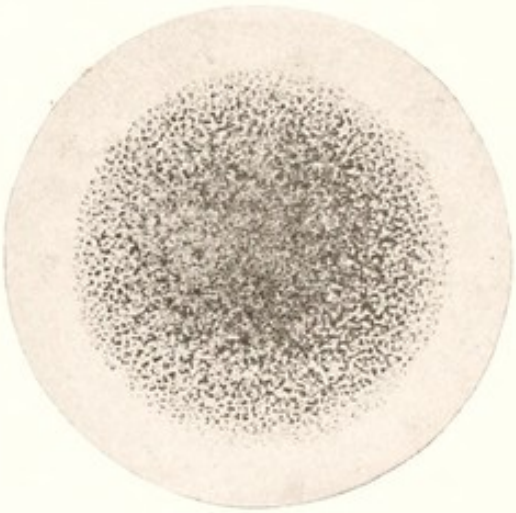


Fig. 113.—Micrococcus tetragenus; colony twenty-four hours old upon the surface of an agar-agar plate. × 100 (Heim).

Pathogenesis.—The introduction of tuberculous sputum or of a minute quantity of a pure culture of this coccus into white mice usually causes a fatal bacteremia in which these organisms are found in small numbers in the heart's blood, but are numerous in the spleen, lungs, liver, and kidneys.

Japanese mice and white mice are highly susceptible to the organism and die three or four days after inoculation.

House-mice, field-mice, and rabbits are comparatively immune. Guinea-pigs may die of general septic infection, though local abscesses result from subcutaneous inoculation.

The tetracocci, when present, probably hasten the tissue-necrosis in tuberculous cavities, aid in the formation of abscesses of the lung and contribute to the production of the hectic fever.

An interesting contribution to the relationship of this coccus to human pathology has been made by Lartigau,* who succeeded in demonstrating that the tetracoccus may be the cause of a pseudo-membranous angina, 3 cases of which came under his observation.

* "Phila. Med. Jour.," April 22, 1899.

Bezancon* has isolated this organism from a case of meningitis. Forneaca† has reported a case of generalized tetragenous septicemia.

Bacillus Pyocyaneus (Gessard)

General Characteristics.—A minute, slender, actively motile, flagellated, non-sporogenous, chromogenic and feebly pathogenic, aërobic or facultative anaërobic, liquefying bacillus, staining by ordinary methods, but not by Gram's method.

In some cases pus has a peculiar bluish or greenish color, which depends upon the presence of *Bacillus pyocyaneus* of Gessard.‡

Distribution.—The bacillus appears to be a rather common saprophyte, being found in feces, manure, and water. It easily

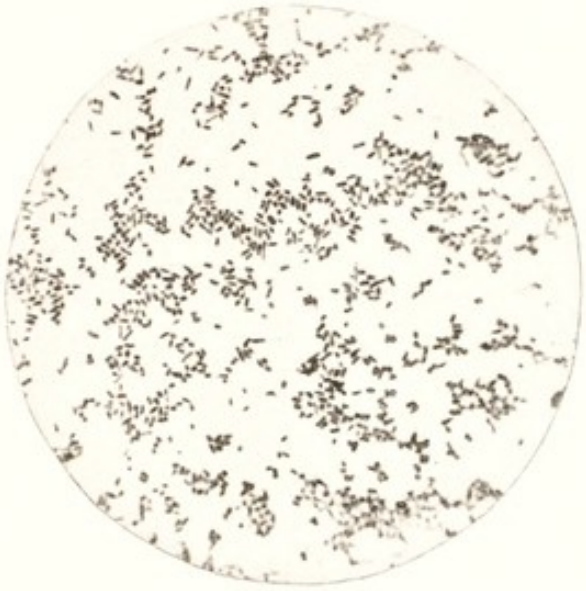


Fig. 114.—Bacillus pyocyaneus, from an agar-agar culture. × 1000 (Itzerott and Niemann).

takes up its residence upon the skin and mucous membranes, and has been found in the perspiration. It sometimes occurs as a saprophyte upon the surgical dressings applied to wounds, and sometimes invades the tissues through wounds, to occasion dangerous infections.

Morphology.—It is a short, slender organism with rounded ends, measuring 0.3 × 1 to 2 μ, according to Flügge; 0.6 × 2 to 6 μ, according to Ernst, and 0.6 × 1 μ, according to Charrin. It is quite pleomorphous, which probably accounts for the difference in measurements. It is occasionally united in chains of four or six. It is actively motile, has one terminal flagellum, and does not form spores.

It closely resembles a harmless bacillus found in water, and

* "Semaine Medicale," 1898.
† "Riforma Medica," 1903.
‡ "De la Pyocyanine et de son Microbe," Thèse de Paris, 1882.

known as Bacillus fluorescens liquefaciens, from which Ruzicka* thinks it has probably descended.

Staining.—It stains well with the ordinary staining solutions, but not by Gram's method.

Isolation.—The isolation of the organism is simple, the ordinary plate method being a satisfactory means of securing it from pus or other discharges.

Cultivation.—Colonies.—The superficial colonies upon gelatin plates are small, irregular, slightly greenish, ill-defined, and produce a distinct fluorescence of the neighboring medium.

Microscopic examination shows the superficial colonies to be rounded and coarsely granular, with serrated or slightly filamentous borders. They are distinctly green in the center and pale at the

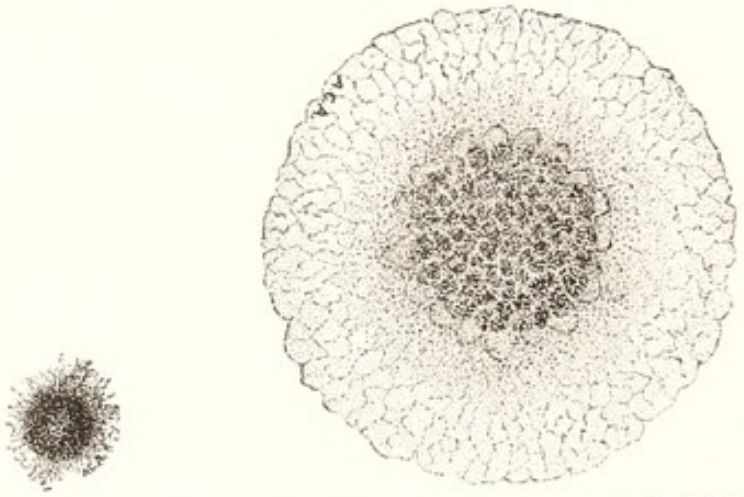


Fig. 115.—Bacillus pyocyaneus. Colonies upon gelatin (Abbott).

edges. The colonies sink into the gelatin as the liquefaction progresses. Four or five days must elapse before the medium is all fluid.

Gelatin Punctures.—In gelatin puncture cultures the chief development of the organisms occurs at the upper part of the tube, where a deep saucer-shaped liquefaction forms, slowly descending into the medium, and causing a beautiful fluorescence. At times a delicate scum forms on the surface, sinking to the bottom as the culture ages, and ultimately forming a slimy sediment.

Agar-agar.—Upon agar-agar the growth developing all along the line of inoculation at first appears bright green. The green color depends upon a soluble pigment (fluorescin) which soon saturates the culture-medium and gives it the characteristic fluorescent appearance. As the culture ages, or if the medium upon which it grows contains much peptone, a second blue pigment (pyocyanin) develops, and the bright green fades to a deep blue-green, dark blue, or in some cases to a deep reddish-brown color. This pigment has

* "Centralbl. f. Bakt. u. Parasitenk.," July 15, 1898, p. 11.

been made the subject of a careful investigation by Jordan.* Its formula, according to Ledderhose,† is $C_{14}H_{14}N_2O$.

A well-known feature of the growth upon fresh agar-agar, upon which much stress has recently been laid by Martin,‡ is the formation of crystals in fresh cultures. Crystal formation in cultures of other bacteria usually takes place in old, partially dried agar-agar, but Bacillus pyocyaneus often produces crystals in a few days upon fresh media. Freshly isolated bacilli show this power more markedly than those which have been for some time part of the laboratory stock of cultures and frequently transplanted.

Bouillon.—In bouillon the organism produces a diffuse cloudiness, a fluorescence, and sometimes an indefinite thin pellicle on the surface.

Potato.—Upon potato a luxuriant greenish or brownish, smeary layer is produced.

Milk.—Milk is coagulated and peptonized. It is slightly acid for the first day or two, then becomes alkaline again.

Metabolic Products.—Apart from the pyocyanin and fluorescin, the former blue, the latter green, cultures of this organism frequently turn red brown. This suggested the formation of a third pigment, but the work of Boland§ has shown this to be a transformation product of pyocyanin common in old cultures.

The organism produces a curdling ferment, a fibrin- and casein-dissolving ferment, a gelatin-dissolving ferment, and a bacteriolytic ferment, the *pyocyanase* of Emmerich and Löw.

It also produces, under favorable conditions, a toxin which has been studied by Wassermann, who found it fatal in doses of 0.2 to 0.5 cc. when intraperitoneally injected into guinea-pigs. The animals show peritonitis and punctiform hemorrhages on the serous membranes.

Bullock and Hunter‖ found that Bacillus pyocyaneus also produces a hemolytic substance, *pyocyanolysin*, by which corpuscles of man, oxen, sheep, apes, rabbits, cats, rats, dogs, and mice are dissolved. The peculiar substance was produced in greatest quantity in virulent cultures three or four weeks old. Jordan** believes that this hemolytic property depends solely upon the intense alkali formed in old cultures. Gheorghewski†† found a leukocyte-destroying substance in the cultures.

In addition to the metabolic pigments mentioned, the organism produces toxins. Wassermann‡‡ found that filtrates of old cultures were more toxic for guinea-pigs than the endotoxins made by lysis

* "Journal of Experimental Medicine," 1899, vol. IV.
† "Deutsche Zeitschr. f. Chirurgie," 1888, Bd. XXVIII.
‡ "Centralbl. f. Bakt.," April 6, 1897, XXI, p. 473.
§ "Centralbl. f. Bakt.," 1899, Bd. XXV, p. 897.
‖ Ibid., XXVIII, 1900, p. 865.
** Ibid., Bd. XXXIII, Ref. 1903.
†† "Ann. de l'Inst. Pasteur," 1899, XIII.
‡‡ "Zeitschrift für Hygiene, 1896, XXII.

of dead bacteria. The organism thus produces both endo- and exotoxins.

Pathogenesis.—The bacillus is pathogenic for the small laboratory animals, but different cultures differ greatly in virulence. One cc. of a virulent bouillon culture, injected into the subcutaneous tissue of a guinea-pig, causes rapid edema, suppurative inflammation, and death in a short time (twenty-four hours). Sometimes the animal lives for a week or more, then dies. There is a marked hemorrhagic subcutaneous edema at the seat of inoculation. The bacilli can be found in the blood and in most of the tissues. Rats and mice behave similarly to guinea-pigs when inoculated subcutaneously.

Rabbits are less susceptible and subcutaneous injections rarely cause death. Intraperitoneal injection may be followed by fatal infection if the bacillus be highly virulent or if it be not virulent, recovery may occur. Intravenous inoculation causes fever, albuminuria, diarrhea and death in a day or two. If the dose be smaller or the virulence of the culture less, a subacute disturbance characterized by wasting, palsy and convulsions may occur. If the animal dies, nephritis can usually be found, and perhaps explains the symptoms.

Dogs are susceptible to infection by B. pyocyaneus, the symptoms bearing a considerable resemblance to rabies.

Blum* reports a case of pyocyaneus infection with endocarditis in a child.

Lartigau,† in his study of "The Bacillus Pyocyaneus as a Factor in Human Pathology," sums up what is known about this rôle of the organism as follows:

"The Bacillus pyocyaneus, like many pathogenic micro-organisms, is occasionally found in a purely saprophytic rôle in various situations in the human economy. It has been found in the saliva by Pansini, in sputum by Frisch, and in the sweat by Eberth and Audanard. Abelous demonstrated its presence in the stomach as a saprophyte. Its existence in suppurating wounds has long been known, and Koch early detected its presence in tuberculous cavities, regarding it as an organism incapable of playing any pathologic rôle. The etiologic relation of the organism to certain cases of purulent otitis media in children was pointed out by Martha, Maggiora and Gradenigo, Babes, Kossel, and others. H. C. Ernst obtained it from a pericardial exudate during life. G. Blumer demonstrated its presence in practically pure cultures in a case of acute angina simulating diphtheria; Jadkewitsch, B. Motz, and Le Noir obtained the bacillus in cases of urinary infection. The cases of Triboulet, Karlinski, Oettinger, Ehlers, and Barker are interesting instances of its rôle in cutaneous lesions.

"In addition to these lesions, other morbid processes have been associated in some cases with the bacillus of blue pus, such as meningitis and bronchopneumonia, by Monnier; diarrhea of infants, by Neumann, Williams, Thiercelin and Lesage, and other observers; dysentery, by Calmette and by Lartigau; and general infection, by Ehlers, Neumann, Oettinger, Karlinski, Monnier, Krannhals, Calmette, Finkelstein, and L. F. Barker."

Nine additional cases of human infection are reported by Perkins.‡

* "Centralbl. f. Bakt. u. Parasitenk.," Feb. 10, 1899, XXV, No. 4.

† "Phila. Med. Jour.," Sept. 17, 1898.

‡ "Jour. of Med. Research," 1901, vol. VI, p. 281.

Immunity.—Immunity against pyocyaneus infection develops after a few inoculations with attenuated or sterilized cultures. These are easily prepared, the thermal death-point determined by Sternberg being 56°C. It also follows injection of either the endotoxin or the exotoxin. In the immunity resulting from the treatment with bacterio-vaccines the serum of the animal becomes agglutinative and bactericidal; in the immunity resulting from treatment with the exotoxin, antitoxin is produced.

Bacillus Proteus Vulgaris (Hauser)

General Characteristics.—An actively motile, flagellated, non-sporogenous, non-chromogenic, liquefying, aërobic and optionally anaërobic, doubtfully pathogenic, aërogenic bacillus, easily cultivated on artificial media and readily stained by the ordinary methods, though not by Gram's method.

This bacillus was first found by Hauser* in decomposing animal infusions, usually in company with two closely allied forms, Proteus mirabilis and Proteus zenkeri, which, as the experiments and observations of Sanfelice and others show, may be identical with it. According to Kruse, it is quite probable that the mixed species formerly called *Bacterium termo* was largely made up of the proteus.

Distribution.—The organism is a common saprophyte and is very abundant in water, earth, and air. It is to be expected wherever putrefactive change is in progress. It is a common mistake for the novice to look upon it as a member of the Bacillus coli group.

Morphology.—The bacilli are variable in size and shape—pleomorphic—and are named *proteus* from this peculiarity. Some differ very little from cocci, some are more like the colon bacillus in shape, others form long filaments, and occasional spirulina forms are met with. True spirals are never found. All of the forms mentioned may be found in pure cultures of the same organism. The diameter of the bacillus is usually about 0.6 μ, but the length varies from 1.2 μ or less to 4 μ or more. No spores are formed. The organisms are actively motile. The long filaments frequently form loops and tangles. Flagella are present in large numbers. Upon one of the long bacilli as many as one hundred have been counted. Involution forms are frequent in old cultures.

Staining.—The bacilli stain well by the ordinary methods. Gram's method usually fails.

Cultivation.—The proteus is easily cultivated and grows well in all the artificial media.

Colonies.—Upon gelatin plates a typical phenomenon is observed in connection with the development of the colonies, for the most advantageous observation of which the medium used for making the cultures should contain 5 instead of 10 per cent. of gelatin. Kruse† describes the phenomenon as follows:

* "Ueber Faulnissbakterien," Leipzig, 1885.
† Flügge's "Die Mikroorganismen."

"At the temperature of the room, rounded, saucer-shaped depressions, with a whitish central mass surrounded by a lighter zone, are quickly formed. Under low magnification the center of each is seen to be surrounded by radiations extending in all directions into the solid gelatin, and made up of chains of bacilli. Between the radiations and the granular center bacteria are seen in active motion. Upon the surface the colony extends as a thin patch, consisting of a layer of bacilli arranged in threads, sending numerous projections from the periphery. Under certain conditions the wandering of the processes can be directly observed under the microscope. It depends not only upon the culture-medium, but, in part, upon the culture itself. Entire groups of bacilli or single threads, by gradual extension and circular movement, detach themselves from the colony and wander about upon the plate. From the radiated central part of the colony peculiar zooglea are formed, having a sausage or screw shape, or wound in spirals like a corkscrew. The younger colonies, which have not yet reached the surface of the gelatin, are more compact, rounded or nodular, later covered with hair-like projections, and becoming radiated like the superficial colonies."

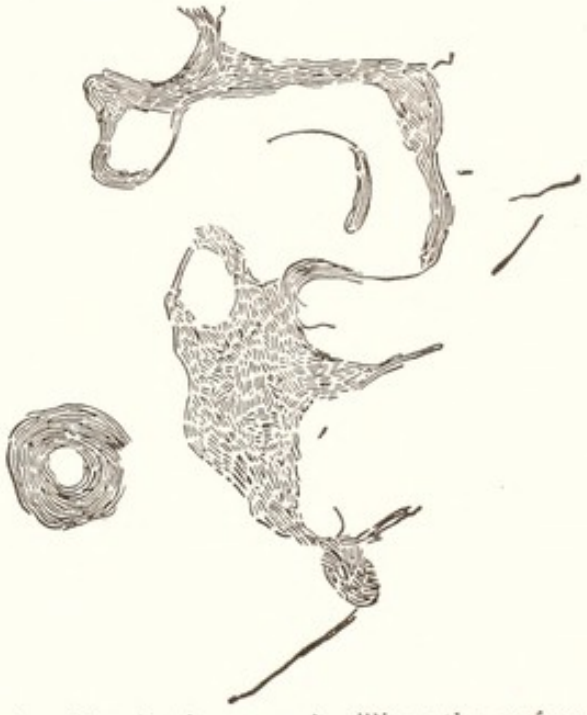


Fig. 116.—Swarming islands of proteus bacilli on the surface of gelatin; × 650 (Hauser).

If the culture-medium be concentrated, or the culture have been frequently transplanted, the phenomenon is less marked or may not occur.

Bouillon.—In this medium the organism grows rapidly, and quickly clouds the fluid. A pellicle soon forms upon the surface and a mucilaginous sediment occurs later.

Gelatin Punctures.—Puncture cultures in gelatin are not characteristic. A stocking-like liquefaction occurs in the gelatin and extends so rapidly that the entire medium is liquefied in a few days. Anaërobic cultures do not liquefy.

Agar-agar.—Upon agar-agar the bacillus forms a moist, thin, transparent, rapidly extending layer which rarely reaches the sides of the tube. Upon agar-agar plates ameboid movement of the colonies sometimes occurs.

Potato.—Upon potato the growth occurs in the form of a smeary patch of soiled appearance.

Milk is coagulated.

Metabolic Products.—The bacillus usually produces alkalies. Indol and phenol are formed from the peptone of the culture-media. Nitrates are reduced to nitrites, and then partly reduced to ammonia. In most culture-media not containing sugar the bacillus produces a disagreeable odor.

In culture-media containing either grape- or cane-sugar fermentation occurs both in the presence and in the absence of oxygen. Milk-sugar is not decomposed.

Pathogenesis.—It is a question whether or not Bacillus proteus is to be ranked among the pathogenic bacteria. Small doses are harmless for the laboratory animals; large doses produce abscesses. A toxic substance resulting from the metabolism of the organism seems to be the cause of death when considerable quantities of a culture are injected into the peritoneal cavity or blood-vessels. The bacilli do not seem able to multiply in the healthy animal body, but can do so when previous disease or injury of its tissues has taken place.

The *proteus* has been secured in cultures from wound and puerperal infections, purulent peritonitis, endometritis, and pleurisy. When the local lesion is limited, as in endometritis, the danger of toxemia is slight; but when widespread, as the peritoneum, it may prove serious. Bacillus proteus has also been found in acute infectious jaundice and in acute febrile icterus, or Weil's disease.

Bordoni-Uffredizzi has shown that the proteus quite regularly invades the tissues after death, though it appears unable to maintain an independent existence in the tissues during life, and is probably of importance only when present in association with other bacteria. It at times grows abundantly in the urine, and may produce primary inflammation of the bladder. The inflammatory process may also extend from the bladder to the kidney, and so prove quite serious.

Epidemics of meat-poisoning have been thought to depend upon Bacillus proteus. One of them was studied by Wesenberg,* who cultivated the organism from the putrid meat by which 63 persons were made ill. Silverschmidt† and Pfuhl‡ have made similar investigations with similar results.

Amebæ and Suppuration

The process of suppuration is not confined to bacterial microorganisms, but is shared to a limited extent by the protozoa. Thus,

* "Zeitschrift für Hygiene," etc., 1898, xxviii.
† Ibid., 1899, xxx.
‡ Ibid., 1900, xxxv.

Entamœba histolytica (*q.v.*) is, to all appearances, the sole excitant of the abscesses of the liver secondary to dysentery. It is true that these are cold abscesses and necrotic rather than distinctly purulent in character, yet it seems best to speak of the organism in this connection.

Entamœba buccalis (Prowazek*) is a small ameba that has been found in purulent exudates in the oral tissues of persons with carious teeth. It is at present thought to be the cause of Riggs' disease or pyorrhea alveolaris.

Amœba kartulisi (Doflein†) appears to be capable of exciting suppuration. It was found by Kartulis in the pus from an abscess of the right side of the lower jaw. The patient was a man aged forty-three years who had been operated upon for the removal of a piece of bone. It is 30 to 38 μ in diameter, is actively motile. Its coarse protoplasm contains red and white blood-corpuscles. Kartulis‡ found the same organism five times in other cases, and Flexner§ found it also.

Amœba mortinatalium, described by Smith and Weidman,‖ was found in distributed small purulent foci in the kidneys and other organs of a still-born fetus.

Miscellaneous Organisms of Suppuration Described More Fully Elsewhere

Before leaving the subject, attention must be directed to other bacteria that under exceptional circumstances become the cause of suppuration. Among these are the pneumococcus of Fränkel and Weichselbaum, the typhoid bacillus, and the Bacillus coli communis. These organisms are considered under separate and appropriate headings, to which the reader is advised to refer.

* "Arbeiten a. d. Kaiserl. Gesundh. Amt.," 1904, XXI, 1, Bull. p. 42.
† "Die Protozoa als Krankheitserreger," Jena., 1901, p. 30.
‡ "Centralbl. f. Bakt. u. Parasitenk." 1903, XXXIII, p. 471.
§ "Bulletin of the Johns Hopkins Hospital," 1892, XXV.
‖ "University of Pennsylvania Medical Bulletin," Sept., 1910.

CHAPTER II

MALIGNANT EDEMA

Bacillus Œdematis Maligni (Koch)

General Characteristics.—A motile, flagellated, sporogenous, anaërobic, liquefying, aërogenic, non-chromogenic, pathogenic bacillus of the soil, readily stained by the ordinary methods, but not by Gram's method.

This organism was originally found by Pasteur* in putrescent animal infusions and called by him (1875) Vibrion septique. It was later more carefully studied and described by Koch.†

It is supposed that this bacillus was among the organisms whose introduction into wounds in the days of pre-antiseptic surgery, commonly occasioned the then prevalent "Hospital gangrene."

Distribution.—The organism is widely distributed in nature, being commonly present in garden earth. It is also found in dust, in waste water from houses, and sometimes in the intestinal contents of animals.

Morphology.—The bacillus of malignant edema is a large rod-shaped organism with rounded ends, measuring 2 to 10 μ by 0.8 to 1.0 μ. It is usually motile, and possesses many flagella. It produces oval endospores centrally situated and giving a barrel shape to the parent bacillus.

Staining.—The bacillus stains well with ordinary cold aqueous solutions of the anilin dyes, but not by Gram's method.

Cultivation.—The organism is a strict anaërobe, but under conditions by which provision is made for the removal of oxygen, grows well both at the room temperature and at that of the incubator. It is not difficult to secure in pure culture, being most easily obtained from the edematous tissues of guinea-pigs and rabbits inoculated with garden earth.

Colonies.—The colonies which develop upon the surface of gelatin kept under anaërobic conditions appear to the naked eye as small shining bodies with liquid, grayish-white contents. Under the microscope they appear filled with a tangled mass of long filaments which under a high power exhibit active movement. The edges of the colony have a fringed appearance, much like the colonies of the hay or potato bacillus.

Gelatin.—In gelatin tube cultures the characteristic growth cannot be observed unless the tube be placed under anaërobic

* "Bull. Acad. Med.," 1877 and 1881.
† "Mittheilungen aus dem kaiserl. Gesundheitsamte," I, 53.

conditions. The best preparation, therefore, is made by heating the gelatin to expel any air it may contain, inoculating it while still liquid, and solidifying it in cold (iced) water. In such a tube the bacilli develop in globular circumscribed areas of cloudy liquefaction which contain a small amount of gas. In gelatin to which a little grape-sugar has been added the gas production is marked.

Agar-agar.—The growth takes place in the form of a cloudy stream, in the lower part of deep punctures in recently heated agar-agar, from which the air has been expelled. If the agar-agar contains 1 per cent. of glucose, it is soon split up by the gas formation. Such cultures give off a very disagreeable odor.

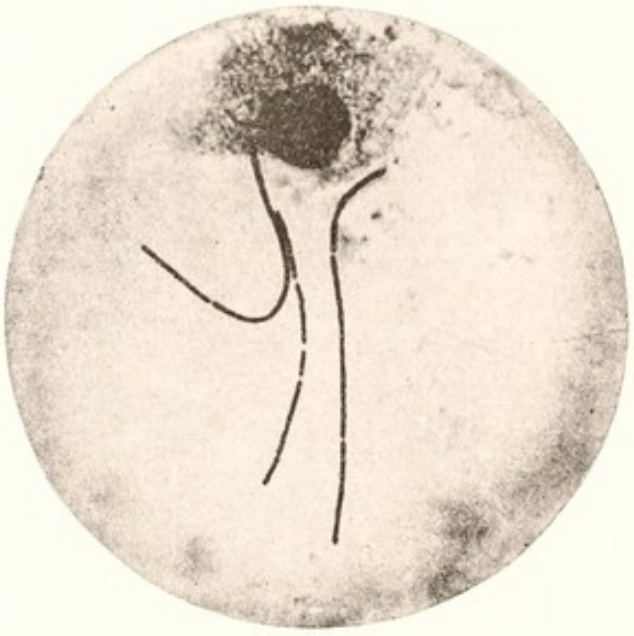


Fig. 117.—Bacillus of malignant edema, from the body-juice of a guinea-pig inoculated with garden earth. × 1000 (Fränkel and Pfeiffer).

Bouillon.—In deep tubes of recently heated bouillon a diffuse turbidity occurs in about twenty-four hours. After the third day the upper half clears, the bacilli and spores sedimenting or moving away from the oxygen. The culture gives off a very disagreeable odor.

In glucose or other sugar bouillon in the fermentation tube, considerable gas is formed.

The gas is partly inflammable, partly not.

Milk.—Milk is slowly coagulated.

Potato.—The bacillus grows upon the surface of potato if kept under anaërobic conditions.

Blood-serum.—Upon coagulated blood-serum, and upon coagulated egg-white, growth occurs under anaërobic conditions, both media being slowly digested and softened.

Vital Resistance.—The bacilli themselves soon succumb when exposed to the air. They are destroyed in a few moments by heating

to 60°C. The spores, on the other hand, resist drying and exposure to the atmosphere well and can be kept alive for years in garden earth. The complete destruction of the spores requires exposure to 90°C. for a half hour. Moist heat at 100°C. kills them in a few minutes.

Metabolic Products.—Of the toxic products of the organism nothing definite is known. It decomposes albumin, forming fatty acids, leucin, hydroparacumaric acid, and an oil with an offensive odor. Among the gases formed, carbonic acid, hydrogen, and marsh gas have been detected.

Pathogenesis.—When introduced beneath the skin, the bacillus is pathogenic for a large number of animals—mice, guinea-pigs, rabbits, horses, dogs, sheep, goats, pigs, calves, chickens, and pigeons. Cattle seem to be immune.

Günther points out that the simple inoculation of the bacillus upon an abraded surface is insufficient to produce infection, because the

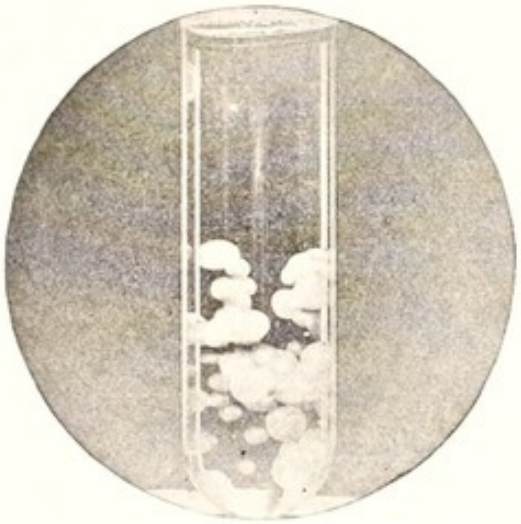


Fig. 118.—Bacillus œdematis, dextrose gelatin culture (Günther).

presence of oxygen is detrimental to its growth. When the bacilli are deeply introduced beneath the skin, infection occurs.

Mice, guinea-pigs, and rabbits sicken and die in about forty-eight hours.

Washed spores of the bacillus are quickly taken up by phagocytes and destroyed without producing infection. Salt-solution suspensions of such spores quickly infect, however, if mixed with some tissue-injuring agent such as lactic acid, or if combined with a harmless micro-organism such as Bacillus prodigiosus by which the phagocytic activity of the leukocytes is distracted through preference.

Lesions.—In the blood the bacilli are few because of the loosely combined oxygen it contains. The great majority of the bacilli occupy the subcutaneous tissue, where very little oxygen is present and the conditions of growth are good. The autopsy shows a marked subcutaneous edema containing immense numbers of the

bacilli. If the animal be permitted to remain undisturbed for some time after death, the bacilli spread to the circulatory system and reach all the organs.

Brieger and Ehrlich* have reported 2 cases of malignant edema in man. Both occurred in typhoid fever patients subcutaneously injected with musk, the infection no doubt resulting from impurities in the therapeutic agent.

Grigorjeff and Ukke† have observed another interesting case of typhoid fever with intestinal ulcerations, through which infection by the bacillus of malignant edema took place. The case was characterized by interstitial emphysema of the subcutaneous tissue of the neck and breast, gas bubbles in the muscles, and a transformation of the entire liver into a spongy porous mass of a grayish-brown color. The spleen was enlarged and soft, and contained a few gas-bubbles. Though the writers consider this organism to be the bacillus of malignant edema, the general impression one receives from the description of the lesions suggests that it was Welch's Bacillus aërogenes capsulatus.

Immunity.—Cornevin found that the passage of the bacillus through white rats diminished its virulence, and that the animals of various species that recovered were immune against subsequent infection with the virulent organisms. Roux and Chamberland‡ found that the filtered cultures were toxic and that animals could be immunized by injection with this toxic filtrate.

GASEOUS EDEMA

Bacillus Aerogenes Capsulatus (Welch)

General Characteristics.—A large, stout, non-motile, non-flagellate, sporogenous, non-chromogenic, purely anaërobic, markedly aërogenic, doubtfully pathogenic bacillus, easily cultivated in artificial media, readily stained by the ordinary methods and by Gram's method.

This disease is caused by an interesting micro-organism described by Welch, and subsequently studied by Welch and Nuttall,§ Welch and Flexner,‖ and others. Welch said at the meeting of the Society of American Bacteriologists held at Philadelphia, December 30, 1904, that he believed this organism to be identical with Kline's Bacillus enteritidis sporogenes,** and that it belongs to the butyric acid group. It is probably also identical with Bacillus phlegmone emphysematose of Fränkel.†† In many systematic writings the organism is now called Bacillus welchii. English writers identify it with Bacillus

* "Berliner klin. Wochenschrift," 1882, No. 44.
† "Militär-medizin. Jour.," 1898, p. 323.
‡ "Ann. de l'Inst. Pasteur," 1887.
§ Bull. of the Johns Hopkins Hospital," July and Aug., 1892, vol. VIII, No. 24.
‖ "Jour. of Experimental Medicine," Jan., 1896, vol. I, No. 1, p. 6.
** Centralbl. f. Bakt. u. Parasitenk, 1895, XVIII, 737.
†† "Centralbl. f. Bakt.," etc., Bd. XIII, p. 13.

perfringens of Veillon and Zuber,* and Besson describes it under this name. Pending final decision upon the identity of these organisms, it is here called by the name originally given it by Welch who first secured it from the body of a man dying suddenly of aortic aneurysm with a peculiar gaseous emphysema of the subcutaneous tissues and internal organs, and a copious formation of gas in the blood-vessels. The blood was thin and watery, of a lac color, and contained many large and small gas bubbles, and many bacilli, which were also obtained from it and the various organs, especially in the neighborhood of the gas bubbles, in nearly pure culture. The coloring-matter of the blood was dissolved out of the corpuscles and stained the tissues a deep red.

Distribution.—It is believed that the natural habitat of the bacillus is the soil, but there is reason to think that it commonly occurs in the intestine, and may occasionally be found upon the skin.

Fig. 119.—Bacillus aërogenes capsulatus (from photograph by Prof. Simon Flexner).

Morphology.—The bacillus is a large organism, measuring 3–5 μ in length, about the thickness of the anthrax bacillus, with ends slightly rounded, or, when joined, square. It occurs chiefly in pairs and in irregular groups, but may also occur in chains. In culture media it is usually straight, with slightly rounded ends. In old cultures the rods may be slightly bent, and involution forms occur. The bacillus varies somewhat in size, especially in length, in different culture-media. It usually appears thicker and more variable in length in artificial cultures than in the blood of animals.

The bacillus is not motile and has no flagella.

Dunham† found that spores were produced upon blood-serum, and especially upon Löffler's blood-serum bouillon mixture. The spores

* Archiv de méd. expér. et d'anat. path., 1898, x, 517.

† "Bull. of the Johns Hopkins Hospital," April, 1897, p. 68.

resist desiccation and exposure to the air for ten months. They stain readily in hot solutions of fuchsin in anilin water, and are not decolorized by a moderate exposure to the action of 3 per cent. solution of hydrochloric acid in absolute alcohol. They are oval, and are usually situated near the middle of the bacillus, which is distended because of the large size of the spore and bulges at the sides.

Staining.—The organism stains well with the ordinary stains, and retains the color well in Gram's method. When stained with methylene-blue a granular or vacuolated appearance is sometimes observed, due to the presence of unstained dots in the cytoplasm.

Usually in the body-fluids and often in cultures the bacilli are surrounded by distinct capsules—clear, unstained zones. To demonstrate this capsule to the best advantage, Welch and Nuttall devised the following special stain:

A cover is thinly spread with the bacilli, dried, and fixed without overheating. Upon the surface prepared, glacial acetic acid is dropped for a few moments, then allowed to drain off, and at once replaced by a strong aqueous solution of gentian violet, which is poured off and renewed several times until the acid has been replaced by the stain. The specimen is then examined in the coloring solution, after soaking up the excess with filter-paper, the thin layer of coloring fluid not interfering with a clear view of the bacteria and their capsules. After mounting in Canada balsam the capsules are not nearly so distinct. The width of the capsule varies from one-half to twice the thickness of the bacillus. Its outer margin is stained, leaving a clear zone immediately about the bacillus.

Cultivation.—The bacillus is *anaërobic* and *aërogenic*. It grows upon all culture media at the room temperature, though better at the temperature of incubation.

Gelatin.—It grows in ordinary neutral or alkaline gelatin, but better in gelatin containing glucose, in which the characteristic gas production is marked. Soft media, made with 5 instead of 10 per cent. of the crude gelatin, is said to be better than the standard preparation.

There is no distinct liquefaction of the medium, but in 5 per cent. gelatin softening can sometimes be demonstrated by tilting the tube and observing that the gas bubbles change their position, as well as by noticing that the growth tends to sediment.

Agar-agar.—In making agar-agar cultures careful anaërobic precautions must be observed. The tubes should contain considerably more than the usual quantity of the medium, which should be boiled and freshly solidified before using. The implantation should be deeply made with a long wire. The growth takes place slowly unless such tubes are placed in a Buchner's jar or other anaërobic device. The deeper colonies are the largest. Sometimes the growth takes place within 10–12 mm. of the surface; at others, within 3–4 cm. of it. After repeated cultivation the organisms seem to become

accustomed to the presence of oxygen, and will grow higher up in the tube than when freshly isolated.

Colonies.—The colonies seen in the culture-media are grayish-white or brownish-white by transmitted light, and sometimes exhibit a central dark dot. At the end of twenty-four hours the larger colonies do not exceed 0.5–1.0 mm. in diameter, though they may subsequently attain a diameter of 2–3 mm. or more. Their first appearance is as little spheres or ovals, more or less flattened, with irregular contours, due to the presence of small projecting prongs, which are quite distinct under a lens. The colonies may appear as little irregular masses with projections.

After several days or weeks, single, well-shaped colonies may attain a large size and be surrounded by projections, either in the form of little knobs or spikes or of fine branchings—hair-like or feathery. Their appearance has been compared to thistle-balls or powder-puffs and to thorn-apples. When the growth takes place in the puncture, the feathery projections are continuous. Bubbles of gas make their appearance in plain agar as well as in sugar-agar, though, of course, less plentifully. They first appear in the line of growth; afterward throughout the agar, often at a distance from the actual growth. Any fluid collecting about the bubbles or at the surface of the agar-agar may be turbid from the presence of bacilli. The gas-production is more abundant at 37°C. than at the room temperature.

The agar-agar is not liquefied by the growth of the bacillus, but is often broken up into fragments and forced into the upper part of the tube by the excessive gas-production.

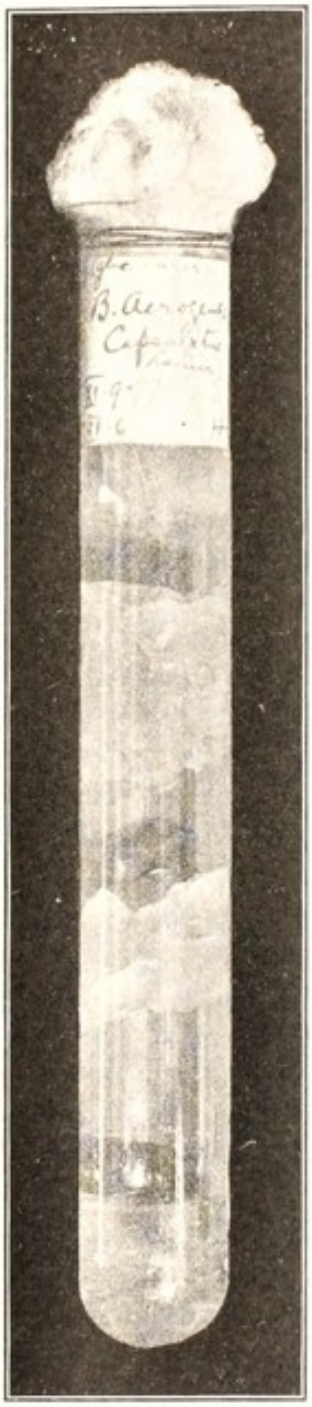


Fig. 120.—Bacillus aërogenes capsulatus, with gas production (from photograph by Prof. Simon Flexner).

Bouillon.—In bouillon, growth does not occur in tubes exposed to the air, but when the tubes are placed in Buchner's jars, or kept under anaërobic conditions, it occurs with abundant gas-formation, especially in glucose-bouillon, and the formation of a frothy layer on the surface. The growth is rapid in development, the bouillon becoming clouded in two to three hours. After a few

days the bacilli sediment and the bouillon again becomes clear. The reaction of the bouillon becomes strongly acid.

Milk.—In milk the growth is rapid and luxuriant under anaërobic conditions, but does not take place in cultures exposed to the air. The milk is coagulated in from twenty-four to forty-eight hours, the coagulum being either uniform or firm, retracted, and furrowed by gas bubbles. When litmus has been added to the milk, it becomes decolorized when the culture is kept without oxygen, but turns pink when it is exposed to the air.

Potato.—The bacillus will also grow upon potato when the tubes are inclosed in an anaërobic apparatus. There is a copious gas-development in the fluid at the bottom and sides of the tube, so that the potato becomes surrounded by a froth. After complete absorption of the oxygen a thin, moist, grayish-white growth takes place upon the surface of the medium.

Vital Resistance.—The vital resistance of the organism is not great. Its thermal death-point was found to be 58°C. after ten minutes' exposure. Cultures made by displacing the air with hydrogen are less vigorous than those in which the oxygen is absorbed from the air by pyrogallic acid. It was found that in the former class of cultures the bacillus died in three days, while in the absorption experiments it was kept alive at the body temperature for one hundred and twenty-three days. It is said to live longer in plain agar than in sugar-agar. To keep the cultures alive it has been recommended to seal the agar-agar tube after two or three days' growth.

Metabolic Products.—The bacillus is unable to make use of the uncombined oxygen of the atmosphere, and derives its oxygen supply entirely from carbohydrates in the medium in which it grows. It causes fermentation of most carbohydrates with the evolution of much gas and some acid. It coagulates milk.

Simonds* divides the organisms known as B. aërogenes capsulatus or B. welchii into four groups according to their metabolic activities as follows:

1. Organisms that ferment inulin and glycerin with production of gas and increase of acidity. Do not form spores in media containing either substance. Produce strong hemolysins, and are pathogenic for guinea-pigs, even after many months cultivation upon artificial media.

2. Organisms that produce acid and gas from glycerin but not from inulin. Form spores in inulin but not in glycerin broth. Hemolytic and pathogenic powers variable.

3. Organisms that produce acid and gas from inulin but not from glycerin. Form spores in glycerin but not in inulin broth. Hemolysis and pathogenicity variable.

4. Organisms that do not produce acid or gas from either inulin or glycerin and from spores in both inulin and glycerin broths.

* Jour. Infectious Diseases, 1915, XVI, 32.

Pathogenesis.—The pathogenic powers of the bacillus are limited, and while in some infected cases it seems to be the cause of death, its power to do mischief in the body seems to depend entirely upon the pre-existence of depressing and devitalizing conditions predisposing to its growth.

Being anaërobic, the bacilli are unable to live in the circulating blood, though they grow in old clots and in cavities, such as the uterus, etc., where little oxygen enters, and from which they enter the blood and are distributed.

In support of these views Welch and Nuttall show that when 2.5 cc. of a fresh sugar-bouillon culture are injected into the ear-vein of a healthy rabbit, it usually recovers. After similar injection with but 1 cc. of the culture, a pregnant rabbit carrying two dead embryos, died in twenty-one hours. It seems that the bacilli were first able to secure a foothold in the dead embryos, and there multiplied sufficiently to bring about the subsequent death of the mother.

After death, when the blood is no longer oxygenated, the bacilli grow rapidly, with marked gas-production, which in some cases is said to cause the body to swell to twice its natural size. The effect upon guinea-pigs does not differ from that upon rabbits, though gaseous phlegmons are sometimes produced.

Pigeons, when subcutaneously inoculated in the pectoral region, frequently die in from seven to twenty-four hours, but may recover. Gas-production causes the tissues to become emphysematous.

Intraperitoneal inoculation sometimes causes fatal purulent peritonitis of laboratory animals.

Sources of Infection.—The infection seen in man usually occurs from wounds into which earth has been ground, as in the case of a compound, comminuted fracture of the humerus, with fatal infection, reported by Dunham, or in wounds and injuries in the neighborhood of the perineum.

Among the twenty-three cases reported by Welch and Flexner* we find wounds of the knee, leg, hip, and forearm, ulcer of the stomach, typhoid ulcerations of the intestine, strangulated hernia with operation, gastric and duodenal ulcer, perineal section, and aneurysm, as conditions in which external or gastro-intestinal infection occurred.

Dobbin,† P. Ernst,‡ Graham, Stewart and Baldwin,§ and Krönig and Menge‖ have studied cases of puerperal sepsis and sepsis following abortion either caused by the bacillus or in which it played an important rôle.

Williams** has found the bacillus in a case of suppurative pyelitis.

* "Journal of Experimental Medicine," Jan., 1896, vol. 1, No. 1.
† "Bull. Johns Hopkins Hospital," Feb., 1897, No. 71, p. 24.
‡ "Virchow's Archiv," Bd. CXXXIII, Heft 2.
§ "Columbus Med. Jour.," Aug., 1893.
‖ "Bakteriologie des weiblichen Genitalkanals," Leipzig, 1897.
** "Bull. Johns Hopkins Hospital," April, 1896, p. 66.

The symptoms following infection are quite uniform, consisting of redness and swelling of the wound, with rapid elevation of temperature and rapid pulse. The wound usually becomes more or less emphysematous, and discharges a thin, dirty, brownish, offensive fluid that contains gas bubbles and is sometimes frothy. The patients occasionally recover, especially when the infected part can be amputated, but death is the common outcome. After death the body begins to swell almost immediately, may attain twice its normal size and be unrecognizable. Upon palpation a peculiar crepitation can be felt in the subcutaneous tissue nearly everywhere, and the presence of gas in the blood-vessels is easy of demonstration. The gas is inflammable, and as the bubbles ignite explosive sounds are heard.

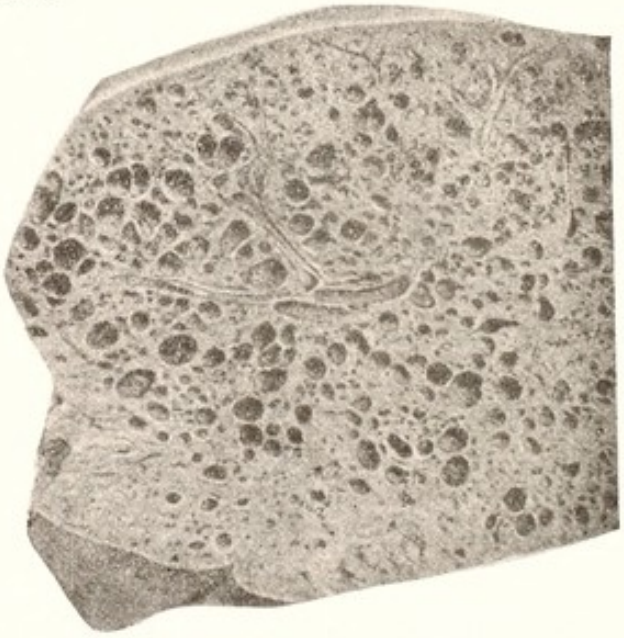


Fig. 121.—"Frothy liver" from Bacillus aërogenes capsulatus infection (Aschoff).

At the autopsy the gas bubbles are found in most of the internal organs, sometimes so numerously as to justify the German term "Schaumorgane" (frothy organs). The liver is especially apt to show this condition When such tissues are hardened and examined microscopically, the bubbles appear as spaces in the tissue, their borders lined with large numbers of the bacillus. There are also clumps of bacilli without gas bubbles, but surrounded by tissue, whose nuclei show a disposition to fragment or disappear, and whose cells and fibers show signs of disintegration and fatty change. In discussing these changes Ernst concluded that they were ante-mortem and due to the irritation caused by the bacillus. The gas-production he regards as post-mortem.

In the internal organs the bacillus is usually found in pure culture, but in the wound it is usually mixed with other bacteria. On this account it is difficult to estimate just how much of the damage before death depends upon the activity of the gas bacillus. That

gas-production after death has nothing to do with pathogenesis during life is shown by injecting into the ear-vein of a rabbit a liquid culture of the gas bacillus, permitting about five minutes' time for the distribution of the bacilli throughout the circulation, and then killing the rabbit. In a few hours the rabbit will swell and its organs and tissues be riddled with the gas bubbles.

At times, however, as in a case of Graham, Stewart and Baldwin, there is no doubt but that the bacillus produces gas in the tissues of the body during life. These observers, in a case of abortion with subsequent infection, found the patient "emphysematous from the top of her head to the soles of her feet" several hours before death.

In this case, in which the bacillus was found in pure culture, it would indeed be difficult to doubt that the fatal issue was due to Bacillus aërogenes capsulatus.

An excellent review of the early literature of the subject is to be found in "A Contribution to the Knowledge of the Bacillus Aërogenes Capsulatus," by W. T. Howard, Jr.*

* "Contributions to the Science of Medicine by the Pupils of W. H. Welch," 1900, p. 461.

CHAPTER III

TETANUS

Bacillus Tetani (Flügge)

General Characteristics.—A motile, flagellated, sporogenous, liquefying, obligatory anaërobic, non-chromogenic, aërogenic, toxic, pathogenic bacillus of the soil, staining by ordinary methods and by Gram's method. Its chief morphologic characteristic is the occurrence of a large round spore at one end.

The bacillus of tetanus was discovered by Nicolaier* in 1884, and obtained in pure culture by Kitasato† in 1889. It is universally acknowledged to be the cause of tetanus or "lock-jaw."

Distribution.—The tetanus bacillus is a common saprophyte in garden earth, dust, and manure, and is a constant parasite in the intestinal contents of herbivorous animals.

The relation of the bacillus to manure is interesting, but it is most probable that manured ground, because it is richer, permits the bacilli to flourish better than sterile ground. The common occurrence of the bacilli in the excrement of herbivorous animals is to be explained through the accidental ingestion of earth with the food cropped from the ground. The spores of the bacillus thus reaching the intestine seem able to develop because of appropriate anaërobic conditions. Verneuil has observed that tetanus rarely occurs at sea except upon cattle transports.

Le Dantec‡ has shown that the tetanus bacillus is a common organism in New Hebrides, where the natives poison their arrows by dipping them into a clay rich in its spores.

Morphology.—The tetanus bacillus is a long, slender organism measuring 0.3 to 0.5 × 2 to 4 μ (Flügge). Its most striking characteristic is an enlargement of one end, which contains a large round spore. The bacilli in which no spores are yet formed have rounded ends and seldom unite in chains or pairs. They are motile and have many flagella arising from all parts of the surface (petrichia).

Staining.—The bacilli stain readily with ordinary aqueous solutions of the anilin dyes and by Gram's method.

Isolation.—The method usually employed for the isolation of the tetanus bacillus was originated by Kitasato, and based upon the observation that its spores can resist exposure to high temperatures for considerable periods of time. After finding by microscopic examination that the bacilli were present in pus, Kitasato spread it upon the surface of an ordinary agar-agar tube and incubated it for

* "Deutsche med. Wochenschrift," 1884, 42.

† Ibid., 1889, No. 31.

‡ See abstracts in the "Centralbl. f. Bakt. u. Parasitenk.," IX, 286; XIII, 351.

Fig. 122.—Bacillus tetani. × 1000 (Fränkel and Pfeiffer).

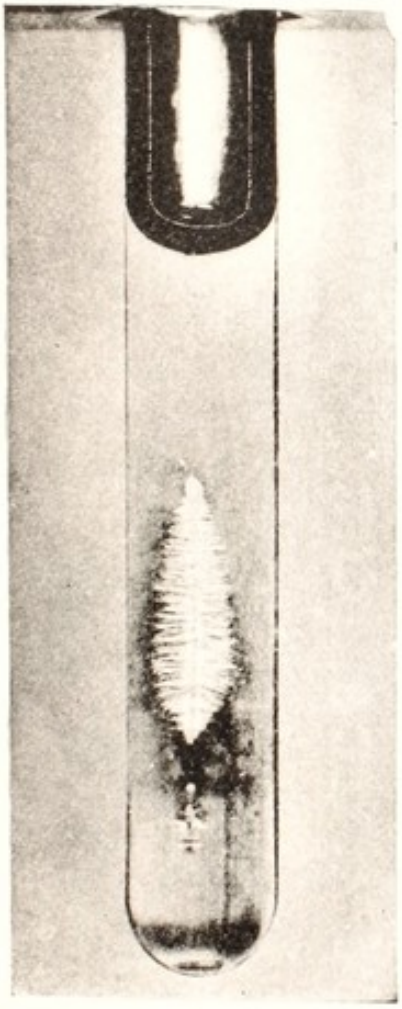


Fig. 123.—Bacillus tetani; six-day-old puncture culture in glucose-gelatin (Fränkel and Pfeiffer).

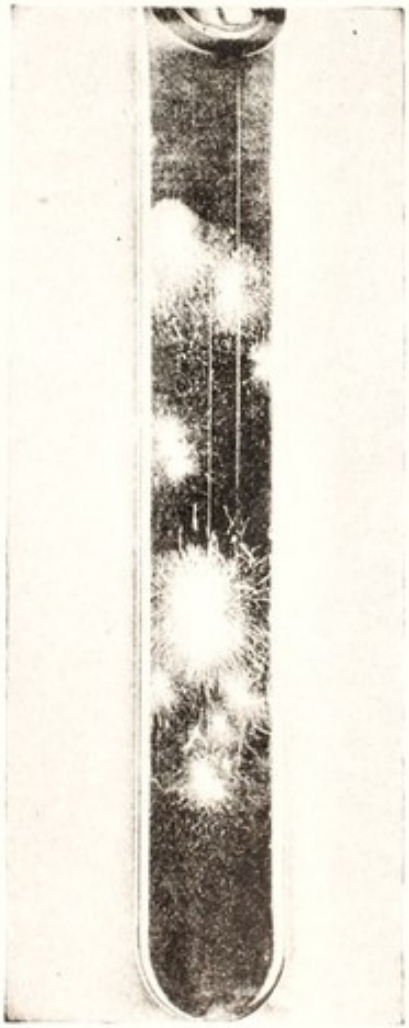


Fig. 124.—Bacillus tetani; culture four days old in glucose-gelatin (Fränkel and Pfeiffer).

twenty-four hours, during which time all of the contained micro-organisms, including the tetanus bacillus, increased in number. He then exposed it for an hour to a temperature of 80°C., by which all fully developed bacteria, tetanus as well as the others, and the great majority of the spores, were destroyed. As scarcely anything but the tetanus spores remained alive, their subsequent growth gave a fairly pure culture.

Cultivation.—The tetanus bacillus is difficult to cultivate because it will not grow where the smallest amount of free oxygen is present. It is hence a typical obligatory anaërobe. Farran* and Grixoni

Fig. 125.—Bacillus tetani; five-day-old colony upon gelatin containing glucose. × 1000 (Fränkel and Pfeiffer).

believe it to have originally been an optional anaërobe, and it is said by these writers that the organism can gradually be accustomed to oxygen so as to grow in its presence. When this is achieved, it loses its virulence.

The general methods for the cultivation of anaërobic organisms, are given under the appropriate heading (Anaërobic Cultures), and need not be repeated here.

The **colonies** of the tetanus bacillus, when grown upon gelatin plates in an atmosphere of hydrogen, resemble those of the well-known hay bacillus. There is a rather dense, opaque central mass surrounded by a more transparent zone, the margins of which consist of a fringe of radially projecting bacilli. Liquefaction occurs slowly.

* "Centralbl. f. Bakt. u. Parasitenk.," July 15, 1898, p. 28.

Gelatin.—The growth occurs deep in the puncture, and is arborescent. Liquefaction begins in the second week and causes the disappearance of the radiating filaments. The liquefaction spreads slowly, but may involve the entire mass of gelatin and resolve it into a grayish-white syrupy liquid, at the bottom of which the bacilli accumulate. The growth in gelatin containing glucose is rapid.

Agar-agar.—The growth in agar-agar punctures is slower, but similar to the gelatin cultures except for the absence of liquefaction.

Bouillon.—The organism can be grown in bouillon without difficulty, when once habituated to the medium. The bouillon should be heated to drive off the air, then rapidly cooled and the transplantation made. If there be a depth of 10 cm. the bacilli grow readily in the lower half of the fluid. If the surface be covered with liquid paraffin before the final sterilization and inoculation, they grow throughout the entire medium. The organism attains its maximum development at a temperature of 37°C. Gas is given off from the cultures, and they have a peculiar odor, very characteristic, but difficult to describe. The bouillon is clouded and contains a sediment.

In bouillon containing sugar considerable gas is formed in the fermentation tube. Both CO_2 and H_2S are formed.

Milk is favorable for the development of the tetanus bacillus. There is no coagulation. Litmus milk is acidified.

Potato.—Upon potatoes under strict anaërobic conditions the bacilli grow but slightly.

Vital Resistance.—The tetanus spores may remain alive in dry earth for many years. Sternberg says they can resist immersion in 5 per cent. aqueous carbolic acid solutions for ten hours, but fail to grow after fifteen hours. A 5 per cent. carbolic acid solution, to which 0.5 per cent. of hydrochloric acid has been added, destroys them in two hours. They are destroyed in three hours by 1 : 1000 bichlorid of mercury solution, but when to such a solution 0.5 per cent. of hydrochloric acid is added, its activity is so increased that the

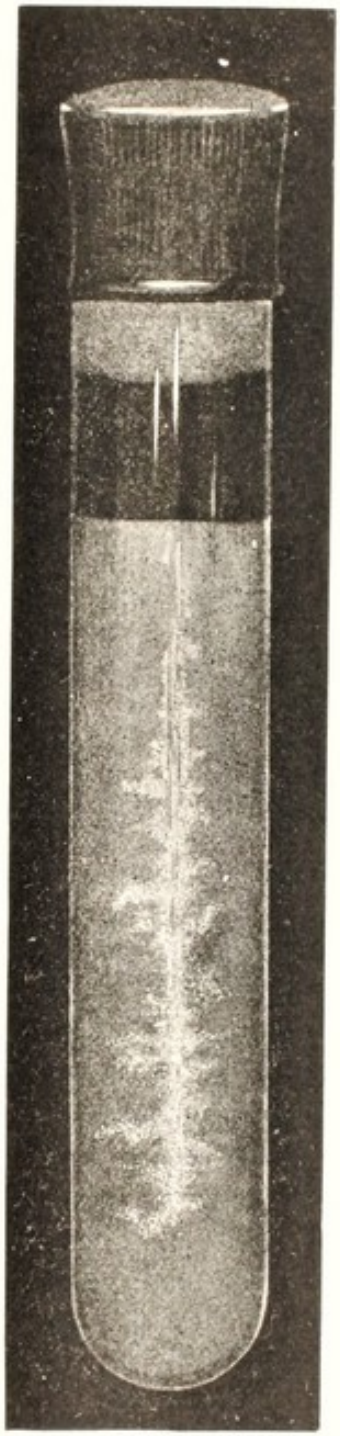


Fig. 126.—Tetanus bacillus; glucose-agar culture, five months old (Curtis).

spores are destroyed in thirty minutes. According to Kitasato,* exposure to streaming steam for from five to eight minutes is certain to kill tetanus spores, and this statement has found its way into most of the text-books without discussion. Theobald Smith,† however, has studied several cultures of the organism and finds that its resistance to heat is much greater, and that in one case seventy minutes' exposure to streaming steam did not kill all of the spores.

Metabolic Products.—Bouillon cultures of the tetanus bacillus contain acids, proteolytic ferment, and several toxic substances, of which tetanospasmin and tetanolysin are best known. The toxic products are apparently all soluble. No endotoxin is known to be formed.

The most ready method of preparing the toxins for experimental study is to cultivate the bacilli in freshly prepared neutral or slightly alkaline sugar-free bouillon under conditions of most strict anaërobiosis, at a temperature of 37°C., and then filter the culture through porcelain. Field‡ found the highest degree of toxicity about the sixth or seventh day. It may attain a toxicity so great that 0.000005 c.c. will cause the death of a mouse. The average culture has such toxicity that 0.001 c.c. is fatal to a guinea-pig. Knorr§ gives some interesting comparisons of the susceptibility of different animals, as follows:

1 gram of horse is destroyed by.....	*x* toxin
1 gram of goat is destroyed by.....	2*x* toxin
1 gram of mouse is destroyed by.....	13*x* toxin
1 gram of rabbit is destroyed by.....	2,000*x* toxin
1 gram of hen is destroyed by.....	200,000*x* toxin

The toxin is very unstable, and is easily destroyed by heat above 60°C. It is also quickly destroyed by light, especially direct sunlight. Flexner and Noguchi‖ found that 5 per cent. of eosin added to the toxin destroyed it through the photodynamic power of the stain. It is also easily destroyed by electric currents. The best method of keeping it is to add 0.5 per cent. of phenol, and then store it in a cool, dark place, in bottles completely filled and tightly corked. It will not keep its strength in liquid form under the best conditions.

To keep it for experimental purposes it is advisable to precipitate the toxin from the bouillon by supersaturation with ammonium sulphate, which causes it to float upon the liquid in the form of a sticky brown scum that can be skimmed off and dried. Such dry precipitate retains its activity for months.

From cultures of tetanus bacilli grown in various media, and from the blood and tissues of animals affected with the disease, Brieger succeeded in separating "tetanin," "tetanotoxin," tetanospasmin," and a fourth substance to which no name is given. All were very

* "Zeitschrift für Hygiene," XII, p. 225.
† "Jour. Amer. Med. Assoc.," March 21, 1908, vol. L, No. 12, p. 931.
‡ "Proc. N. Y. Path. Soc.," March, 1904, p. 18.
§ "Münch. med. Wochenschrift," 1898, p. 321.
‖ "Studies from the Rockefeller Institute," 1905, V.

poisonous and productive of tonic convulsions. Later Brieger and Fränkel isolated an extremely poisonous toxalbumin from sugar-bouillon cultures of the bacillus. Ehrlich* later discovered a new poisonous element to which he applied the name *tetanolysin*.

The purified toxin of Brieger and Cohn was fatal to mice in doses of 0.00000005 gram. Lambert† considers the tetanus toxin to be the most poisonous substance that has ever been discovered.

Fermi and Pernoss‡ found most toxin produced in agar-agar cultures, less in gelatin cultures, and least in bouillon cultures.

Ehrlich§ found two poisons in the tetanus toxin, one of which was convulsive and was in consequence called *tetanospasmin*, the other hemolytic and called *tetanolysin*. When tetanus toxin is added to defibrinated blood, the tetanolysin is absorbed by the corpuscles, many of which are dissolved, while the tetanospasmin remains unchanged.

Dönitz‖ and Wassermann and Takaki** have found that the tetanus toxin has a specific affinity for the central nervous system, with whose cells it combines *in vitro* and becomes inert.

Roux and Borrel†† have found that when tetanus toxin is injected into the brain substance a very much smaller dose will cause death than is necessary when the poison is absorbed from the subcutaneous tissues.

Like most of the bacterial toxins, the tetanus poison is only effective when produced in or injected into the tissues and absorbed into the circulation. It is harmless when given by the digestive tract, Ramon‡‡ having administered by the mouth 300,000 times the fatal hypodermic dose without producing any symptoms.

One of the most interesting peculiarities about the toxin is the comparative uniformity of the period intervening between its administration and the appearance of the symptoms—erroneously called the incubation period. This varies within a narrow margin, inversely, with the size of the dose. Thus, according to Behring, the effect of varying doses of the toxin upon mice becomes evident according to the size of the dose in from twelve to thirty-six hours, thus:

13 lethal doses	symptoms in 36 hours
110 lethal doses	symptoms in 24 hours
333 lethal doses	symptoms in 20 hours
1300 lethal doses	symptoms in 14 hours
3600 lethal doses	symptoms in 12 hours

The local action of the toxin is very painful and associated with spasm of the muscular fibers with which it comes in contact. Pit-

* "Berliner klin. Wochenschrift," 1898.
† "New York Med. Jour.," June 5, 1897.
‡ "Centralbl. f. Bakt.," etc., XV, p. 303.
§ "Berliner klin. Wochenschrift," 1898, No. 12, p. 273.
‖ "Deutsche med. Wochenschrift," 1897, p. 428.
** "Berliner klin. Wochenschrift," 1898, 35.
†† "Ann. de l'Inst. Pasteur," 1898 XII.
‡‡ "Deutsche med. Wochenschrift," Feb. 24, 1898.

field,* thinking that it might be useful in the treatment of certain paralytic affections, injected a minute quantity of it into the calf of his leg and experienced the severe spasmodic local effects of the poison for twelve hours.

It has been the belief of most physiologists that tetanus toxin acts solely upon the motor cells of the spinal cord, and causes the tonic spasms as strychnin does. The affinity of the toxin for the nervous tissues has been made the subject of careful investigations by Marie and Morax† and Meyer and Ransom.‡ The former found that the absorption of tetanus toxin took place partly through the peripheral nerves because of specific affinity between the toxin and the axis cylinder substance; the latter found the toxin carried to the central nervous system solely by the motor nerves, the action depending upon the integrity of the axis cylinder. They believe that the toxin is absorbed by the axis cylinder endings, and reaching the corresponding spinal nerve center by that route spreads to the corresponding center in the other half of the cord and outward, resulting in generalized tetanus. When intoxication is produced through the circulation, the poison is taken up by the nerve endings in all parts of the body, and the disease is not localized, but general. Antitoxin, unlike the toxin, does not travel by the nerve route, but is found only in the blood and lymph. Zupnik§ has brought forward evidence that this view is incorrect and that there are two distinct actions caused by the toxin. He differentiates between *tetanus ascendens* and *tetanus descendens*. The former always follows the intramuscular introduction of the toxin, and depends upon its direct action upon the muscle itself. It explains the familiar phenomenon of rigidity making its first appearance in that member into which the inoculation was made. The ascending tetanus gradually ascends from muscle to muscle. He thinks the absorption of the poison by the muscle-cells depends upon their normal metabolic function, as when their nerves are severed, the fixation of the toxin and the occurrence of the tonic spasm does not occur.

Tetanus descendens results from the entrance of the toxin into the circulation from the cellular tissue and its distribution in the blood. Under these conditions Zupnik believes it acts upon the central nervous system, especially upon the spinal cord, manifesting itself in extreme reflex excitability with irregular motor discharges resulting in clonic spasms.

There are, therefore, two forms of spasm in tetanus: the *tonic* convulsions, seeming to depend upon local action and fixation of the toxin, and the *clonic* convulsions, depending upon the centric action. The latter are the more dangerous for the sufferer.

* "Therapeutic Gazette," March 15, 1897.

† "Ann de l'Inst. Pasteur," 1902, XVI, p. 818; and "Bull. de l'Inst. Past.," 1903, I, p. 41.

‡ "Arch. f. exper. Path. u. Pharmak.," 1903, XLIX.

§ "Wiener klin. Wochenschrift," Jan. 23, 1902.

The lockjaw or *trismus* and the *opisthotonos* that are so characteristic of the affection depend, according to Zupnik's view, upon a loss of equilibrium among the muscles affected. They occur only in descending tetanus and depend upon spasm of muscles without equally powerful opposing groups. The stronger muscles of the jaw are those that close it; the stronger muscles of the back, those of the erector group. This view is exactly the opposite of Meyer and Ransom,* who believe that the tetanus toxin is absorbed only along the nerve trunks, and found that section of the spinal cord prevented the ascent of tetanus from the lower extremities. Injection of the toxin into a posterior nerve-root produced tetanus dolorosus. Injection of the toxin into a posterior nerve-root together with section of the spinal cord produced exaltation of the reflex irritability—"Jactationstetanus." Injection in sensory nerves does not produce tetanus dolorosus because the transportation of the poison along these trunks is so slow.

The *tetanolysin* is a hemolytic component of the toxic bouillon, and is entirely separate and distinct from the tetanospasmin or convulsive poison. It probably takes no part in the usual clinical manifestations of tetanus.

Pathogenesis.—The work of Kitasato has given us very complete knowledge of the biology of the tetanus bacillus and completely established its specific nature.

When a white mouse is inoculated with an almost infinitesimal amount of tetanus culture, or with garden earth containing the tetanus bacillus, the first symptoms come on in from one to two days, when the mouse develops typical tetanic convulsions, first beginning in the neighborhood of the inoculation, but soon becoming general. Death follows sometimes in a very few hours. In rabbits, guinea-pigs, mice, rats, and other small animals the period of incubation is from one to three days. In man the period of incubation varies from a few days to several weeks, and averages about nine days.

The disease is of much interest because of its purely toxic nature. There is usually a small wound with a slight amount of suppuration and at the autopsy the organs of the body are normal in appearance, except the nervous system, which bears the greatest insult. It, however, shows little else than congestion either macroscopically or microscopically.

The conditions in the animal body are in general unfavorable to the development of the bacilli, because of the loosely combined oxygen contained in the blood, and they grow with great slowness, remaining localized at the seat of inoculation, and never entering the blood. Doubtless most cases of tetanus are mixed infections in which the bacillus enters with aërobic bacteria, that aid its growth by absorbing the oxygen in the neighborhood. The amount of poison produced must be exceedingly small and its power tremen-

* "Archiv. f. exper. Path. u. Pharmak.," 1903, Bd. XLIX, p. 396.

dous, else so few bacilli growing under adverse conditions could not produce fatal toxemia. The toxin is produced rapidly, for Kitasato found that if mice were inoculated at the root of the tail, and the skin and the subcutaneous tissues around the inoculation afterward either excised or burned out, the treatment would not save the animal unless the operation were performed *within an hour after the inoculation.*

Some incline to the view that the toxin is a ferment, and the experiments of Nocard* might be adduced in support of the theory. He says: "Take three sheep with normal tails, and insert under the skin at the end of each tail a splinter of wood covered with the dried spores of the tetanus bacillus; watch these animals carefully for the first symptoms of tetanus, then amputate the tails of two of them 20 cm. above the point of inoculation, . . . the three animals succumb to the disease without showing any sensible difference."

The circulating blood of diseased animals is fatal when injected into susceptible animals because of the toxin it contains; and the fact that the urine is also toxic to mice proves that the toxin is excreted by the kidneys.

Two classes of infected wounds are particularly apt to be followed by tetanus—namely, those into which soil has been carried by the injuring implement and those of considerable depth. The infecting organism reaches the first class in large numbers, but finds itself under aërobic and other inappropriate conditions of growth. It reaches the second class in smaller numbers, but finds the conditions of growth better because of the depth of the wound.

The severity of the wound has nothing whatever to do with the occurrence of tetanus, pin-pricks, nail punctures, insect stings, vaccination, and a variety of other mild injuries sometimes being followed by it.

An interesting fact has been presented by Vaillard and Rouget,† who found that if the tetanus spores were introduced into the body freed from their poison, they were unable to produce the disease because of the promptness with which the phagocytes took them up. If, however, the toxin was not removed, or if the body-cells were injured by the simultaneous introduction of lactic acid or other chemic agent, the spores would immediately develop into bacilli, begin to manifacture toxin, and produce the disease. This suggests that many wounds may be infected by the tetanus bacillus though the surrounding conditions rarely enable it to develop satisfactorily and produce enough toxin to cause disease.

In very rare cases tetanus may possibly occur without the previous existence of a wound, as in the case reported by Kamen, who found the intestine of a person dead of the disease rich in Bacillus tetani. Kamen is of the opinion that the bacilli can grow in the

* Quoted before the Académie de Médicine, Oct. 22, 1895.

† See "Centralbl. f. Bakt. Infekt., u. Parasitenk.," vol. XVI, p. 208.

intestine and be absorbed, especially where imperfections in the mucosa exist.

Montesano and Montesson,* unexpectedly found the tetanus bacillus in pure culture in the cerebro-spinal fluid of a case of paralytic dementia that died without a tetanic symptom.

Immunity.—All animals are not alike susceptible to tetanus. Men, horses, mice, rabbits, and guinea-pigs are susceptible; dogs much less so. Cattle suffer chiefly after castration, accouchement, or abortion. Most birds are scarcely at all susceptible either to the bacilli or to their toxin. Amphibians and reptiles are immune, though it is said that frogs can be made susceptible by elevation of their body-temperature.

The injection of the toxic bouillon or of the redissolved ammonium sulphate precipitate, in progressively increasing doses, into animals, causes the formation of antibodies (antitoxin) by which the effects of both the tetanospasmin and the tetanolysin are destroyed. The purely toxic character of the disease makes it peculiarly well adapted for treatment with antitoxin, and at the present time our sole therapeutic reliance is placed upon it. The mode of preparing the serum and the system of standardization are discussed in the section upon Antitoxins in the part of this work that treats of the Special Phenomena of Infection and Immunity.

Antitoxin.—Numerous cases of the beneficial action of antitoxin are on record, but, as Welch† has pointed out, the antitoxin of tetanus is a disappointment in the treatment of tetanus. Moschcowitz,‡ in his excellent literary review of the subject, has shown that its use has reduced the death-rate from about 80 to 40 per cent., and that it therefore cannot be looked upon as a failure.

Irons§ has analyzed 225 cases of tetanus treated with antitoxic serum and found the mortality 20 per cent. lower than in cases otherwise treated. He says that it is important that the full effect of the antitoxin be immediately obtained, the best method of using it being that outlined by Park in which 3000 units are given intraspinously at the earliest possible moment after the symptoms appear, and 10,000 to 20,000 units given intravenously at the same time. On the following day the intraspinous injection of 3000 should be repeated. On the fourth or fifth day, 10,000 units should be given subcutaneously. By these means a high antitoxic content of the blood and juices is maintained.

The use of antitoxic serums must not replace other non-specific modes of treatment such as local treatment of the wound and the administration of sedatives, etc. The result of its experimental injection, in combination with the toxin, into mice, guinea-pigs, rabbits, and other animals is perfectly satisfactory, and affords protec-

* "Centralbl. f. Bakt. u. Parasitenk.," Dec., 1897, Bd. XXII, Nos. 22, 23, p. 663.
† "Bulletin of the Johns Hopkins Hospital," July and August, 1895.
‡ "Annals of Surgery," 1900, XXXII, 2, pp. 219, 416, 567.
§ "Jour. Am. Med. Asso.," 1914, LXII, 2025.

tion against almost any multiple of the fatal dose, but the quantity needed, in proportion to the body-weight, to save an animal from the unknown quantity of toxin being manufactured in its body increases so enormously with the day or hour of the disease as to make the dose, which increases millions of times where that of diphtheria antitoxin increases but tenfold, a matter of difficulty and uncertainty. Nocard also called attention to the fact that the existence of tetanus cannot be known until a sufficient toxemia to produce spasms exists, and that therefore it is impossible to attack the disease in its inception or to begin the treatment until too late to effect a cure. At this point it is well to recall Nocard's experiment with the sheep, in whose blood so much toxin was already present when symptoms first appeared that the amputation of their infected tails could not save them.

The explanation of this inability of the antitoxin to effect a cure when administered after development of the symptoms of tetanus is probably found in a ready fixation of the toxin in the bodies of the infected animals. This is well shown by the experiments of Dönitz,* who found that if a mixture of toxin and antitoxin were made before injection into an animal, twelve minimum fatal doses were neutralized by 1 cc. of a 1 : 2000 dilution of an antitoxin. If, however, the antitoxin was administered four minutes after the toxin, 1 cc. of a 1 : 600 dilution was required; if eight minutes after, 1 cc. of a 1 : 200 dilution; if fifteen minutes after, 1 cc. of a 1 : 100 dilution. He found that similar but slower fixation occurred with diphtheria toxin.

It was found by Roux and Borrel† that doses of tetanus antitoxin absolutely powerless to affect the progress of the disease, when administered in the ordinary manner by subcutaneous injection, readily saved the animal if the antitoxin were injected into the brain substance.

Chauffard and Quénu,‡ who injected the antitoxin into the cerebral substance, found that such administration brought about an apparent cure in one case.

Their observations were followed by an attempt to apply the method in human medicine, and patients with tetanus were trephined and the antitoxin injected beneath the dura and into the cerebral substance. The results have not, however, been satisfactory, and as the method cannot be looked upon as itself free from danger, it has been abandoned.

The only means of treating the disease to be recommended at present is the intraspinous, intravenous and subcutaneous injection of large and frequently repeated doses of the antitoxic serum. There can be little doubt but that the administration must be so free as to load up the patient's blood with the antitoxin in hopes that its pres-

* Reference 18, in "Jour. of Hygiene," vol. II, No. 2, in Ritchie's article.
† "Ann. de l'Inst. Pasteur," 1898, No. 4.
‡ "La Presse méd.," No. 5, 1898.

ence there may detach the toxic molecules from their anchorage to the nerve cells.

Prophylactic Treatment.—While tetanus antitoxin is extremely disappointing, in practice, for the cure of tetanus, it is most satisfactory for its prevention. "An ounce of prevention is better than a pound of cure," and if the surgeon would administer a prophylactic injection of tetanus antitoxin in every case in which the occurrence of tetanus was at all likely, the disease would rarely develop.

Bacilli Resembling the Tetanus Bacillus

Tavel* has called attention to a bacillus commonly found in the intestine, sometimes in large numbers in the appendix in cases of appendicitis, and looked upon by one of his colleagues, Fräulein Dr. von Mayer, as the probable common cause of appendicitis. He calls it the "Pseudo-tetanus-bacillus."

The bacillus measures 0.5 by 5–7μ, is rather more slender than the tetanus bacillus, and its spores are oval, situated at the end of the rod, and cause a slight bulging rather pointed at the end. The bacillus is provided with not more than a dozen flagella—usually only four to eight—thus differing markedly from the tetanus bacillus, which has many. The flagella are easily stained by Löffler's method without the addition of acid or alkali. The organism does not stain so well by Gram's method as the true tetanus bacillus. The bacillus is a pure anaërobe.

The growth in bouillon is rather more rapid than that of the tetanus bacillus. It will not grow in gelatin. The growth in agar-agar is very luxuriant and accompanied by the evolution of gas. Upon obliquely solidified agar-agar the colonies are round, circumscribed, and often encompassed by a narrow, clear zone, which is often notched. The spores are killed at 80°C.

The organism produced no symptoms in mice, guinea-pigs, and rabbits even when 2–5 cc. of a culture were subcutaneously introduced.

Sanfelice† and Lubinski‡ have observed a bacillus in earth and meat-infusions that is morphologically and culturally like the tetanus bacillus, but differs from it in not possessing any pathogenic powers.

Kruse§ has also described a bacillus much like the tetanus micro-organism that grows aërobically. It is not pathogenic.

* "Centralbl. f. Bakt.," etc., March 31, 1898, XXIII, No. 13, p. 538.
† "Zeitschrift für Hygiene," vol. XIV.
‡ "Centralbl. f. Bakt. u. Parasitenk.," XVI, 19.
§ Flügge, "Die Mikroorganismen," vol. II, p. 267.

CHAPTER IV

ANTHRAX

Bacillus Anthracis (Koch)

General Characteristics.—A non-motile, non-flagellated, sporogenous, liquefying, non-chromogenic, pathogenic, aërobic bacillus staining by the ordinary methods and by Gram's method.

The disease of herbivora known as anthrax, "splenic fever," "*Milzbrand*," and "*charbon*," is a dreaded and common malady in France, Germany, Hungary, Russia, Persia, and the East Indian countries. In Siberia the disease is so common and malignant as to

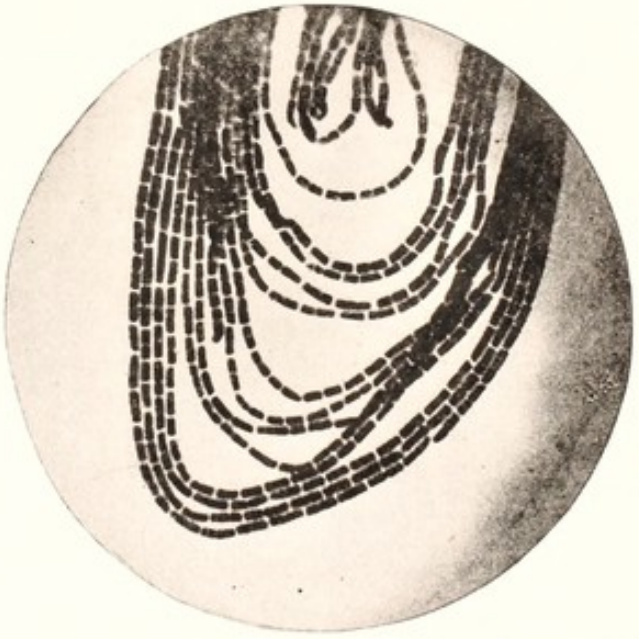


Fig. 127.—Bacillus anthracis; colony three days old upon a gelatin plate; adhesive preparation. × 1000 (Fränkel and Pfeiffer).

deserve its popular name, "Siberian pest." Certain districts, as the Tyrol and Auvergne, in which it seems to be endemic, serve as foci from which the disease spreads in summer, afflicting many animals, and ceasing its depredations only with the advent of winter. It is not rare in the United States, where it seems to be chiefly a disease of the summer season.

Herbivorous animals are most frequently affected, especially cows and sheep. Carnivorous animals are less often affected, though not immune. Among laboratory animals, white mice, house-mice, guinea-pigs, and rabbits are highly susceptible; rats, scarcely

susceptible; birds, reptiles and amphibians usually immune. Man is susceptible in varying degree.

Anthrax was one of the first infectious diseases proved to depend upon a specific micro-organism. As early as 1849 Pollender* discovered small rod-shaped bodies in the blood of animals suffering from anthrax, but the exact relation which they bore to the disease was not pointed out until 1863, when Davaine,† by a series of interesting experiments, proved their etiologic significance to most unbiased minds. The final confirmation of Davaine's conclusions and actual proof of the matter rested with Koch,‡ who, observing

Fig. 128.—Bacillus anthracis; showing the capsules. From a case of human infection. Magnified 1000 diameters (Schwalbe).

that the bacilli bore spores, cultivated them successfully outside the body, and produced the disease by the inoculation of pure cultures.

Morphology.—The anthrax bacillus is a large rod-shaped organism, of rectangular form, with slightly rounded corners. It measures 5 to 20 μ in length and from 1 to 1.25 μ in breadth. It has a pronounced tendency to form long threads, in which, however, the individuals can usually be made out, the lines of junction of the component bacilli giving the thread somewhat the appearance of a bamboo rod. In preparations made by staining blood or other animal juices the bacilli often appear surrounded by transparent capsules. Such are not found in specimens made from artificial cultures.

Sporulation.—The formation of endospores is prolific in the pres-

* "Vierteljahrsschr. für ger., Med.," 1855, Bd. VIII.
† "Compte-rendu," 1863, lvii.
‡ "Beiträge zur Biol. d. Pflanzen," 1876, II.

ence of oxygen. When oxygen is withheld spore-formation does not occur. In the bodies of experiment animals spore-formation is unusual and its occurrence signifies the local presence of abundant oxygen. On account of this peculiarity of the organism, the dead body of an animal is less dangerous as a source of infection than the discharges from living animals. As, however, the wool, hair and hides of infected animals are always soiled by the discharges, these are a menace to all that handle them and ought not be used. Each spore has a distinct oval shape, is transparent, situated at the center of the bacillus in which it occurs. It does not alter the contour of the bacillus. When a spore is placed under conditions favorable to its development, it increases in length and ruptures at the end,

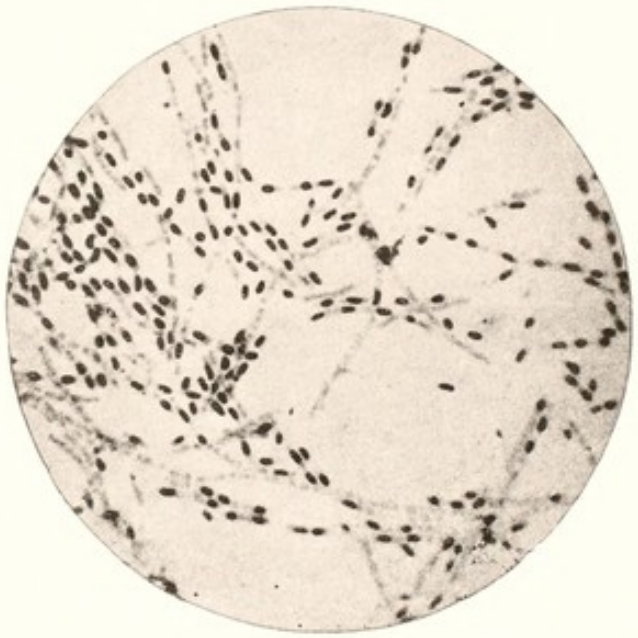


Fig. 129.—Bacillus anthracis, stained to show the spores. × 1000 (Fränkel and Pfeiffer).

from which the new bacillus escapes. The spores of the anthrax bacillus, being large and readily obtainable, form excellent subjects for the study of spore-formation and germination, for the study of the action of germicides and antiseptics, and for staining.

Motility.—The bacilli are not motile and have no flagella.

Staining.—They stain well with ordinary solutions of the anilin dyes, and can be beautifully demonstrated in the tissues by Gram's method and by Weigert's modification of it. Picrocarmin, followed by Gram's stain, gives a beautiful, clear picture. The spores can be stained by any of the special methods (*q.v.*).

Isolation.—The bacillus of anthrax is one of the easiest organisms to secure in pure culture from the tissues and excreta of diseased animals. Its luxurious vegetation, the typical appearance of its colonies, and its infectivity for the laboratory animals combine to make possible its isolation either by direct cultivation from the tis-

sues, by the plate method, or by the inoculation into animals and recovery of the micro-organisms from their blood.

Cultivation.—Colonies.—Upon the surface of a gelatin plate the bacillus forms beautiful and highly characteristic colonies. To the naked eye they appear first as minute round, grayish-white dots. Under the microscope they are egg-shaped, slightly brown and granular. Upon the surface of the medium, they spread out into flat, irregular, transparent tufts like curled wool, and from a tangled center large numbers of curls, made up of parallel threads of bacilli, extend upon the gelatin. Before the colony attains to any considerable size liquefaction sets in. Beautiful adhesion preparations can

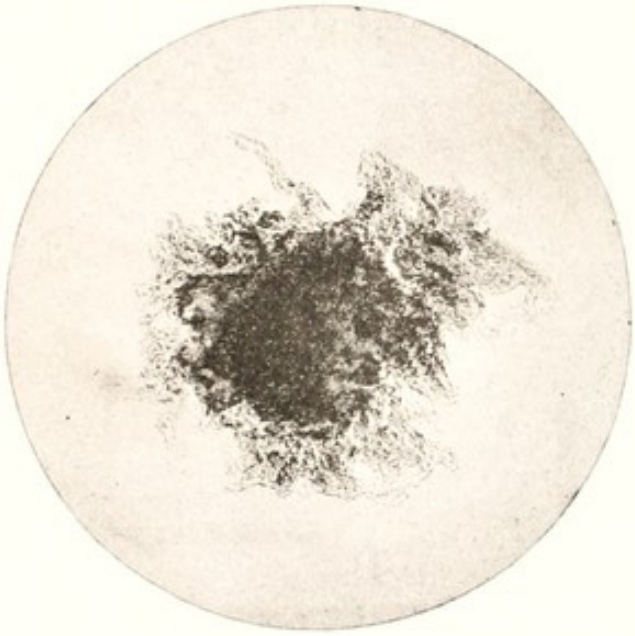


Fig. 130.—Bacillus anthracis; colony upon a gelatin plate. × 100 (Fränkel and Pfeiffer).

be made if a perfectly clean cover-glass be passed once through a flame and laid carefully upon the gelatin, the colonies being picked up entire as the glass is carefully removed. Such a specimen can be dried, fixed, and stained in the same manner as an ordinary cover-glass preparation.

Gelatin Punctures.—In gelatin puncture cultures the growth is even more characteristic than are the colonies. The bacilli begin to grow along the entire track of the wire, but develop most luxuriantly at the surface, where oxygen is plentiful and where a distinct shaggy pellicle is formed. From the deeper growth, fine filaments extend from the puncture into the surrounding gelatin, with a beautiful arborescent effect.

Liquefaction progresses from above downward until ultimately the entire gelatin is fluid and the growth sediments.

Agar-agar.—Upon agar-agar characteristic appearances are few. The growth takes place along the line of inoculation, forming a

grayish-white, translucent, slightly wrinkled layer with irregular edges, from which curls of bacillary threads extend upon the medium. When the culture is old, the agar-agar usually becomes brown in color. Spore-formation is luxuriant.

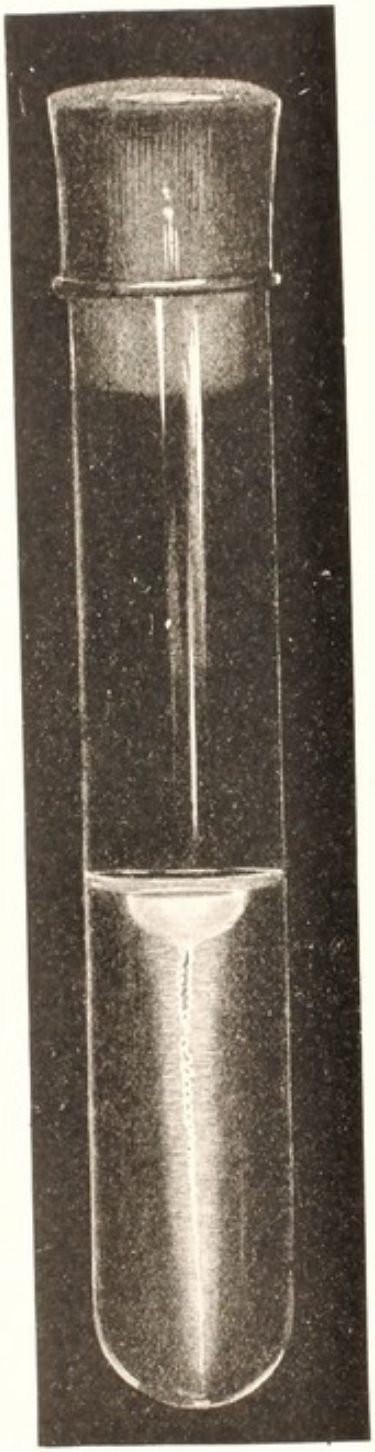


Fig. 131.—Bacillus anthracis; gelatin stab culture, showing characteristic growth with commencing liquefaction and cupping (from evaporation) at the surface of the medium (Curtis).

Bouillon.—In bouillon the anthrax bacillus grows chiefly upon the surface, where a thick felt-like pellicle forms. From this, fuzzy extensions descend into the clear bouillon below. After a few days some wooly aggregations can be seen in the bottom of the tube. In the course of time the growth ceases and the surface pellicle sinks. If, by shaking, it is caused to sink prematurely, a new, similar surface growth takes its place. Spore-formation is rapid at the surface.

Potato.—Upon the potato the growth is white, creamy, and rather dry. Sporulation is marked.

Blood-serum.—Blood-serum cultures lack characteristic peculiarities; the culture-medium is slowly liquefied.

Milk.—The anthrax bacillus grows well in milk, which it coagulates and acidulates. Later the coagulum is peptonized and dissolved, leaving a clear whey.

Vital Resistance.—The bacillus grows between the extremes of 12° and 45°C., best at 37°C. The exposure of the organism to the temperature of 42° to 43°C. slowly diminishes its virulence.

When dried upon threads, the spores retain their vitality for years, and are highly resistant to heat and disinfectants. The spores of anthrax are killed by five minutes' exposure to 100°C. It is said by some that spores subjected to 5 per cent. carbolic acid can subsequently germinate when introduced into susceptible animals, their resistance to this strength carbolic solution being so great that they are not destroyed by it under twenty-four hours. They are killed in two hours by exposure to 1 : 1000 bichlorid of mercury solution.

Metabolic Products.—The anthrax bacillus produces a curdling

ferment. Iwanow* found that the organism forms acetic, formic, and caproic acids, but it produces no important change of reaction in the medium in which it grows. It generates no indol. Its proteolytic enzyme is active, digesting both casein and fibrin.

It is doubtful whether the anthrax bacillus produces any important toxic substance. Hoffa† isolated a basic substance from anthrax cultures and called it *anthracin;* Hankin and Wesbrook,‡ an albumose fatal in large doses and immunizing in small ones. Brieger and Fränkel§ isolated a tox-albumin from the tissues of animals dead of anthrax. Martin‖ separated protalbumose, deuteroalbumose, peptone, an alkaloid, leucin, and tyrosin. The albumoses were not very poisonous, but the alkaloid was capable of producing death after the development of somnolence. The animals were edematous. Marmier** isolated a toxin of non-albuminous nature and immunizing power. Conradi†† in an elaborate research failed to find that the anthrax bacillus produced any soluble extracellular or intracellular poison capable of affecting susceptible animals, and concludes that it is highly improbable that the anthrax bacillus produces any toxic substances at all.

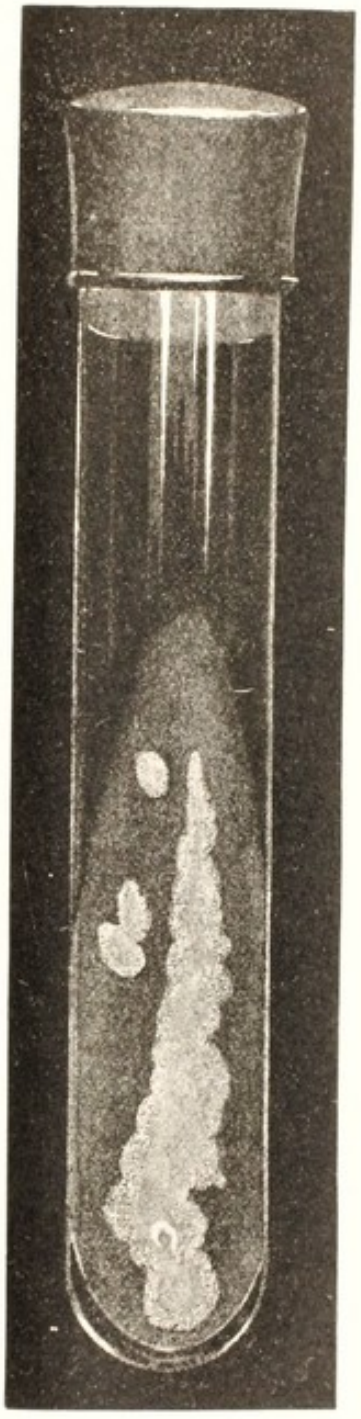


Fig. 132.—Bacillus anthracis; glycerin agar-agar culture (Curtis).

Pathogenesis.—Avenues of Infection.—Infection usually takes place through the *respiratory tract*, through the *alimentary canal*, or through the *skin*. It may take place through the *placenta*.

I. The Respiratory Tract.—The inhalation of the spores of the anthrax bacillus is possible whenever such are present in the atmosphere. The effect produced will depend upon the number of spores inhaled and the resistance or susceptibility of the animal. In man, a resisting animal, an-

* "Ann. de l'Inst. Pasteur," 1892.
† "Ueber die Natur. des Milzbrandgifts," Wiesbaden, 1886.
‡ "Ann. de l'Inst. Pasteur," 1892, No. 9.
§ "Ueber Ptomaine," Berlin, 1885–1886.
‖ "Proceedings of the Royal Society," May 22, 1890.
** "Ann. de l'Inst. Pasteur," 1895, p. 533.
†† "Zeitschrift für Hygiene," June 14, 1899.

thrax is rarely so caused except the number of bacilli be great, when it results in a disturbance at first localized in the lungs, and much resembling pneumonia. From the lungs generalized infection may later occur and destroy life. This form of infection is of occasional occurrence among men whose occupation occasionally brings them into contact with the hair or hides of animals dead of anthrax, and is often spoken of as "wool-sorters' disease."

Anthrax in cattle probably results from the inhalation or ingestion of the spores of the bacilli from the pasture. Interesting discussions arose concerning the infection of the pastures. It was argued that, the bacilli being inclosed in the tissues of the diseased animals, infection of the pasture must depend upon the distribution of the germs from buried cadavers, either through the activity of earthworms, which ate of the earth surrounding the corpse and deposited the spores in their excrement (Pasteur), or to currents of moisture in the soil. Koch seems, however, to have demonstrated the fallacy of both theories by showing that the conditions under which the bacilli find themselves in buried cadavers are opposed to fructification or sporulation, and that in all probability the bacteria suffer the same fate as the cells of the buried animals, and disintegrate, especially if the animal be buried at a depth of two or three meters.

Fränkel points out particularly that no infection of the soil by the dead animal could be worse than the pollution of its surface by the bloody stools and urine, rich in bacilli, discharged upon it by the animal before death, and that it is the live, and not the dead, animals that are to be blamed for the infection.

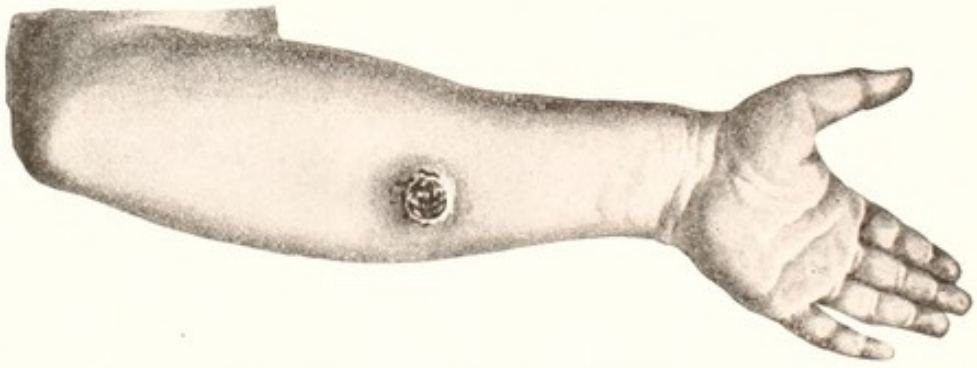


Fig. 133.—Anthrax carbuncle or malignant carbuncle (Lexer).

II. The Alimentary Tract.—When the bacilli are taken into the stomach they are probably destroyed by the acid gastric juice. The spores, however, are able to endure the acid, and pass uninjured into the intestine, where the suitable alkalinity enables them to develop into bacilli, surround the villi with thick networks of bacillary threads, separate the covering epithelial cells, enter the lymphatics, and then the blood, and effect general infection.

III. The Skin.—The bacillus frequently enters the body through wounds, cuts, scratches, and perhaps occasionally fly-bites, though

from the work of Nuttall* it is pretty clear that flies play little part in the transmission of the disease. Under these conditions the organisms at once find themselves in the lymphatics or capillaries, and may cause immediate general infection. In human beings a "malignant pustule" is apt to follow local infection, and may recover or ultimately cause death by general infection.

The *malignant pustule* usually makes its appearance upon the face, hands or arms. The first symptom is a reddish papule that extends and becomes vesicular. At the point of infection necrosis is rapid, and within forty-eight hours there may be a brownish eschar surrounded by a crop of secondary vesicles, beyond which there is edema

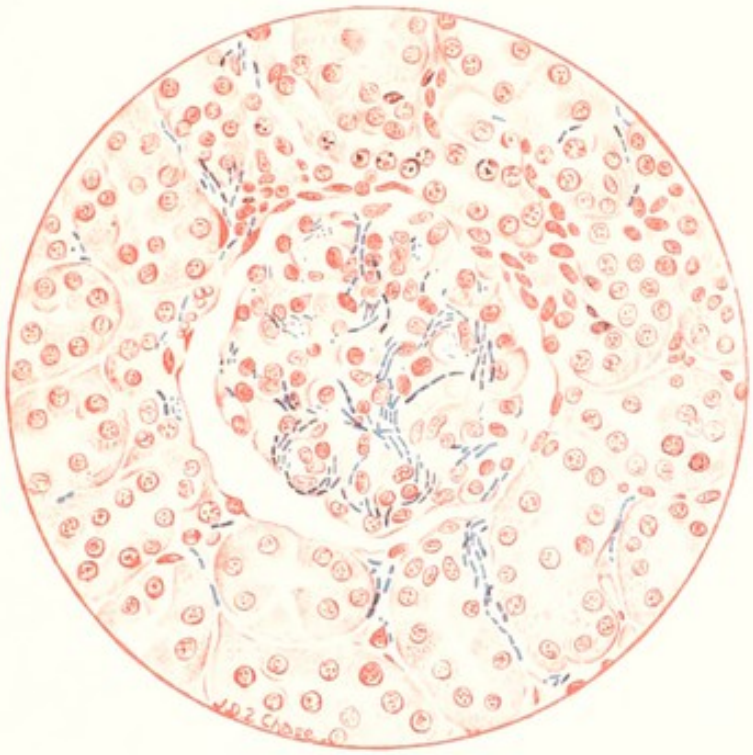


Fig. 134.—Anthrax bacilli in glomeruli of kidney.

or brawny swelling. According to the susceptibility of the patient the disease may soon localize, the slough detach and recovery set in, or the edema and swelling may continue, blood invasion occur and death ensue. Heinemann,† in compiling statistics of the fatality of malignant pustule, shows that the danger of the lesion is greatly mitigated by complete excision. Koch found the death-rate among 1473 cases to be 38.8 per cent., but Heinemann's statistics upon 2255 cases show the deaths to be only 5.8 per cent.

Lesions.—The disease as seen in the laboratory is accompanied by few marked lesions. The ordinary experimental inoculation is made by cutting away a little of the hair from the abdomen of a guinea-pig or rabbit, or at the root of a mouse's tail, making a little

* "Johns Hopkins Hospital Reports," 1899.
† "Deutsche Zeitschrift für Chirurgie," 1912, CXIX, 201.

subcutaneous pocket by a snip with sterile scissors, and introducing the spores or bacilli with a heavy platinum wire, the end of which is flattened, pointed, and perforated. An animal inoculated in this way dies, according to the species, in from twenty-four hours to three days, suffering from weakness, fever, loss of appetite, and a bloody discharge from nose and bowels. There is much subcutaneous edema near the inoculation wound. The abdominal viscera are injected and congested. The spleen is enlarged, dark in color, and of mushy consistence. The liver is also somewhat enlarged. The lungs are usually slightly congested.

When organs which present no appreciable changes to the naked eye are subjected to a microscopic examination, the appropriate staining methods show the capillary and lymphatic systems to be almost universally occupied by bacilli, which extend throughout their meshworks in long threads. Most beautiful bacillary threads can be found in the glomeruli of the kidney and in the minute capillaries of the intestinal villi. In the larger vessels, where the blood-stream is rapid, no opportunity is afforded for the formation of the threads, and the bacteria are relatively few, so that the burden of bacillary obstruction is borne by the minute vessels. The condition is thus a pure bacteremia.

Death from anthrax seems to depend more upon the obstruction of the circulation by the multitudes of bacilli in the capillaries, and upon the appropriation of the oxygen destined to support the tissues, by the bacilli, than upon intoxication by the metabolic products of bacillary growth.

Virulence.—The anthrax bacillus maintains its virulence almost without modification because of the prolific formation of spores and their remarkable resisting powers. By artificial means, however, the formation of spores can be inhibited and the bacilli attenuated. This was first achieved by Pasteur* by cultivation at temperatures above the optimum, at which no spores were formed. Toussaint† found that the addition of 1 per cent. of carbolic acid to blood of animals dead of anthrax destroyed the virulence of the bacilli; Chamberland‡ and Roux found the virulence destroyed when 0.1–0.2 per cent. of bichromate of potassium was added to the culture medium; Chauveau used atmospheric pressure to the extent of six to eight atmospheres and found the virulence diminished; Arloing§ found that direct sunlight operated similarly; Lubarsch, that the inoculation of the bacilli into immune animals, such as the frog, and their subsequent recovery from its blood, diminishes the virulence.

Vaccination.—Pasteur‖ early realized the importance of some prac-

*"Rec. de méd vet.," Paris, 1879, p. 193.
† "Compte-rendu Acad. des Sci. de Paris," XCI, 1880, p. 135.
‡ "Ann. de l'Inst. Pasteur," 1894, p. 161.
§ "Compte-rendu de l'Acad. des Sci.," Paris, 1892, CXIV, p. 1521.
‖ "Rec. de Méd. vet.," Paris, 1879, p. 193.

tical measure for the protective vaccination of cattle against the disease, and devoted himself to investigating the problem. He found that the inoculation of attenuated bacilli into cows and sheep, and their subsequent reinoculation with mildly virulent bacilli, afforded them immunity against highly virulent organisms.

The protective inoculations prepared by Pasteur consisted of two cultures of diminished virulence, to be employed one after the other, each rendering the vaccinated animals more immune. The cultures were prepared, that is, attenuated by cultivation at 42°C. for a sufficient length of time, the bacilli forming no spores and gradually losing their virulence at this temperature. The *first vaccine* was kept from fifteen to twenty days at 42°C. It killed mice and guinea-pigs one day old, but was without action on guinea-pigs of adult size. The second vaccine only remained at the temperature of 42°C. for from ten to twelve days and killed mice, guinea-pigs and occasionally rabbits.

The second vaccine is administered from two to three weeks after the first is given, by hypodermic injection into the tissues of the neck or flank. Of each broth culture about 1 cc. is administered. The animals frequently become ill.

Pasteur demonstrated the value of his method in 1881 at Pouilly-le-Fort, in a manner so convincing to the entire world that it was immediately put into practice in France. Roger* says that between 1882 and 1894 there were 1,788,677 sheep vaccinated, with a mortality of 0.94 per cent., the previous death-rate having been 10 per cent. There were also 200,962 cattle vaccinated, with a reduction of the death-rate from 5 per cent. to 0.34 per cent.

Slight protection against anthrax can be afforded in other ways. Hüppe found that the simultaneous inoculation of bacteria not at all related to anthrax will sometimes cause the animal to recover. Hankin found in the cultures chemic substances, especially an albuminose, that exerted a protective influence. Rettger† prepared "prodigiosus powder" from potato cultures of B. prodigiosus, which when injected into guinea-pigs during experimental anthrax infection prolonged life or induced recovery.

Serum Therapy.—In 1890 Ogata and Jasuhara showed that the blood of experiment animals convalescent from anthrax possessed an antitoxic substance of such strength that 1:800 parts per body-weight would protect a mouse. Similar results have been attained by Marchoux.‡ Serum therapy in anthrax is, however, of no practical importance either for prophylaxis or treatment, as vaccinating the animals is far cheaper and more satisfactory.

Bacteriologic Diagnosis.—When it is desired to have a bacteriologic diagnosis of anthrax made where no laboratory facilities are at

*Les Maladies Infectieuse, II, p. 1489.
† "Jour. of Infectious Diseases," Nov. 25, 1905, vol. II, No. 4, p. 562.
‡ "Ann. de l'Inst. Pasteur," November, 1895, IX, No. 11, pp. 50–75.

hand, an ear of the dead animal can be inclosed in a bottle or fruit jar and sent to the nearest laboratory where diagnosis can be made. The ear contains so little readily decomposable tissue that it keeps fairly well, drying rather than rotting. It contains enough blood to enable a bacteriologist to make a successful examination.

Sanitation.—As every animal affected with anthrax is a menace to the community in which it lives—to the men who handle it as well as the animals who browse beside it—such animals should be killed as soon as the diagnosis is made, and, together with the hair and skin, be burned, or if this be impracticable, Fränkel recommends that they be buried to a depth of at least 1½–2 meters, so that the sporulation of the bacilli is made impossible. The dejecta should also be carefully disinfected with 5 per cent. carbolic acid solution. As the pastures and barnyards are certainly infected wherever an animal has been the victim of anthrax, all other susceptible animals upon the farm, and all such upon neighboring farms, should at once be vaccinated.

Cases of human anthrax must be treated by isolation, careful dressing of the lesions when external, the dressings being burned as soon as removed. The expectoration, urine and feces should be disinfected with care. The patient should be defended from flies, and the nurse and others who come into contact with the patient should be warned of the dangerous character of the infection.

Bacilli Resembling the Anthrax Bacillus

Bacilli presenting the morphologic and cultural characteristics of the anthrax bacillus, but devoid of any disease-producing power, are occasionally observed. Of these, Bacillus anthracoides of Hüppe and Wood,* Bacillus anthracis similis of McFarland,† and Bacillus pseudoanthracis‡ have been given special names. What relationship they bear to the anthrax bacillus is uncertain. They may be entirely different organisms, or they may be individuals whose virulence has been lost through unfavorable environment.

* "Berliner klin. Wochenschrift," 1889. 16.
† "Centralbl. f. Bakt.," vol. XXIV, No. 26, p. 556.
‡ "Hygienische Rundschau," 1894, No. 8.

CHAPTER V

HYDROPHOBIA, LYSSA, OR RABIES

NEURORRHYCTES HYDROPHOBIÆ (CALKINS)

HYDROPHOBIA, lyssa, or rabies is a specific infectious toxic disease to which dogs, wolves, skunks and cats are highly susceptible, and which, through their saliva, can be communicated to men, horses, cows and other animals. The means of communication is almost invariably a bite, hence the specific infection must be present in the saliva.

The infected animals manifest no symptoms during a varying incubation period in which the wound heals kindly. For human beings this period may be of twelve months' duration; in rare cases may be only a few days; its average duration is about six weeks.

Toward the close of the incubation period an observable alteration occurs in the wound, which becomes reddened, may suppurate, and is painful. The victim has a sensation of horrible dread, which passes into wild excitement, with paralysis of the pharyngeal muscles and inability to swallow. The wild delirium ends in a final stage of convulsion or palsy. The convulsions are tonic, rarely clonic, and finally cause death by interfering with respiration.

During the convulsive period much difficulty is experienced in swallowing liquids, and it is supposed that the popular term "hydrophobia" arose from the reluctance of the diseased to take water because of painful spasms brought on by the attempt.

The infectious nature of rabies seems to have been first demonstrated by Galtier.* Pasteur, Chamberland and Roux† continued the investigation and found that in animals that die of rabies the salivary glands, pancreas and the nervous system contain the infection, and are more appropriate for the experimental purposes than the saliva, which is invariably contaminated with accidental pathogenic bacteria.

The introduction of a fragment of the medulla oblongata of a dog dead of rabies beneath the dura mater of a rabbit causes the development of typical rabies in the rabbit in about six days.

Specific Organism.—It is not yet generally conceded that the pathogenic micro-organism of rabies has been discovered, though there is continually accumulating evidence in favor of the "bodies of Negri."‡ Believing that the evidence at hand is strongly in favor

* "Compte-rendu de l'Acad. des Sciences de Paris," 1879, LXXXIX, 444.
† Ibid., 1881, XCII, 159.
‡ "Zeitschrift für Hygiene," 1903, XLIII, 507; XLIV, 520; 1909, LXII, 421

of the protozoan nature and etiological importance of these bodies, they are tentatively accepted as the cause of the disease and treated accordingly in the text. To these bodies Calkins has given the name Neurorrhyctes hydrophobiæ.

Morphology.—By appropriately staining sections of the cerebrum, cerebellum, pons, basal ganglia, spinal ganglia, and salivary glands, of human beings or animals dead of rabies, it was possible to demonstrate small rounded bodies measuring 4 to 10 μ as a rule, though varying from 1 to 20 μ in the interior of the protoplasmic process of the cells. In experimental infections they are most numerous in the hippocampal convolution. The bodies, when stained by the methods given below, usually appear red in color. They are ovoid in shape, well-circumscribed, and vary in size from invisibility to 20 μ in length. The smaller of them do not show any structural differentiation, but the larger show central condensations that may be nuclear material. The greater number of them lie in the cytoplasm of the nerve cells; some are free. These are the Negri bodies.

Williams and Lowden* are convinced that they are protozoan organisms, that they are the cause of rabies, and that their presence is pathognomonic of rabies. They believe:

1. The smear method of examining the Negri bodies (*vide infra*) is superior to any other method so far published for the following reasons: (*a*) It is simpler, shorter and less expensive; (*b*) the Negri bodies appear much more distinct and characteristic. For this reason and the preceding one its value in diagnostic work is great; (*c*) the minute structure of the Negri bodies can be demonstrated more clearly; (*d*) characteristic staining reactions are brought out.

2. The Negri bodies as shown by the smears, as well as by the sections, are specific to hydrophobia.

3. Numerous "bodies" are found in fixed virus.

4. "Bodies" are found before the beginning of visible symptoms, *i.e.*, on the fourth day in fixed virus, on the seventh day in street virus, and evidence is given that they may be found early enough to account for the appearance of infectivity of the host tissues.

5. Forms similar in structure and staining qualities to the others, but just within the limits of visible structure (at 1500 diameter magnification), have been seen; such tiny forms, considering the evidence they give of plasticity, might be able to pass the coarser Berkefeld filters.

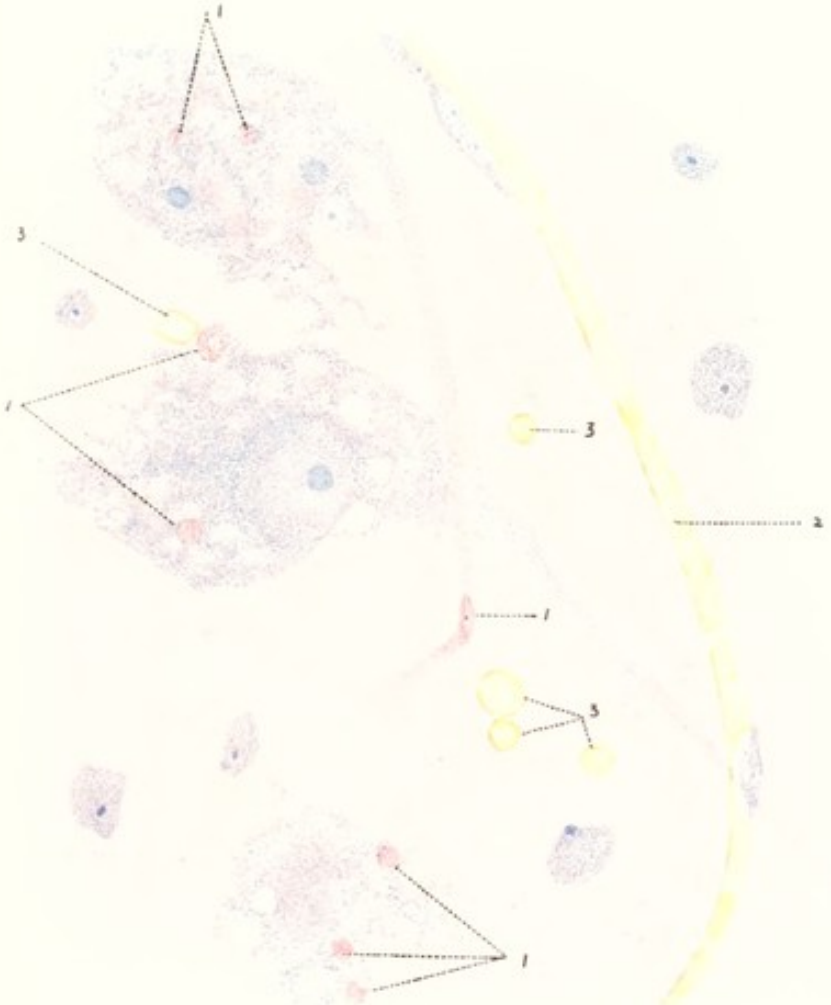
6. The Negri bodies are organisms belonging to the class Protozoa. The reasons for this conclusion are: (*a*) They have a definite characteristic morphology; (*b*) this morphology is constantly cyclic, *i.e.*, certain forms always preponderate in certain stages of the disease, and a definite series of forms indicating growth and multiplication can be demonstrated; (*c*) the structure and staining qualities, as shown especially by the smear method of examination, resemble those of certain known Protozoa, notably of those belonging to the sub-order Microsporidia.

7. The proof that the Negri bodies are living organisms is sufficient proof that they are the cause of hydrophobia; a single variety of living organisms found in such large numbers in every case of a disease, and only in that disease, appearing at the time that the host tissue becomes infective, in regions that are infective, and increasing in those infective areas with the course of the disease can be no other, according to our present views, than the cause of that disease.

One of the objections urged against the bodies of Negri as the specific cause of the disease was the failure of the organism

*"Jour. of Infectious Diseases," 1906, III, 452.

PLATE I



Nerve-cells containing Negri bodies. Hippocampus impression preparation, dog. Van Gieson stain; × 1000. 1, Negri bodies; 2, capillary; 3, free red blood-corpuscles. (Courtesy of Langdon Frothingham.)

to appear elsewhere than in the central nervous system, when the saliva, the salivary glands and the pancreas were known to harbor it. This has now been overcome by the demonstration of the bodies in the salivary glands in precisely the same form as that seen in the nervous system by Manuelian.*

Steinhardt, Poor and Lambert† have endeavored to determine

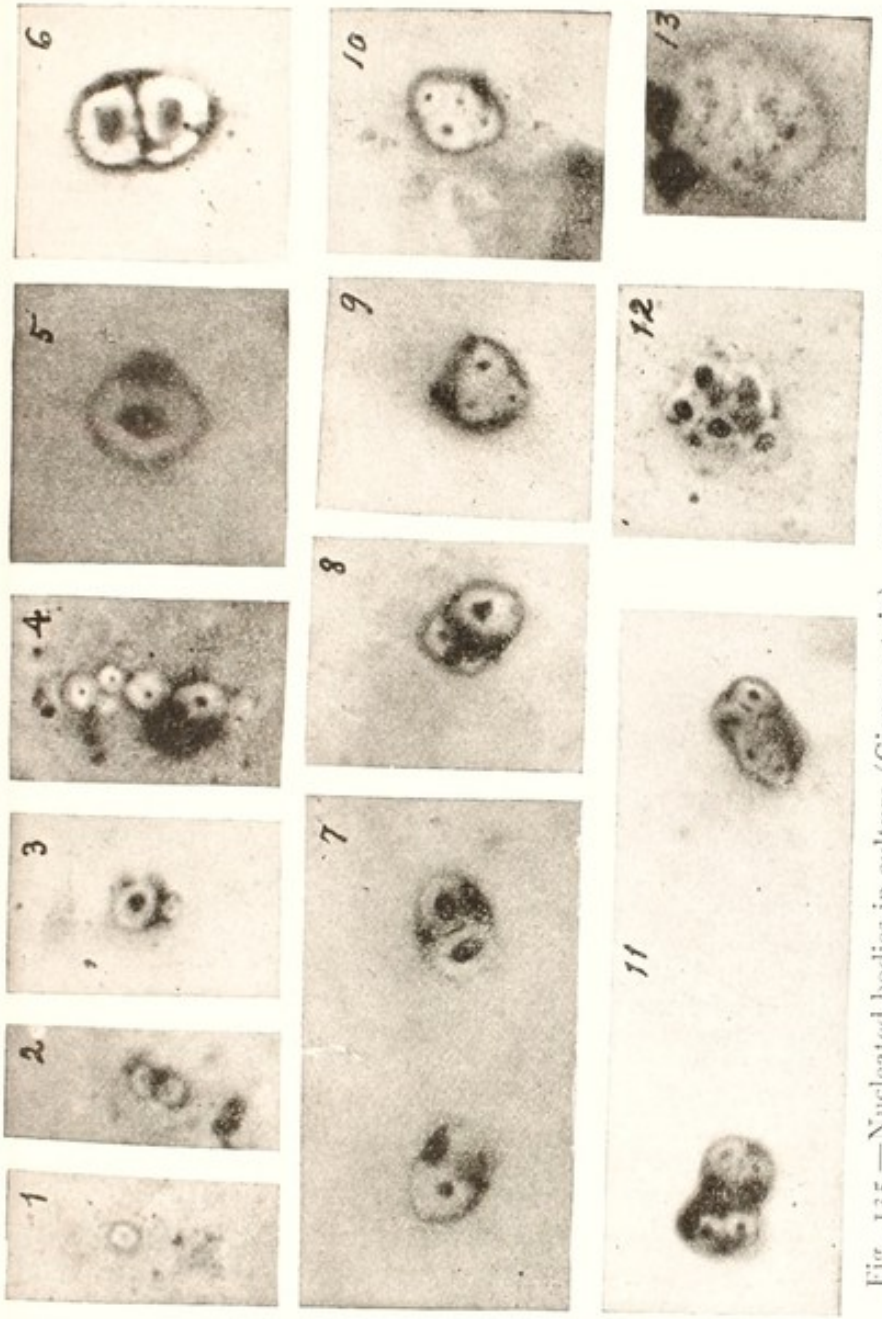


Fig. 135.—Nucleated bodies in culture (Giemsa stain). 1 to 13 represent the nucleated bodies of different stages of development in a culture (second and fourth generations, five days old) prepared from the brain of a rabbit experimentally infected with "fixed" virus. In the original material there were no such forms to be seen either in the films or in sections. All microphotographs were taken from the film preparation stained with Giemsa solution. Magnification uniformly × 1100 (Noguchi, in Journal of Experimental Medicine).

whether Negri bodies are parasitic micro-organisms or degeneration products of the nervous system, and have shown that when cells of the normal guinea-pig brain are incubated in blood plasma, their cytoplasm, when stained by Van Gieson's stain, show small pink-staining bodies surrounded by a blue granular ring, indistinguishable

*"Ann. de l'Inst. Pasteur," 1914, XXVII, 233.
† Jour. of Infectious Diseases, 1912, XI, 459.

from the unstructured Negri bodies observed with great frequency in the rabid guinea-pig brain. In a few instances these forms contained a blue-staining central ring or point, and closely resembled the structured forms of Negri bodies. The normal guinea-pig brain inoculated with rabid material, street or fixed virus, incubated in the same manner, showed the same structures. The brains of guinea-pigs dying of street virus and rabbits dying of fixed virus, incubated in small fragments, gave no development of Negri bodies in blood plasma, beyond the small structured and unstructured forms, although in one preparation the ganglion cells appeared to be living at the end of twenty-one days' incubation.

Cultivation.—Attempts to cultivate Negri bodies were made by Moon,* but the success of his attempts seemed doubtful. The first

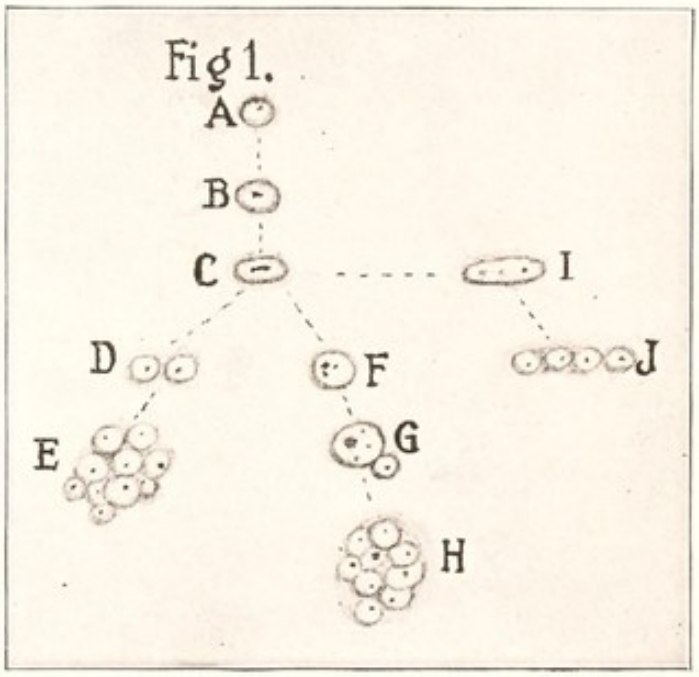


Fig. 136.—From rabbit "fixed-virus" brain; *a*, *b*, *c*, *d*, *f*, and *i*, types of Negri bodies seen at death of rabbit; *e*, *g*, *h*, and *j*, apparent multiplication and segmentation of the bodies after three days at 24°C. Drawing made from smears stained by Giemsa's method and magnified about 2000 diameters (Williams, in Jour. Am. Med. Assoc.).

claim to successful cultivation of the Negri bodies was made by Noguchi.† The cultivation was done according to his already successful method for Spirochæta of various kinds. Large, small and dividing bodies appeared in the culture fluid, after inoculation with a fragment of nervous tissue from various animals with infection following inoculation with street virus and "fixed" virus. But Williams‡ at once pointed out that there is no certainty that the bodies increased in numbers in the cultures, though Noguchi says that they reappear in new cultures "through many generations." Noguchi's

* "Jour. of Infectious Diseases," 1913, XIII, 213.
† "Jour. of Experimental Medicine," 1913, XVIII, 314.
‡ "Jour. Amer. Med. Assoc.," 1913, LXI, 1509.

paper seems more like a preliminary report than a finished work, and future publication on the subject is promised. Two methods of obtaining the virus of rabies freed from the cells of the host and free from contaminating organisms, published by Poor and Steinhardt,* give some promise of permitting the introduction of the bodies of rabies into artificial culture media in a measured quantity of fluid, perhaps containing a known number of organisms, and thus permitting better methods of estimating the growth in artificial culture.

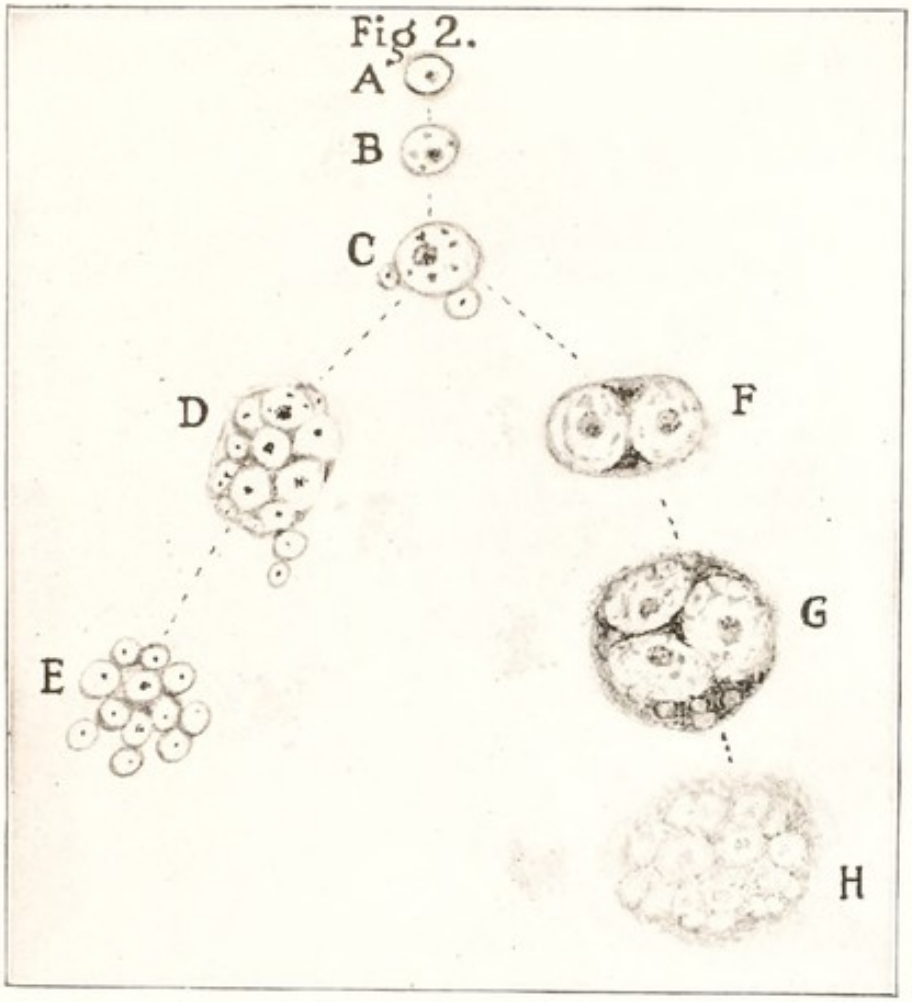


Fig. 137.—From dog "street-virus" brain; *a*, *b*, *c*, and *f*, types of Negri bodies seen at death of dog; *d*, *e*, *g*, and *h*, apparent multiplication and segmentation of the bodies after three days at 24°C. (Williams, in Jour. Am. Med. Assoc.).

Staining.—The Negri bodies are not difficult to stain and find when one is familiar with them or when they are present in the nervous tissue in considerable numbers. To find a few, to find them quickly, and to recognize them unmistakably is, however, a different matter. They stain by all of the Romanowsky modifications, by all of the eosin-methylene blue combinations, and by various other methods.

*"Jour. of Infectious Diseases," 1913, XII, 202.

Williams and Lowden* stained Negri bodies by one of the following methods:

(*a*) Giemsa's solution.—The smears are fixed in methyl alcohol for about 5 minutes. The staining solution recommended is that last used by Giemsa:

Azur II. Eosin	3.0
Azur II	0.8
Glycerin (Merck's chemically pure)	250.0
Methyl alcohol (chemically pure)	250.0

Both the glycerin and methyl alcohol are heated to 60°C. The dyes are put into the alcohol and the glycerin is added slowly, stirring. The mixture is allowed to stand at even temperature over night, and after filtration is ready for use. At the time of use one drop of the stain is added for every cubic centimeter of distilled water made alkaline by the addition of one drop of a 1 per cent. solution of potassium carbonate to 10 cc. of the water.

The stain is poured on the slide and allowed to stand for from one-half to three hours. The longer time brings out the structure better and in twenty-four hours well-made smears are not overstained. After the stain is poured off, the smear is washed in running tap water for from one to three minutes and dried with filter-paper.

By this method the "bodies" are stained blue and the central bodies and chromatoid granules blue, red or azure. The cytoplasm of the nerve cells stains blue also, but the bodies can be seen distinctly within it. For diagnostic purposes the method may be shortened thus:

Methyl alcohol	5 minutes.
Equal parts of Giemsa solution and distilled water	10 minutes.

(*b*) The eosin-methylene blue of Mallory (*q.v.*).

The smears are fixed in Zenkers' solution for one-half hour; after being rinsed in tap water they are placed successively in 95 per cent. alcohol and iodine for one-quarter hour, 95 per cent. alcohol for one-half hour, absolute alcohol one-half hour, eosin solution 20 minutes, rinsed in tap water, methylene blue solution 15 minutes; differentiated in 95 per cent. alcohol, lasting one to five minutes and dried with filter-paper.

With this method the cytoplasm of the "bodies" is magenta, light in the small bodies, darker in the larger; the center bodies and chromatoid granules are a very dark blue, the nerve-cell cytoplasm a light blue, the nucleus a darker blue and the red blood-cells a brilliant eosin pink.

Harris† uses the following method of staining Negri bodies that seems to have the advantages of coloring them so as to bring out their structure, and to do away with the granular precipitate that occurs in most other methods.

Smears of the appropriate material are made upon slides and fixed by the application of methyl alcohol for one minute, are then washed with water to remove the alcohol, placed for from one to three minutes in an old saturated solution of eosin in 96 per cent. alcohol, after which they are washed for two or three seconds with water to remove the excess of eosin. This stains the Negri bodies. Counterstaining is effected by immersing for five to fifteen seconds in a fresh solution of Unna's alkaline methylene blue, after which there is a brief washing in water, decolorization in 95 per cent. alcohol and then the usual treatment with absolute alcohol, xylol and balsam if the preparation is to be covered and preserved, or the spread is blotted and dried if to be examined without a cover. The whole process requires less than five minutes.

Smears that have been dried for several days or weeks cannot be thus stained with satisfaction. The older the eosin solution the more rapidly and intensely it stains. To secure the best results it should not be less than two months old. The methylene blue should not be more than a week or two old, else it will yield an objectionable precipitate.

* "Jour. of Infectious Diseases," 1906, III, 452.
† "Jour. of Infectious Diseases," 1908, V, 566.

Reichel and Engle* stain Negri bodies with the following:

Sat. alc. sol. methylene violet	10 cc.
Sat. alc. sol. fuchsin	7 drops.
Sterile water	40 cc.

The smears of cerebellum or hippocampus are fixed with absolute alcohol and ether and the stain poured on, heated, poured back into the bottle, again poured on, heated and poured back into the bottle, this being done three times, each time for about half a minute. Then wash in water, blot and examine. To examine, a nerve-cell is found with the low power and then examined with the high power. The Negri bodies are brick red. The stain soon fades. Smears kept for any length of time lose the staining reaction.

Luzzani† gives the following method of staining Negri bodies.

The tissue to be stained should be fixed in Zenker's solution, imbedded in paraffine and cut into very thin slices. Mann's stain is used:

1:100 aqueous solution of eosin	45 cc.
1:100 aqueous solution of methylene blue	35 cc.
Distilled water	100 cc.

(The solution of eosin and of methylene blue should be kept separately, and only mixed and diluted at the time of using. The diluted mixture does not keep longer than some days, or at best, a few weeks.)

After the sections are cut, they are fixed to the slides with Mayer's glycerin albumen, the paraffine removed with xylol, the xylol with alcohol, and the alcohol with water. The stain is then applied for some minutes after which the section is rapidly washed in tap water, then in absolute alcohol; when dehydrated in the absolute alcohol, they are washed in a solution of

Absolute alcohol	30 cc.
Saturated solution of caustic soda in absolute alcohol	5 drops.

until they lose the blue color and become entirely red. They are then given a washing in absolute alcohol, plunged into tap water and then washed with distilled water slightly acidified with acetic acid until they turn blue again. The final steps are absolute alcohol, xylol and Canada balsam. The Negri bodies are red, the cells blue.

The method should be as applicable for smears or contact spreads as for sections, and for purposes of diagnosis the hippocampal convolution can be cut across, a clean side touched to the cut surface and removed. Nerve-cells adhere to the glass which is dried and treated as though it had an adhering section of tissue. The Negri bodies are best seen in the processes of the nerve-cells.

Pathology.—It is generally supposed that the activity of the rabic virus is largely confined to the nervous system, and that from the point of admission to the body it ascends the peripheral nerves to effect its final and fatal influence upon the central nervous system. The seat of inoculation has, therefore, much to do with the facility and rapidity with which the symptoms and termination come on.

When the virus enters through the skin of the forearm or lower limb, it has a long way to travel, and the period of incubation is long; when it enters about the face, a correspondingly short distance to go, and a correspondingly brief period of incubation. The occurrence

* Personal communication.

† "Ann. de, l'Inst, Pasteur," 1913, XXXVII, 1039.

of symptoms is accepted as evidence that the central nervous system has been reached.

When as in experimental inoculation the virus is at once placed in the central nervous system, symptoms do not at once develop, hence it is concluded that not only must the essential parasites reach the central nervous system, but they must do so in sufficient numbers before enough damage can be done to produce the symptoms. Under the most favorable conditions of infection, this requires about six days.

The virus is, however, not confined to the nervous system for the saliva is infective, and the salivary glands, pancreas, and perhaps other glands harbor the infective agent. How it reaches these structures has not yet been determined. In them Negri bodies are present but whether they reach the glands through the blood or by way of their nervous connections is not known.

There is no *morbid anatomy* of rabies. Carefully made autopsies upon the bodies of rabid human beings and animals show nothing by which the nature of the disease can be determined. Most interest naturally centers about the brain and spinal cord as being the chief sources of disturbance and chief seats of the virus. There are, however, so few changes as scarcely to merit description. In some cases the meninges are distinctly congested, but in uncomplicated cases there is no meningitis and therefore no inflammatory exudation.

In a few cases there may be scattered minute hemorrhages. In many cases there are no lesions.

The *pathologic histology* of rabies reveals certain fairly constant lesions described in the next section, but they are not now regarded as characteristic of the disease.

Diagnosis of Rabies.—There are three means of arriving at a diagnosis of rabies in cases of suspected "mad-dogs."

The animal having been killed, its head is cut off by an incision through the neck at some distance from the skull, and immediately taken to an appropriate laboratory or carefully packed in plenty of ice and sent to the laboratory by express. The fresher the tissue received by the laboratory worker, the more certain his results can be.

Carefully opening the skull of the dog, the brain is removed to a sterile dish. Good sized bits of tissue are taken from the appropriate portions of the brain and placed in glycerin for future inoculation operations if necessary, small bits of the same tissue are spread upon slides according to the "smear" method of Williams and Lowden, or slides may be spread by the "adhesion" method of Frothingham* who makes an incision into the brain, lays it open in the appropriate areas, and then applies the flat surface of a perfectly clean slide to the flat cut surface of the brain. When the slide is lifted up (not slid off), nerve-cells adhere to it, in which the Negri bodies may

*Jour. Med. Research, 1916, XIV, 477.

later be found. Other parts cut from the appropriate areas of the brain tissue are placed in fixative to prepare for sectioning should that later become desirable.

Williams and Lowden* devised a new technic of examination for Negri bodies that has been of considerable advantage to those engaged in looking for them for assisting in the diagnosis of rabies, as well as in studying the bodies themselves. It may be called the "smear method" to differentiate it from the older and less certain "section method." Briefly, the method is as follows:

Glass slides and cover-glasses are washed thoroughly with soap and water and heated in a flame to get rid of oily substances. A small bit of the gray substance of the brain chosen for examination is placed upon one end of a slide, a cover-glass placed upon it and pressed down so as to spread out the nervous tissue in a thin layer, when the cover is slowly moved to the opposite end of the slide spreading out the nerve-cells and distributing them over the surface. The tissues selected for examination should come from at least three different parts of the gray matter of the central nervous system, first, from the cortex of the brain in the neighborhood of the fissure of Rolando, or in the region corresponding to it; second, from Ammon's horn; third, from the cerebellum.

The smears are dried in the air and then stained as stated above.

Formerly an examination of the spinal sympathetic ganglia was made, and the diagnosis made from what was found in them. This constitutes the least important and most rarely pursued form of diagnostic procedure at the present time. However, we will suppose some sympathetic ganglia secured. The remainder of the animal's head can then be destroyed. With the material thus secured we make the following diagnostic tests:

1. Examination for the Negri bodies.
2. Inoculation of rabbits.
3. Examination for histological changes in the ganglia.

1. **The Negri Bodies.**—As now generally conceded, the discovery of these bodies in the cells of the central nervous system may be taken as positive evidence of the existence of rabies in its transmissible stage.

2. **The Inoculation of Rabbits.**—This is only necessary in highly suspicious cases in which no Negri bodies are found, or in which the investigator is not satisfied that such bodies are specific indications of the disease.

The glycerinated or fresh nervous tissue can be employed. A bit of the tissue is made into a creamy suspension, under aseptic precautions, by adding physiological salt solution, crushing and grinding in a small agate mortar. When it is ready a rabbit is anesthetized, the hair is pulled out over one side of the skull (or if it be preferred,

* "Jour. of Infectious Diseases," 1906, III, 452.

the skin can be shaved), the scalp is washed with an antiseptic solution and an incision about an inch long is made and the skull exposed. With a small trephine a button of bone is cut out and the dura exposed. The suspension of nervous tissue is drawn up in a sterile hypodermic syringe, and one or two drops of it injected beneath the dura mater or deeply into the brain tissue. If the operation be successful the wound heals and no meningitis follows, but at the end of about six days the rabbit becomes paralyzed, "dumb rabies." Several rabbits should be simultaneously inoculated as should a single rabbit develop meningitis, through accident or bad technic, no information is gained, and no diagnosis is possible. The rabid rabbits die in a day or two after the onset of the palsy, and Negri bodies can be found in the brain tissue, which is infectious for other rabbits in endless series.

3. **The Histological Changes in the Nervous System.**—These are now rarely looked for, as experience has shown them to be the least reliable means of making the diagnosis. The chief changes are the "tubercles of Babes,"* which consist of perivascular collections of cells, and collections of newly formed cells about the ganglionic nerve-cells of the brain and cord.

Van Gehuchten and Nelis,† and Ravenel and McCarthy‡ have studied these lesions. Ravenel and McCarthy think that Babes gave undue prominence to the rabid tubercle, which consists of an aggregation of embryonal cells about the central canal of the cord, about the ganglionic nerve-cells, and about the capillary blood-vessels. They think, however, that the lesions of the nerve-ganglion cells are pathognomonic if taken in connection with the clinical manifestations of the disease. The specific changes consist of degeneration, chromatolysis and even total disappearance of the nuclei of the ganglion cells, dilatation of the pericellular space, and invasion not only of this space, but also of the nerve-cells by embryonal cells, and at the same time the appearance of small corpuscles which are hyaline, brownish and in part metachromatic. Spiller§ refused to regard these lesions as pathognomonic of rabies and it is now generally conceded that they are not specific of rabies, and, therefore, not to be looked upon as of more than confirmatory evidence of the disease.

Virulence.—The virus of rabies is variable in virulence to a marked degree. "Street virus," or that obtained from rabid dogs, is so variable that before scientific study with it is possible, it must be standardized. This is done by passage through rabbits, the technic of the inoculation being the same as that given in the section on "Diagnosis." After being passed successively from rabbit to

* Ann. de l'Inst. Pasteur, 1896, VI, 209.
† "Univ med. Mag." Jan., 1901.
‡ "Archiv. de Biologie," 1900, XVI.
§ "Pathological Society of Philadelphia," March, 1901.

rabbit from twenty to thirty times, a maximum virulence is attained and the virus is said to be "fixed." Pasteur found that the virulence of the nervous tissue was diminished by inspissation, by drying under aseptic precautions in a sterile jar over calcium chloride. There is some doubt whether this results in actual diminution in the virulence of the organisms as Pasteur thought, or whether the virulence is diminished by dilution, *i.e.*, by effecting the destruction of many of the organisms. There seems to be no means of determining this at present. The diminution of virulence is in proportion to the length of time the nervous tissue is dried.

Prophylaxis.—To prevent rabies, means must be devised for preventing dog-bites. In an island community like England, rabies may be successfully eliminated by destroying all animals suspected of having the disease, muzzling the dogs for a time, and denying admission to new dogs until they have spent a long enough period in quarantine to exclude the possibility of their being infected with the disease.

Upon continents it seems unlikely that rabies can ever be completely eradicated as it is not only a disease of dogs, but also of wolves, foxes, skunks and other wild animals by which dogs may be bitten.

However, it is the dog that is the common distributor and to which attention must be directed.

All rabid animals should at once be killed, and all others known to have been bitten by them also killed so soon as the diagnosis of rabies in the first animal is confirmed. If the bitten animals cannot for any reason be killed, they should be carefully confined until the incubation period is long past. All stray dogs and cats should be destroyed because not being under any observation, their condition is not known. Dogs in general should be muzzled when abroad.

Immunity to rabies may be brought about in human beings by the method of active immunization given below, but as rabies is a somewhat rare disease of human beings, it does not seem worth while to advise immunization except when there is some particular danger of its occurrence. Such danger obtains when human beings have been attacked and bitten by rabid animals or by dogs running at large, whose health is a matter of doubt. Recovery from rabies in human beings is practically unknown. Any individual, therefore, that is bitten under suspicious circumstances may be in danger of developing an almost certainly fatal malady. This is not to be construed to mean that every person bitten by a certainly rabid dog must necessarily contract rabies, for there are accidents and circumstances attending the transmission of diseases of infectious nature, but whether certain or not, the danger of rabies is great in such cases and they ought to receive immediate care and attention. Many content themselves with an attempted destruction of the introduced virus by applying the actual cautery, or caustics, or

powerful germicides to the wounds made by the dog's teeth, and Lambert who worked upon this matter experimentally came to the conclusion that though a few cases might thus be saved, the method was too unreliable to be recommended. The long period of incubation of human rabies (from 15 to 250 days and averaging 40 days) is the source of salvation for many infected persons, for it makes it possible to effect immunization during that period and so inhibit the development of the disease itself.

Immunization against Rabies.—Pasteur* observed that the virulence of the virus was less in animals that had been dead for some time than in those just killed, and by experiment found that when the nervous system of an infected rabbit was dried in a sterile atmosphere its virulence attenuated in proportion to the length of time it was kept. A method of attenuating the virulence was thus suggested to Pasteur, and the idea of using attenuated virus as a protective vaccine soon followed. After careful experimentation he found that by inoculating a dog with much attenuated, then with less attenuated, then with moderately strong virus, it developed an immunity that enabled it to resist infection with an amount of virulent material that would certainly kill an unprotected dog.

It is remarkable that this method, based upon limited accurate biologic knowledge, and upon experience with very few micro-organisms, should find absolute confirmation as our knowledge of immunity, toxins, and antitoxins progressed. Pasteur introduced the unknown poison-producers, attenuated by drying and capable of generating only a little poison, accustomed the animal first to them and then to stronger and stronger ones until immunity was established.

For the treatment of infected cases exactly the same method is followed as for the production of immunity. Indeed, the treatment of a patient bitten by a rabid animal is simply the production of immunity during the prolonged incubation period of the affection, so that the disease may not develop. The patient, to be successfully treated, must come under observation early.

The Attenuation Method.—To protect human beings from the development of hydrophobia after they have been bitten by rabid animals, it is necessary to use material of standard or known virulence. This can be prepared, according to the directions of Högyes,† by the passage of virus from a rabid animal through from 21 to 30 rabbits.

For this purpose some of the hippocampal tissue of the dog is made into an emulsion with sterile salt solution and injected subcutaneously into a rabbit. As soon as this animal dies, its spinal cord is removed, a similar emulsion made with a fragment of it, and a second rabbit inoculated, and so on through the series until a standard virulence is attained and the virus is said to be "fixed."

* "Compt.-rendu de l' Acad. de Sciences de Paris," XCII, 1259; XCV, 1187, XCVIII, 457, 1229; CI, 765; CII, 459, 835; CIII, 777.

† See Kraus and Levaditi, "Handbuch der Immunitätsforschung," I.

It has a much higher degree of virulence than the "street virus" taken from the rabid dog, but its virulence does not vary. In most laboratories the "fixed virus" is obtained from other laboratories and kept passing through rabbits. In this manner uniformity of dosage and virulence is most easily maintained.

The technic of obtaining the rabbit's cord given by Oshida* is the one now generally employed. As given by Stimson,† it is performed at the Hygienic Laboratory as follows: "The rabbit, when completely paralyzed, is killed with chloroform and nailed to a board, back uppermost, and thoroughly wetted down with an aseptic solution (1 per cent. trikresol). An incision is made through the skin from the forehead nearly to the tail and the skin laid back on each side, the ears being cut close to the head. An area 1 inch wide is seared with a hot iron around the occiput and nuchal region and ear openings. The skull is then transversely divided in the center of the seared areas by means of bone-

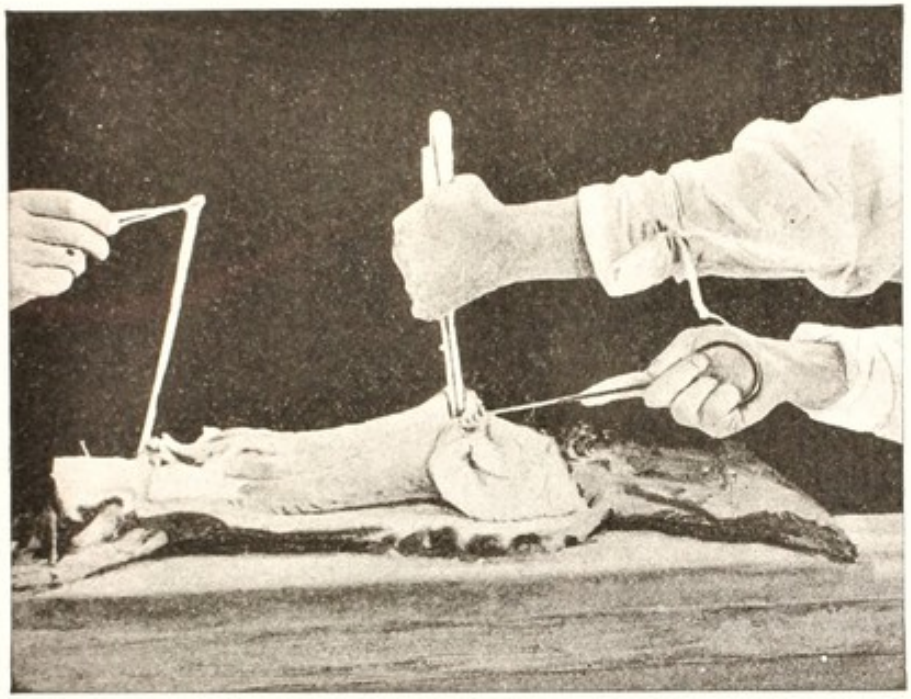


Fig. 138.—Removal of the spinal cord from a rabbit (Stimson, Bull. No. 65, Hygienic Laboratory).

cutting forceps. The neck is dissected loose from the skin and a large square of sterile gauze is inserted beneath it. The lumbar region is dissected up for a few inches and a similar piece of gauze placed beneath it. Then a piece of telegraph wire about 14 inches long, bent into a handle at one end and having a small wisp of cotton twisted about the other end, is used to push the cord out of its canal. The spine is steadied by a pair of lion-jawed forceps.

An assistant catches the cord with forceps as it emerges from the cervical opening and lifts it out. The spinal nerves are torn off during this procedure, and the membranes stripped off, leaving a clean sterile cord. A silk ligature with one long end is placed around the upper end, and another, just below the middle of the cord, which is then cut into two pieces just above the lower ligature. A small piece is cut off of the upper end of the upper portion and placed in a tube of bouillon, which is incubated as a test for sterility. The cords are hung in the drying bottle over sticks of caustic potash or calcium chloride.

The longer the cord dries, the more the virulence of the micro-organisms attenuates.

When the cord has reached the necessary attentuation, 1 cm. of

* "Centralbl. f. Bakt. u. Parasitenk.," 1901, XXIX, Orig., 988.

† "Facts and Problems of Rabies," Hygienic Laboratory, Bulletin No. 65, June, 1910, Washington, D. C.

it is emulsified with 3 cc. of sterile 0.8 per cent. salt solution and is ready for use. There can be no absolute accuracy of dosage. The injection material made in the laboratory under strict aseptic precautions can be used with perfect safety for many hours subsequently if kept cold, and can be packed in ice and sent by express to the physician to use at the home of his patients.

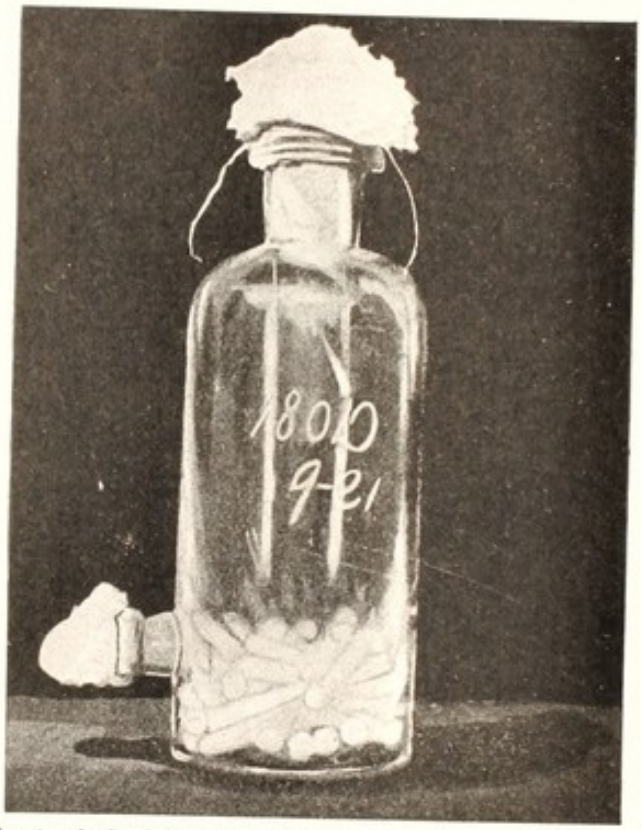


Fig. 139.—Method of drying the spinal cord of a rabbit for the purpose of attenuation (Stimson, Bull. No. 65, Hygienic Laboratory).

As the transfer of the cord to glycerin preserves the virulence for some time at whatever degree it had when so transferred, it is now customary to keep on hand, in glycerin, in the laboratory, spinal cords of rabbits dried one, two, three, four days, and so on through the whole series, always available for furnishing vaccines of all required strengths, independently of new experimental rabbits, and also makes it possible for one rabbit cord to furnish material for several cases. The treatment of a patient bitten by a rabid animal, and in danger of acquiring rabies, requires numerous injections with material of varying virulence, as shown in the following tabulations:

PASTEUR'S ORIGINAL SCHEME (Marx)

Light schema			ntense schema		
Day of treatment	Age of dried cord	Amount of injected emulsion	Day of treatment	Age of dried cord	Amount of injected emulsion
	Days	*cc.*		*Days*	*cc.*
First	14	3	First	14	3
	13	3		13	3
Second	12	3		12	3
	11	3		11	3
Third	10	3		10	3
	9	3	Second	9	3
Fourth	8	3		8	3
	7	3		7	3
Fifth	6	2	Third	6	2
	6	2		6	2
Sixth	5	2	Fourth	5	2
Seventh	5	2	Fifth	5	2
Eighth	4	2	Sixth	4	2
Ninth	3	1	Seventh	3	1
Tenth	5	2	Eighth	4	2
Eleventh	5	2	Ninth	3	1
Twelfth	4	2	Tenth	5	2
Thirteenth	4	2	Eleventh	5	2
Fourteenth	3	2	Twelfth	4	2
Fifteenth	3	2	Thirteenth	4	2
Sixteenth	5	2	Fourteenth	3	2
Seventeenth	4	2	Fifteenth	3	2
Eighteenth	3	2	Sixteenth	5	2
			Seventeenth	4	2
			Eighteenth	3	2
			Nineteenth	5	2
			Twentieth	4	2
			Twenty-first	3	2

(From Bulletin No. 65, Hygienic Laboratory, June, 1910, U. S. Public Health and Marine-Hospital Service.)

The system of treatment at present used at the Hygienic Laboratory is shown in the following tables:

SCHEME FOR MILD TREATMENT

Day	Cord	Amount injected			Day	Cord	Amount injected		
		Adult	Five to ten years	One to five years			Adult	Five to ten years	One to five years
	Injections	*cc.*	*cc.*	*cc.*		*Injections*	*cc.*	*cc.*	*cc.*
1	8 − 7 − 6 = 3	2.5	2.5	2.0	12	4 = 1	2.5	2.5	2.5
2	5 − 4 = 2	2.5	2.5	1.5	13	4 = 1	2.5	2.5	2.5
3	4 − 3 = 2	2.5	2.5	2.0	14	3 = 1	2.5	2.5	2.0
4	5 = 1	2.5	2.5	2.5	15	3 = 1	2.5	2.5	2.0
5	4 = 1	2.5	2.5	2.5	16	2 = 1	2.5	2.0	1.5
6	3 = 1	2.5	2.5	2.0	17	2 = 1	2.5	2.0	2.5
7	3 = 1	2.5	2.5	2.0	18	4 = 1	2.5	2.5	2.5
8	2 = 1	2.5	1.5	1.0	19	3 = 1	2.5	2.5	2.5
9	2 = 1	2.5	2.0	1.5	20	2 = 1	2.5	2.5	2.0
10	5 = 1	2.5	2.5	2.5	21	2 = 1	2.5	2.5	2.0
11	5 = 1	2.5	2.5	2.5					

SCHEME FOR INTENSIVE TREATMENT

Day	Cord	Amount injected			Day	Cord	Amount injected		
		Adult	Five to ten years	One to five years			Adult	Five to ten years	One to five years
	Injections	*cc.*	*cc.*	*cc.*		*Injections*	*cc.*	*cc.*	*cc.*
1.....	8—7—6=3	2.5	2.5	2.5	12....	3=1	2.5	2.5	2.0
2.....	4—3=2	2.5	2.5	2.0	13....	3=1	2.5	2.5	2.0
3.....	5—4=2	2.5	2.5	2.5	14....	2=1	2.5	1.5	2.0
4.....	3=1	2.5	2.5	2.0	15....	2=1	2.5	2.5	2.0
5.....	3=1	2.5	2.5	2.0	16....	4=1	2.5	2.5	2.5
6.....	2=1	2.5	2.0	1.5	17....	3=1	2.5	2.5	2.5
7.....	2=1	2.5	2.5	2.0	18....	2=1	2.5	2.5	2.0
8.....	1=1	2.5	1.5	1.0	19....	3=1	2.5	2.5	2.0
9.....	5=1	2.5	2.5	2.5	20....	2=1	2.5	2.5	2.5
10.....	4=1	2.5	2.5	2.5	21....	1=1	2.5	2.5	2.0
11.....	4=1	2.5	2.5	2.5					

(From Bulletin No. 65, Hygienic Laboratory, June, 1910, U. S. Public Health and Marine-Hospital Service.)

The Dilution Method—Högyes,* of Budapest, believes that Pasteur was mistaken in supposing that the drying was of importance in attenuating the virus, and thinks that dilution is the chief factor. He makes an emulsion of rabbit's medulla (1 gram of medulla to 10 cc. of sterile broth) as a stock solution, to be prepared freshly every day, and uses it for treatment, the first dilution used being 1 : 10,000; then on succeeding days 1 : 8000, 1 : 6000, 1 : 5000, 1 : 2000, 1 : 1000, 1 : 500, 1 : 250, 1 : 200, 1 : 100, and finally the full strength, 1 : 10.

Cabot† found the dilution method attended with danger to the animal immunized, which was not true of the dried-cord method of Pasteur.

The Inspissation Method.—A new method of carrying out the dilution method, suggested by Harris and Shackell,† seems to be devoid of danger to the patient and bids fair to recommend itself on the ground of greater accuracy than former methods. It depends upon Shackell's method of desiccation:‡

> The material to be dried is placed in the bottom of a Schubler's vacuum desiccating jar, in the upper part of which is a separate dish containing sulphuric acid. The temperature is reduced by placing the jar, half submerged, in a salt and ice mixture, and after thorough solidification of the material has resulted, a rapid vacuum is produced by a Geryk pump to less than 2 mm. of mercury. During the process of desiccation, the temperature in the lower half should be kept several degrees below 0°C. Unless the sulphuric acid be repeatedly shaken to prevent saturation with water, the time required for complete desiccation will be unduly prolonged.

By this method brains and cords may be desiccated *in toto*, with-

* "Acad. des Sciences de Buda-Pest," Oct. 17, 1897; "Centralbl. f. Bakt. u. Parasitenk.," 1887, II, 579.

† "Journal of Experimental Medicine," 1899, vol. IV, No. 2.

‡ Lab. Sec. Amer. Pub. Health Asso., Sept. 6, 1910.

out destruction of virulence, in from twenty-four to thirty-six hours. The material thus dried is like chalk and easily pulverized. It is, however, highly hygroscopic and if permitted to absorb water becomes leathery and loses virulence rapidly.

In a later paper Harris* found that the more thoroughly and rapidly the material is frozen, the greater will be the amount of virulence remaining after desiccation. A new method suggested is as follows:

"The brain or cord is ground in a porcelain mortar, with the addition of water drop by drop until a thick smooth paste is formed. Carbon dioxide snow is then collected from a tank in the ordinary manner and is added in small amounts to the paste which should be stirred thoroughly meanwhile to prevent the material freezing in a solid mass. Freezing occurs rapidly and when complete the material is very brittle and easily reducible to a fine powder. During the pulverization more snow is added from time to time to prevent thawing. When the material is thoroughly pulverized, it is transferred to a small beaker with an excess of snow and placed in the bottom of a Schubler's vacuum jar which has previously been half immersed in a mixture of salt and ice and become thoroughly cold. A beaker of sulphuric acid is then placed on wire gauze in the upper part of the jar in such manner that there is free access of air between the frozen material and the sulphuric acid. The acid is placed in the upper part because if placed below, it soon freezes at the low temperature. The vacuum should measure less than 2 mm. of mercury. During desiccation the temperatures should not be allowed to rise above −15°C. The jar should be rotated gently several times daily to mix the water and the acid. A single brain will become thoroughly dry in from thirty-six to forty-eight hours.

The object in thoroughly pulverizing the virus is two-fold. It results in a more complete mixture, so that all parts contain an equal amount of virulence. Secondly, it permits of more rapid drying and an easy transfer into smaller containers for subsequent tests. To avoid any absorption of moisture, the dry powder is transferred from the beaker to small glass tubes the ends of which are sealed in a flame. The transfer is effected in a moisture-free atmosphere by covering the top of the beaker with rubber dam held in place by adhesive strips. A small puncture is made in the rubber large enough to admit the tube, and through this the tubes are inserted and filled. From 20 to 100 mg. is a convenient amount put into each tube. If the tube has a diameter of 4 mm., each millimeter of powder will weigh approximately 2 mg.

Harris believes that the use of desiccated virus in anti-rabic immunization of animals and persons offers many advantages over other methods.

Harris† reports that 182 patients have been injected with the virus thus prepared for the purpose of immunizing them against hydrophobia. No deaths have occurred and no complications developed. It is thus to all appearances a safe and efficient method and is especially economical to the laboratory in time, labor and money. Material can be prepared two or three times a year and put aside in the cold to be used only when needed and as one rabbit

* "Jour. of Infectious Diseases," 1912, X, 369.
† "Jour. of Infectious Diseases," 1913, XIII, 155.

furnishes enough material to immunize 20–25 patients, the initial cost is negligible. The work can be undertaken in any hospital or municipal laboratory without increasing the staff or the expense. To be able to prepare at one time enough material for from six to twelve months' use and to have this always ready for any number of patients is such a lessening of labor and anxiety as only those who have followed the classic method of drying cords can appreciate.

If the conclusion of Harvey and McKendrick* be correct, and "the immunizing power of any given portion of a rabies cord is a function of the unkilled remnant of the rabies virus which is contained in that cord," one should be able to find out with mathematical certainty how many minimum infective doses will produce a definite degree of immunity. For this purpose they suggest that the virulence of the virus is expressed in "units," one unit being the smallest amount which, when injected intra-cerebrally into a full-grown rabbit, will produce paresis on the seventh day.

Specific Treatment.—Babes and Lepp† thought that the serum of animals that had received repeated injections of the crushed nervous tissue of rabid animals was neutralizing or destructive to the rabies virus *in vitro*, called it "antirabic serum," and believed that it conferred a defensive power upon other animals. Marie,‡ however, found it to be a simple neurotoxic serum and inert in its action upon the virus. It is never used in the treatment of rabies, at present.

* "Theory and Practice of Anti-rabic Immunization," Calcutta, 1907.
† "Ann. de l'Inst. Pasteur," 1889, III.
‡ "Compt.-rendu Soc. Biol.," June 18, 1904, LVI, p. 1030.

CHAPTER VI

ACUTE ANTERIOR POLIOMYELITIS

Acute anterior poliomyelitis, atrophic spinal paralysis, infantile palsy, "spinale Kinderlähmung," is an acute infectious disease, largely confined to the first three years of life, and characterized by fever, destruction of cells in the gray matter of the central nervous system, palsy and rapid atrophy of the palsied muscles. It is of sporadic and occasionally of epidemic occurrence in all parts of the world. Although infectious, its transmissibility is so slight as to make contagiousness a matter of doubt.

The essential cause is in doubt, though it is possible that it is a minute coccoid organism that may be capable of artificial cultivation. It is certain that there is an infectious agent and that it is filterable through the Berkefeld filters. Probably the best account of the history and epidemiology of the disease has been compiled by Wickman.*

The disease was investigated bacteriologically by various workers, and it went through the usual experience of having various micro-organisms isolated and described, to be afterward abandoned as accidental and unimportant agents. The modern studies of the subject, by modern methods of investigation, were begun by Landsteiner and Popper.† Their method of procedure was to emulsify the spinal cord of a fatal case of the disease, in a nine-year-old child, in physiological salt solution, and inject it into the peritoneal cavities of monkeys. One monkey became ill and died on the eighth day; the other became paralyzed on the seventeenth day after the inoculation. A similar emulsion of the cord of the paralyzed monkey failed to infect other monkeys into which it was injected. Knöpfelmacher,‡ and Strauss and Huntoon§ were also able to infect one monkey with human virus, but could carry the infection no further.

Flexner and Lewis‖ made careful experiments upon 81 monkeys inoculated with the disease. They found the incubation period to vary from 4 to 33 days, the average being 9.82 days. During this period there were prodromal symptoms such as nervousness and excitability, fatigue, tremor of the face and limbs, shifting gaze

* "Beiträge zur Kentniss der Heine-Medinischen Krankheit," Berlin, 1907.
† "Zeitschrift für Immunitätsforschung," 1909, II, 377.
‡ "Med. Klin.," 1909, V, 1671.
§ "New York Med. Jour.," 1910, XCI, 64.
‖ "Journal of the Amer. Med. Assoc.," 1909, LIII, 1639, and "Jour. Medical Research," 1910, XII, 227.

when the attention was attracted, and a wrinkled and mobile rather than smooth and placid face. The onset of the disease is sudden, with or without the given signs, and consists of paralysis. In general, any of the larger voluntary muscle groups may be affected; other groups may be weak or partially paralyzed. The paralysis may be of all grades of completeness. There may be some anesthesia; occasionally there was evidence of pain. The animals may die or they may recover. In the latter case the paralysis sometimes entirely disappears; more frequently it persists and the paralyzed member gradually stiffens and is deformed by contractures.

In the dead monkeys, or those that were killed for study, the chief lesions were in the gray matter of the central nervous system and consisted of edema, diffuse livid injections of the blood-vessels and punctiform and pin-head-sized hemorrhages. When healing sets in, the lesions are firmer, paler, non-circumscribed, and raised somewhat above the level of the surrounding gray and white matter.

The chief histological changes were also in the gray matter especially in the cord, where they occurred in either the anterior or posterior horns, but more frequently and more extensively in the anterior horns. There was a high degree of cellular infiltration of the perivascular spaces, edema of the spaces, and hemorrhage into the spaces. From the spaces the cells often passed into the ground substance. But independent foci of small cells, edema and hemorrhage also existed in the nervous tissue. The nerve cells often showed degeneration which consisted of hyaline transformation and necrosis leading to loss of the tigroid substance, cell-processes, nuclei, etc. Often the cell was surrounded by lymphocytes or invaded by polymorphonuclear leukocytes. Sometimes the nerve-cells had disappeared and the leukocytes taken their places. Ultimately, a part of the nervous elements would be removed and replaced by an indefinite cellular tissue, containing many compound granular corpuscles.

The monkeys were infected by various methods, the first being the direct inoculation of the brain by a needle introduced through the opening made by a small trephine. They found, however, that the virus readily finds its way to the nervous system when introduced subcutaneously, and less readily when introduced intraperitoneally. The blood of the infected animal contains the virus at the beginning of the attack but how richly was not determined. The crebro-spinal fluid also contains it at the time the palsy appears. The vaso-pharyngeal mucosa also contains it, and can convey it to other animals.

The virus readily passed through Berkefeld filters, and the clear filtrate thus obtained, when injected into monkeys by the intracerebral or subcutaneous routes, regularly produced the disease in an infectious form so that it was clear that the lesions were in-

fectious and not toxic in character though brought about by filtered fluid.

The virus resists freezing but is readily destroyed by heating to 45°–50°C. for half an hour.

Various attempts were made by Kraus and Wernicke,* Lentz and Huntemüller† and Marks‡ to infect rabbits with the virus, but

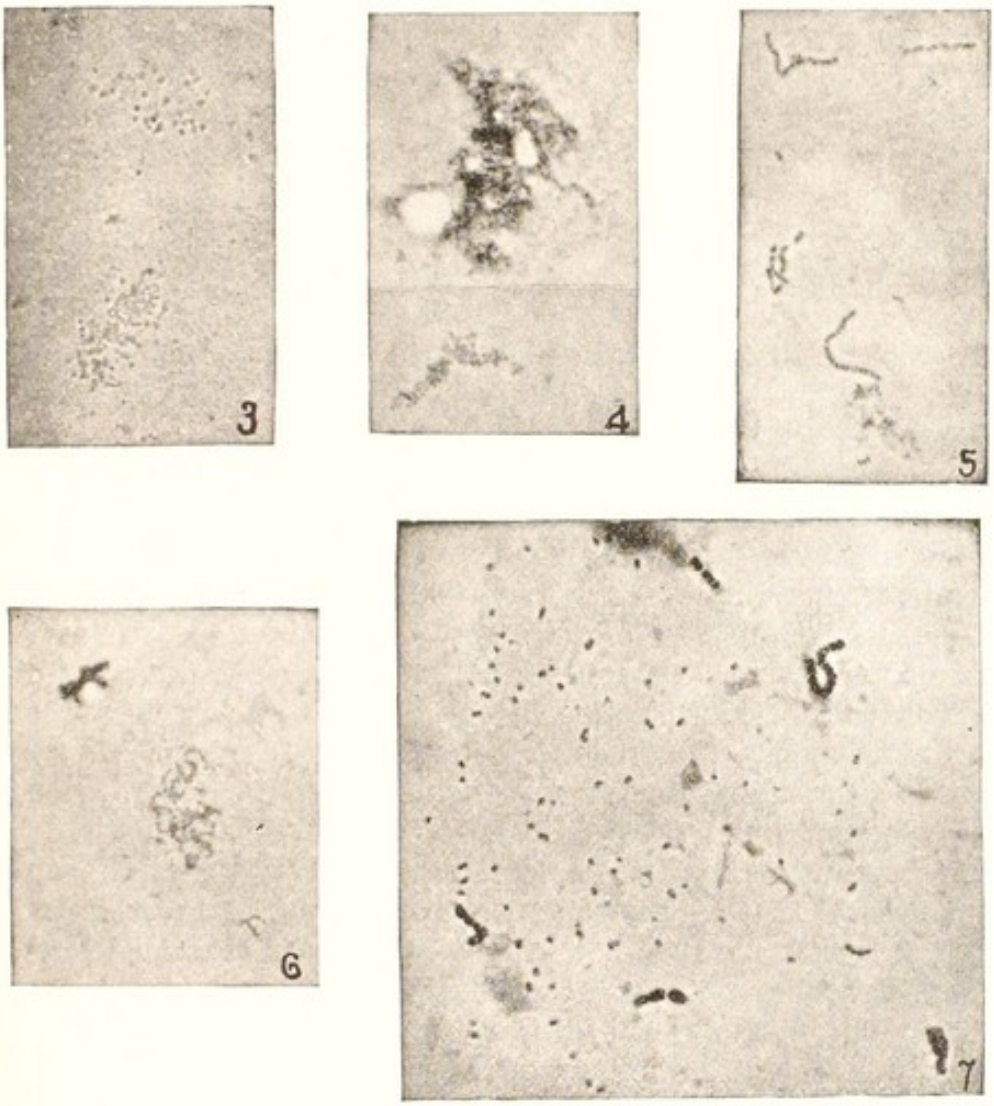


Fig. 140.—Micro-organism causing epidemic poliomyelitis. 3, Separate globoid bodies, × 1000; 4, aggregated masses of globoid bodies, × 1000; 5, chains and pairs of globoid bodies, × 1000; 6, chains of globoid bodies compared with *Streptococcus pyogenes*, × 1000; 7, agar fragment showing pairs of globoid bodies compared with *Streptococcus pyogenes*, × 1000 (Flexner and Noguchi, in Journal of Experimental Medicine).

though some successes were reported, there seems to be no development in the rabbit of lesions or disturbances resembling the characteristic lesions and symptoms of acute anterior poliomyelitis in man and the monkey.

* "Deutsche med. Wochenschrift," 1909, XXXV, 1825; 1910, XXXVI, 693.
† "Zeitschrift für Hygiene," 1910, LXVI, 481.
‡ "Jour. Exp. Med.," 1911, XIV, 116.

In 1912, Rosenau and Brues* reported that in 50 per cent. of their experiments, the virus of acute anterior poliomyelitis was transmitted from monkey to monkey by the bite of the stable fly Stomoxys calcitrans, and expressed the belief that it was a biological and not a mechanical transfer, and that the virus underwent some change and development in the flies. These results were confirmed by Anderson and Forst,† but failed to be confirmed by other workers and later could not be successfully repeated by the same investigators.

Howard and Clark‡ worked over the subject of transmission of the disease by insects, and investigated the house-fly Musca domestica; the bed-bug, Cimex lectularius; the lice, Pediculus capitis and Pediculus vestimenti; various mosquitoes, Culex pipiens, Culex solicitans and Culex cantator, and found that only one of these insects, the common house-fly, Musca domestica, can carry the virus in an active state for several days both upon the surface of its body and in its gastro-intestinal tract. None of the suctorial insects withdrew the virus with the blood of the infected monkeys to which they were applied.

Flexner and Noguchi§ made experiments upon the cultivation of the micro-organism supposed to be the infective agent. The technic employed was much like that employed for the cultivation of Treponema pallidum (*q.v.*), and resulted in an undoubted quantitative increase in the infectiveness of the virus. Further, they were now able, for the first time, to describe an organism that might be the specific infectious agent. It is a globoid body measuring from 0.15–0.3 μ, arranged in pairs, chains and indefinite masses. Its small size makes it barely visible and able to penetrate the pores of the Berkefeld filters.

This organism they were able to stain both by the methods of Giemsa and Gram. Having come to recognize it in the culture, they were subsequently able to find it in sections of tissue from the lesions of poliomyelitis, and conclude that "The micro-organism exists in the infectious and diseased organs; it is not, so far as is known, a common saprophyte, or associated with any other pathological condition; it is capable of reproducing, on inoculation, the experimental disease in monkeys, from which animals it can be recovered in pure culture. And besides these classical requirements, the micro-organism withstands preservation and glycerination as does the ordinary virus of poliomyelitis within the nervous organs. Finally, the anaërobic nature of the micro-organism interposes no obstacle to its acceptance as the causative agent, since the living tissues are devoid of free oxygen and the virus of poliomyelitis has not yet been detected in the circulating blood or cerebro-spinal fluid

* "Monthly Bull. of the State Board of Health of Massachusetts," 1912, VII, 314.

† "Public Health Reports," 1913, XXVIII, 833.

‡ "Jour. Exp. Med.," 1912, XVI, 850.

§ "Jour. Exp. Med.," 1913, XVIII, 461.

of human beings, in which the oxygen is less firmly bound; nor need it, even should the micro-organism be found sometimes to survive in these fluids."

From these discoveries it is now certainly well established that acute anterior poliomyelitis is an infectious disease, occasioned by a minute anaërobic organism, of globoid form, capable of resisting the bactericidal effects of glycerin for months, and capable of passing through the pores of a Berkefeld filter. When nervous or other tissue containing it, or pure cultures of it, are introduced into the nervous tissue or into the subcutaneous tissues of certain animals, of which the monkey is the chief one, the disease is readily induced.

The mode of transmission remains to be discussed. From the failure of those who continued the insect experiments to achieve continued success, and because of the short time the infectious agents are in the blood—only the first few days—and the small number that seem to be there, it is well to assume that insects play a doubtful rôle, unless it be the common house-fly, Musca domestica.

Flexner and Clark* have shown that when the virus is introduced into the upper nasal mucosa in monkeys its propagation can be followed from the olfactory lobes of the brain to the medulla oblongata and spinal cord. Since the virus can thus find its way from the nasal mucosa to the deeper nervous tissues, they hold the opinion that it is through this avenue that infection commonly takes place.

During the disease, the infectious agents are upon the nasal mucosa, they may be discharged from the surface into the atmosphere, and inhalation by others may be the means of infection. It is also not impossible that house-flies first visiting the nose of an infected sleeping child, and then some other sleeping child, may carry the organisms.

One attack of the disease confers immunity, and experimental immunization can be effected by a succession of doses beginning with great dilutions and ascending to greater concentrations like the Högyes method in rabies, but as the disease comes on without a preliminary dog-bite, and as the period of incubation is short, and as our first knowledge of it coincides with the appearance of the paralysis when the damage is already done, no practical utilization can be made of our knowledge of the facts of immunity to the disease at the present time.

* "Proc. Soc. Exper. Biol. and Med.," 1912–13, x, 1.

CHAPTER VII

CEREBRO-SPINAL MENINGITIS

Diplococcus Intracellularis Meningitidis (Weichselbaum)

General Characteristics.—A minute non-motile, non-flagellate, non-sporogenous, non-chromogenic, non-liquefying, aërobic, pathogenic coccus, staining by ordinary methods, but not by Gram's method.

Acute cerebro-spinal meningitis may be secondary to various more or less well-localized infections when it depends upon such micro-organisms as may be carried by accident to the meninges. Among these may be mentioned pneumococci, staphylococci, streptococci, Bacillus influenzæ, B. typhosus, B. coli, B. mallei, B. pestis and others.

In addition to these cases, however, there are numerous cases of primary infection of the membranes, either sporadic or epidemic in occurrence. Such constitute the disease known as *cerebro-spinal fever, epidemic cerebro-spinal meningitis*, or "*spotted fever*." It is a very dangerous febrile malady, characterized by high temperature, an irregular exanthem, early meningitis, a moderate degree of contagion, and a high mortality. The cause of this infection is a specific organism known as the *meningococcus*, or Diplococcus intracellularis meningitidis.

As early as 1887 Weichselbaum* carefully described a diplococcus found in 6 cases of cerebro-spinal meningitis that may have been identical with one found by Leichtenstern† in 1885 in the purulent exudate of a case of meningitis, and with a coccus observed as early as 1884 by Celli and Marchiafava.‡ Weichselbaum's studies and description of this coccus seem to have attracted but little attention at first, and references to them are but brief in most of the text-books. The prevailing opinion was that its occurrence in cerebro-spinal meningitis was accidental, as inoculations into animals showed its pathogenic power to be very limited. The careful studies of Jäger,§ Scherer,‖ Councilman, and Mallory and Wright** (embracing 55 cases, in which the cocci were found by culture or by microscopic examination in 38), and of Flatten,†† Schneider,†† Rieger,†† Schmidt,†† Göppert,†† Flügge,†† von Lingelsheim,††

* "Fortschritte der Med.," x, 18 and 19.
† "Deutsche med. Wochenschrift," 1885.
‡ "Gazette degli Ospedali," 1884, viii.
§ "Zeitschrift für Hygiene," xix, 2, 351.
‖ "Centralbl. f. Bakt. u. Parasitenk.," 1895, xvii, 13 and 14.
** "Amer. Jour. Med. Sci.," March, 1898, vol. cxv, No. 5.
†† "Klinisches Jahrbuch," 1906.

Besredka* Flexner† and others have, however, shown the diplococcus of Weichselbaum to be, without doubt, the specific organism.

Distribution.—The distribution of Diplococcus intracellularis in nature is as yet unknown. It has been found in cerebro-spinal meningitis by those who have looked for it, twice has been found in the nose in coryza by Scherer, has been found in the conjunctiva by Carl Fränkel‡ and Axenfeld,§ and in the purulent discharges of rhinitis and otitis by Jäger.‖

Morphology.—The micro-organism is a biscuit-shaped diplococcus having a great resemblance to the gonococcus. This resemblance is further increased by the fact that the cocci are usually found inclosed in the protoplasm of the leukocytes. Weichselbaum,

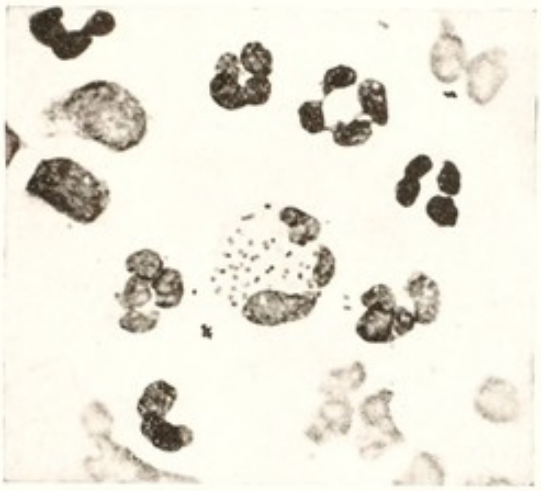


Fig. 141.—Meningococcus in spinal fluid (from Hiss and Zinsser, "Text-Book of Bacteriology," D. Appleton & Co., Publishers).

by whom this was first observed, found it constant in sections of the brain and its membranes, though in the exudate of the disease a good many free cocci may be observed. It was this peculiar relationship to the cells that led Weichselbaum to name the organism Diplococcus intracellularis. Many of the cocci inclosed in the cells are apparently dead and degenerated, as they stain badly and do not grow when the pus is transferred to culture-media.

Identification.—Carl Fränkel, in discussing the micro-organism, points out that its morphologic peculiarities have much in common with the pneumococcus, so that the most refined methods of differentiation should always precede a positive determination. Its resemblance to the gonococcus should also be kept in mind.

Perhaps the greatest difficulty obtains in making a certain

* "Annales de l'Inst. Pasteur," 1906, XX, 4.
† "Jour. Exp. Med.," 1906–07.
‡ "Zeitschrift für Hygiene," June 14, 1899.
§ Lubarsch and Oestertag, "Ergebnisse der allg. Path. u. path. Anat.," III, S. 573.
‖ "Deutsche med. Wochenschrift," 1894, S. 407.

differentiation between the meningococcus and Micrococcus catarrhalis (*q.v.*), especially when such investigations are directed toward discovering the former organism in the nasal discharges. This cannot be done by microscopic examination, but must be achieved through cultivation of the organisms and observation of the cultures. Micrococcus catarrhalis grows well upon nearly all culture-media; meningococci, very sparsely except upon special media. The former organism grows fairly well at room temperatures (20°C. or less); the latter, only at 25°C. and above. The colonies of the former are coarsely granular; those of the latter, finely granular.

Staining.—The organism is easily stained with the usual aqueous solutions of the anilin dyes. It does not stain by Gram's method.

For staining the meningococcus the method of Pick and Jacobsohn* is highly praised by Carl Fränkel, who modifies it by adding three times as much carbol-fuchsin as is recommended in the original instructions, which are as follows: Mix 20 cc. of water with 8 drops of saturated methylene-blue solution; then add 45 to 50 drops of carbol-fuchsin. Allow the fluid to act upon the cover-glass for five minutes. The cocci alone are blue, all else red.

Isolation.—The organism can be secured for cultivation either from the purulent matter of the exudate found at autopsy, or from the fluid obtained by lumbar puncture. To obtain this fluid Park† gives the following directions: "The patient should lie on the right side with the knees drawn up and the left shoulder depressed. The skin of the patient's back, the hands of the operator, and the large antitoxin syringe should be sterile. The needle should be 4 cm. in length, with a diameter of 1 mm. for children, and larger for adults. The puncture is generally made between the third and fourth lumbar vertebræ. The thumb of the left hand is pressed between the spinous processes, and the point of the needle is entered about 1 cm. to the right of the median line and on a level with the thumb-nail, and directed slightly upward and inward toward the median line. At a depth of 3 or 4 cm. in children and 7 or 8 cm. in adults the needle enters the subarachnoid space, and the fluids flow out in drops or in a stream. If the needle meets a bony obstruction, withdraw and thrust again rather than make lateral movements. Any blood obscures microscopic examination. Adults, not too ill, may sit upon a chair or upon the edge of the bed while the spinal puncture is made, as shown in Kolmer's illustration. The fluid is allowed to drop into sterile test-tubes or vials with sterile stoppers. From 5 to 15 cc. should be withdrawn. No ill effects have been observed from the operation."

In making a culture from this fluid Park points out that, as many of its contained cocci are dead, a considerable quantity of the fluid (say about 1 cc.) must be used.

* "Berliner klin. Wochenschrift," 1896, 811.

† "Bacteriology in Medicine and Surgery," Philadelphia, 1899, p. 364.

The cocci have also been cultivated from the nasal discharges in 6 cases studied by Weichselbaum, and in 18 studied by Scherer. Elser* has isolated the organism from the circulating blood of patients suffering from epidemic cerebro-spinal fever. To determine the presence of the coccus in the nasal discharges where other similar cocci may be present, Gram's stain may be used and followed by an aqueous solution of Bismarck-brown. The meningococci will be brown.

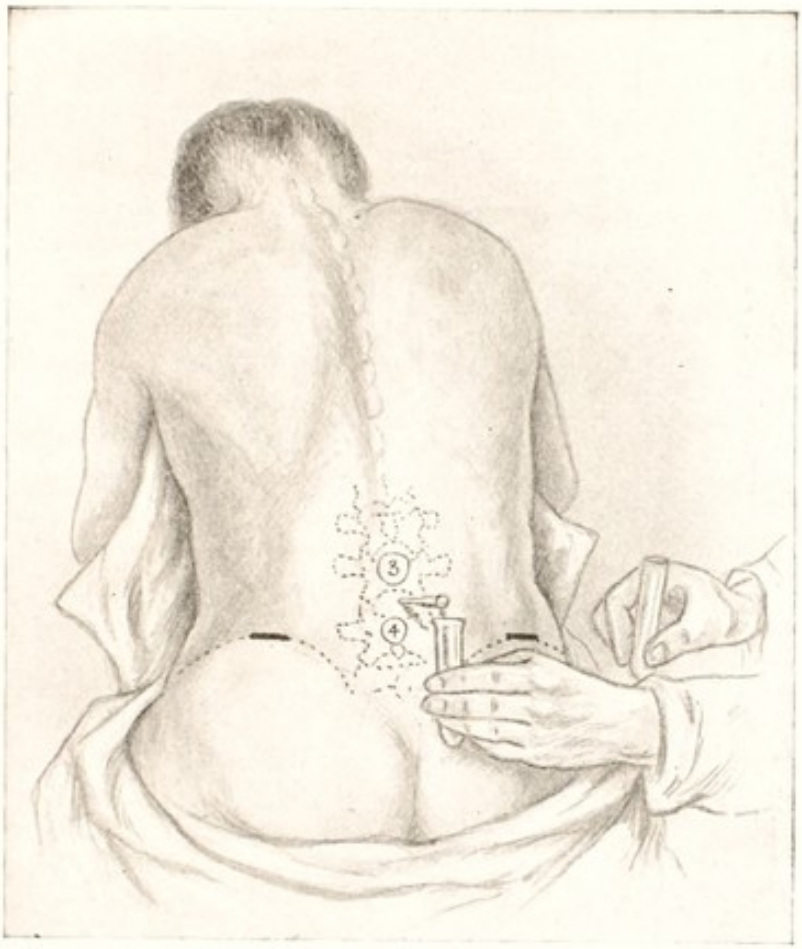


Fig. 142.—Technic of spinal puncture. The patient is sitting on the edge of a chair and is bent forward; the crests of the ilia are indicated by black lines, and are on a level with the spinous process of the fourth lumbar vertebra; the "soft spot" is found just above. The first tube receives the first few drops of fluid, which are usually blood tinged. (Kolmer.)

Cultivation.—The organism was successfully cultivated by Weichselbaum, but does not readily adapt itself to artificial media. It develops upon agar-agar and glycerin agar-agar, upon Löffler's blood-serum mixture, and, according to Goldschmidt,† upon potato. Weichselbaum did not find that it developed upon potato. It does not grow in bouillon or gelatin. The cultures are usually scanty and without characteristic features.

* "Jour. Medical Research," 1906, XIV, 89.
† "Centralbl. f. Bakt. u. Parasitenk.," II, 22, 23.

Flexner* found that the difficulties of cultivation were greatly reduced by the employment of sheep-serum instead of human serum. Sheep-serum water was prepared according to the method of Hiss (sheep-serum 1 part, water 2 parts, sterilized in the autoclave) and mixed with a beef-infusion agar-agar containing 2 per cent. of glucose. The quantity of sheep-serum need not exceed $\frac{1}{20}$ to $\frac{1}{10}$ of the volume of the agar-agar It is added to the sterile melted agar, which is afterward slanted in test-tubes or allowed to congeal on the expanded surface of 16-ounce Blake bottles when mass cultures are to be used. There is nothing characteristic about the cultures. The cocci grow only at the temperature of the body, attain only a sparse development, and form a more or less confluent line of minute, rounded, grayish colonies which are easily overlooked upon opaque media like blood-serum. The general characteristics of the growth are not unlike those of the pneumococcus, streptococcus, and gonococcus.

Colonies.—When grown upon agar-agar plates, the deep colonies scarcely develop at all, appearing under the low-power lens as minute, irregularly rounded, granular masses. The surface colonies are larger, and consist of an opaque yellowish-brown nucleus about which a flat, rounded disk spreads out. The edges may be dentate; the color is grayish or yellowish near the center, becoming less intense as the thin edges are reached; the structure is finely granular.

Vital Resistance.—The vitality of the culture is low, and the cocci die quickly. It becomes necessary, therefore, when studying the organism to transplant it frequently—Park† says every two days. Flexner‡ found that they do not survive beyond two or three days and that transplantations do not succeed unless considerable quantities of the culture are placed upon the surface of the fresh medium, showing that many of the organisms were already dead. This is confirmed by the microscopic appearance of the cultures. Those sixteen to twenty-four hours old stain sharply and uniformly; on the second day many of the cocci show irregularities of size and staining, and after several days no normal-looking cocci can be found. It was found, however, that in carefully preserved cultures of certain strains a few cocci might survive for many months. Vitality is preserved longest when the cultures are kept in the thermostat and *not* taken out when grown, to be kept at room temperature or in a refrigerator. The addition of a small quantity of a calcium salt favors prolonged vitality and will sometimes maintain it for four or five weeks in cultures that would otherwise die in a few days. Sodium chlorid is injurious to the cocci. Flexner attributed the autolysis of the cultures to an enzyme.

The organism is soon killed by drying, by exposure to the sun,

* "Jour. Experimental Med.," 1907, IX, p. 105.
† "Bacteriology in Medicine and Surgery," 1899, p. 362.
‡ Loc. cit.

and by quite moderate variations of temperature. It succumbs to very high dilutions of most germicides in a very short time.

The thermal endurance of the organism is very slight. It will not grow except at 37°C., ceases to grow at 40°C. It is killed in five minutes at 60°C.

Agglutination.—When animals are immunized by repeated injections of the Diplococcus intracellularis, their blood-serum and body-juices become agglutinative. Such serums kept in the laboratory can be used for the identification of the coccus in fresh culture, though the reaction is not exact, since the agglutinability of different strains of cocci is different. The serums have an agglutinating power that varies from 1 : 500 to 1 : 3000 in the hands of different observers.

Metabolic Products.—The meningococcus breaks up dextrose and maltose with the production of acids, but has no similar action upon levulose, saccharose, or inulin. Acid production is unaccompanied by gas evolution. To determine the acid the coccus may be grown upon acetic-fluid agar containing the sugar under examination, and a little litmus or neutral red.

No indol is produced, no gelatin-softening coagulating or other ferments are formed.

The meningococcus produces an endotoxin. Albrech and Ghon* were able to kill white mice with dead cultures. Lepierre† obtained a toxin from bouillon cultures by precipitating them with alcohol.

Pathogenesis.—The results of animal inoculations made with Diplococcus intracellularis meningitidis are disappointing. Subcutaneous inoculations into the lower animals are continually without effect. Intrapleural and intraperitoneal injections of cultures of the organism into mice and guinea-pigs are sometimes fatal, the dead animals showing a serofibrinous inflammation with the presence of the cocci. The intravenous injection of the coccus into rabbits is followed by death without important or conclusive symptoms, and usually without the presence of cocci in the blood.

Weichselbaum endeavored to reproduce the original cerebrospinal meningitis in animals by trephining and injecting the cocci beneath the dura. In this manner he inoculated three rabbits and three dogs. Two of the rabbit injections failed, probably because the injected material escaped at once from the wound. The third rabbit died, and showed marked congestion of the membranes of the brain and a minute softened and hemorrhagic area. In these the cocci were found by culture to be abundant. The three dogs all died with congestion and pus-formation in the membranes and areas of softening in the brain substance. The cocci were recovered from two of the dogs, but the lesions of the third animal, which lived twelve days, contained none.

* "Wiener klin. Wochenschrift," 1901.
† "Jour. de phys. et de path. gén.," v, No. 3.

Flexner* found that in large doses the coccus was always capable of killing small guinea-pigs and mice when injected intraperitoneally. To achieve this, however, the organisms should be suspended in sheep-serum water, not in salt solution, which is an active poison to them.

Bettencourt and Franca† tried to infect monkeys by trephining, by injecting into the spinal canal, and by rubbing the cocci upon the nasal mucous membranes, but without success. Von Lingelsheim and Leuchs‡ and Flexner§ were more successful. Flexner's method was to introduce a hypodermic needle into the spinal canal, wait until a few drops of cerebro-spinal fluid had escaped, and then inject the culture. When thus introduced at a low level of the spinal canal, the diplococci distribute themselves through the meninges in a few hours and excite an acute meningitis, the exudate of which accumulates chiefly in the lower spinal meninges and the meninges of the base of the brain. The inflammation extends, in monkeys, into the membranes covering the olfactory lobes and along the dura mater into the ethmoid plate and nasal mucosa.

The nasal mucous membrane is found in many instances to be inflamed and beset with hemorrhages. Smear preparations from the nasal mucosa show many polymorphonuclear leukocytes containing the cocci in a degenerated form. The cocci were not cultivated from the nasal exudates.

Mode of Infection.—It is not known by what channels infection with Diplococcus intracellularis meningitidis takes place. Weichselbaum supposed it might enter by the nasal, auditory, or other passages, especially the nose, where he constantly found it, and the more recent studies of Goodwin and Sholly‖ have shown the organisms to be of frequent occurrence in the nasal cavities of meningitis patients as well as occasionally in those associated with them. It thus becomes evident that association with the diseased may lead to the infection of the well, and that the cases should be isolated. The same conclusions were reached by Kolle and Wassermann,** who studied the nasal secretions of 112 healthy individuals, not exposed to the disease, without finding any cocci, but found them in the nasopharynx of the father of a child suffering from the disease, and that of another child with suspicious symptoms.

Steel†† has found what may be a variety of the meningococcus in the simple posterior basic meningitis of infants. The organism differs from that of Weichselbaum in having a greater longevity upon culture-media, where it often lives as long as thirty days. It is

* Loc. cit.
† "Zeitschr. f. Hyg. u. Infekt.," XLVI, p. 463.
‡ "Klin. Jahrbuch," 1906, XV, p. 489.
§ Loc. cit.
‖ "Journal of Infectious Diseases," 1906, Supplement No. 2, p. 21.
** "Klinisches Jahrbuch," XV, 1906.
†† "Pediatrics," Nov. 15, 1898.

easily stained by methylene blue, but not by Gram's method. Another similar organism has been described by Elser and Huntoon.*

Bacteriological Diagnosis.—In cases with the clinical symptoms of meningitis, the bacteriological diagnosis is of great assistance in determining the correctness of the diagnosis and the nature of the infection. It is accomplished by means of the lumbar puncture (vide supra) and the study of the cerebro-spinal fluid thus secured. Normal cerebro-spinal fluid is clear, that in meningitis is cloudy. A few cubic centimeters of the fluid can be used for culture and inoculation experiments of as many kinds as are deemed advisable. The remainder is placed in a tube and whirled in a centrifuge. From the sediment, smears are made upon slides and stained by various methods, including Gram's method. If the chief cells appearing in the sediment are lymphocytes, tuberculous meningitis should be thought of and smears stained for tubercle bacilli, and guinea-pigs inoculated. If the cells are polymorphonuclear cells, tuberculous meningitis is usually excluded. If small cocci are found, chiefly in the cells, the next question is their reaction to the Gram stain. If positive to the stain, the pneumococcus should be thought of; if negative to the stain, the meningococcus. If the suspected organism grows readily upon ordinary culture-media, it is not the meningococcus; if it grow only in the special media it is probably the meningococcus. Finally, the agglutinative test with diluted antiserum may be made to perfect the diagnosis.

Specific Therapy.—Kolle and Wassermann† carefully studied antimeningococcus sera for specific opsonins, for bacteriotropic substances, and for other evidences of favorable therapeutic action, but came to no definite conclusions. Flexner and Jobling‡ had better success both in developing the experimental and practical knowledge of the serum. The serum was prepared first with goats and then with horses, the animals being injected with suspensions of the meningococci. The serum is used by injecting it into the spinal canal through a lumbar puncture. The precaution must be taken to permit some of the fluid to escape first, and then replace it by the antiserum, of which not more than 30 cc. must be injected. Several such injections should be made. Tabulations of the results following the employment of Flexner's serum show a large percentage of recoveries.

* "Journal of Medical Research," 1909, XX, 377.
† Loc. cit.
‡ "Jour. Experimental Medicine," 1907, IX, p. 168, and 1908, X, p. 141.

CHAPTER VIII

GONORRHEA

Micrococcus Gonorrhœæ (Neisser)

General Characteristics.—A minute, biscuit-shaped, non-motile, non-sporogenous, non-liquefying, non-chromogenic, non-flagellate, aërobic, strictly parasitic coccus, not stained by Gram's method, cultivable upon special media, and pathogenic for man only.

All authorities now accept the "gonococcus" as the specific cause of gonorrhea. It was first observed in the urethral and conjunctival secretions of gonorrhea and purulent ophthalmia by Neisser* in 1879.

Bumm† found other cocci closely resembling the gonococcus in the inflamed urethra, and points out that neither its shape nor its position in the cells can be regarded as characteristic, but that failure to stain by Gram's method can alone enable us to say with certainty that biscuit-shaped cocci found in urethral pus are gonococci.

Distribution.—The gonococcus is a purely parasitic pathogenic organism. It can be found in the urethral discharges of gonorrhea from the beginning until the end of the disease, and often for many months and even years after recovery from it. After the period of creamy pus has passed, its numbers are usually outweighed by other pyogenic organisms. Wertheim‡ cultivated the gonococcus from a case of chronic urethritis of two years' standing, and proved its virulence by producing experimental gonorrhea in a human being. The organisms are chiefly found within the pus-cells or attached to the surface of epithelial cells, and should always be sought for as diagnostic of gonorrhea, as purulent urethritis is sometimes caused by other organisms, as Bacillus coli communis§ and Staphylococcus pyogenes.

Morphology.—The organisms occur in pairs. Each pair of young cocci is composed of two spherical organisms, but as they grow older the inner surfaces become flattened and separated from one another by a narrow interval, so that they somewhat resemble a coffee-bean. A pair of the cocci resembles the German biscuit, and is described by the Germans as *semmelformig*.

* "Centralbl. f. d. med. Wissenschaft," 1879, No. 28.

† "Der Mikroorganismus der gonorrhoischen Schleimhauterkrankungen," "Gonococcus Neisser," second edition, 1887.

‡ "Archiv f. Gynäkologie," 1892, Bd. XLII, Heft 1.

§ Van der Pluyn and Loag, "Centralbl. f. Bakt. u. Parasitenk.," Feb. 28, 1895, Bd. XVII, Nos. 7, 8, p. 233.

The gonococci are small, the length of one of the coffee-bean cocci being about 1.6 μ, its breadth about 0.8 μ. They are not motile, nor provided with flagella, and are without spores.

Quite as characteristic as the form of the organism is its relation to the cells. In most of the inflammatory exudates the gonococci are contained either in epithelial cells or in leukocytes, very few of them lying free. This intracellular position is supposed to depend upon active phagocytosis of the cocci by the cells. It may not obtain in old lesions.

Staining.—They stain readily with all the aqueous solutions of the anilin dyes—best with rather weak solutions, but not by Gram's method.

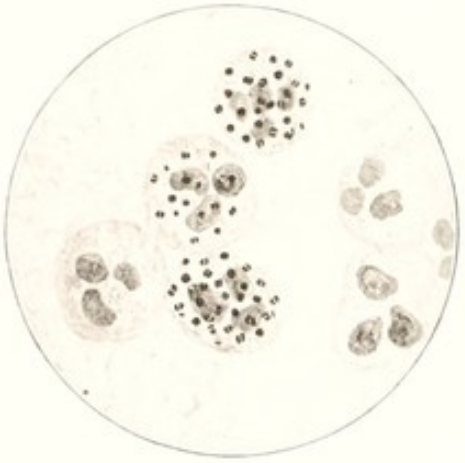


Fig. 143.—Gonococci in urethral pus.

The organisms contained in pus can be beautifully shown by first treating the prepared film with alcoholic eosin, and then with Löffler's alkaline methylene blue. A *differential color test* can be made by staining the film by Gram's method and then with aqueous Bismarck brown, or, what may be still better, with 3 per cent. aqueous solution of *pyronin*. Ordinary pus cocci, taking the Gram's stain, appear blue-black; the gonococci, taking the counter-stain, are brown in the former, purplish red in the latter case.

Isolation and Cultivation.—The organism does not grow upon any of the ordinary culture-media, and grows very scantily upon any artificial medium. Wertheim* succeeded in cultivating it by diluting a drop of gonorrheal pus with *human blood-serum*, mixing this with an equal part of melted 2 per cent. agar-agar at 40°C., and pouring the mixture into Petri dishes, which, as soon as the medium became firm, were stood in the incubator at 37°C. or, preferably, 40°C. In twenty-four hours the colonies could be observed. Those upon the surface showed a dark center, surrounded by a delicate granular zone.

* "Archiv. für Gynäkologie," 1892.

Young* had excellent success with a hydrocele-agar prepared as follows:

"The fluid (hydrocele or ascitic) is obtained sterile, the locality of the puncture being carefully sterilized by modern surgical methods, the sterile trocar covered at its external end with sterilized gauze so as not to be infected by the operator's hand, and the fluid collected in sterile flasks, the sterile stoppers being then replaced. Collecting the fluid in this way we have very rarely had it contaminated, often keeping it several months before using it. The fluid is mixed with ordinary nutrient agar. A number of common slants are put in the autoclave for five minutes. This liquefies the agar and at the same time thoroughly sterilizes the tubes and cotton stoppers. The slants are then put in a water-bath at 55°C. so as not to coagulate the albumin when mixed with the agar. The stopper having been removed from a small flask of hydrocele fluid, the top of the flask is flamed and the albuminous fluid is then poured into an agar tube (the top of which has also been flamed) in proportions a little more than one to two." The medium can be allowed to solidify in tubes or can be poured into Petri dishes.

When one of the colonies was transferred to a tube of human blood-serum, or of one of the above-described mixtures obliquely coagulated, isolated little gray colonies occur, later becoming confluent and producing a delicate smeary layer upon the medium. The main growth is surrounded by a thin, veil-like extension which gradually fades away at the edges. A slight growth occurs in the water of condensation.

Heiman† found that the gonococcus grows best in a mixture of 1 part of pleuritic fluid and 2 parts of 2 per cent. agar. Wright‡ prefers a mixture of urine, blood-serum, peptone, and agar-agar.

Wassermann§ used a mixture of 15 cc. of pig-serum, 35 cc. of water, 3 cc. of glycerin, and 2 per cent. of nutrose. The nutrose is dissolved by boiling and the solution sterilized. This is then added to agar, in equal parts, and used in plates.‖

Laitinen** found agar-agar mixed with one-third to one-half its volume of cyst or ascitic fluid, and bouillon containing 1 per cent. of peptone and 0.5 per cent. of sodium chlorid, mixed with one-third to one-half its volume of cyst or ascitic fluid, very satisfactory. The gonococcus could be kept alive upon these media for two months. Laitinen found that the gonococcus produces acids in the early days of its development, and alkalies subsequently. He was unable to isolate any toxin from the cultures.

Vital Resistance.—Authorities agree that the gonococcus has very slight power of heat endurance. Wertheim found the optimum temperature of cultivation to be 39° to 40°C., and saw no harm result from exposure to 42°C. It is killed in a few minutes at 55°C. The gonococci, though not easily cultivated, are said to resist unfavorable conditions, especially drying, very well. Kratter was

* "Contributions to the Science of Medicine by the Pupils of William M. Welch," Baltimore, 1900, p. 677.

† "Medical Record," Dec. 19, 1886.

‡ "Amer. Jour. Med. Sci.," Feb., 1895.

§ "Berliner klin. Wochenschrift," 1897.

‖ See "Text-Book of Bacteriology," by Hiss and Zinsser, 1910, p. 383.

** "Centralbl. f. Bakt. u. Parasitenk.," June 1, 1898, vol. XII, No. 20, p. 874.

able to demonstrate their presence upon washed clothing after six months, and found that they still stained well. This may not mean that the organisms were still alive.

In artificial culture the gonococcus soon dies, though cultures from different sources differ considerably in this regard. As a rule they survive but a few transplantations, though Young found that one culture had been kept alive by students in his laboratory for more than three months.

Diagnosis.—The diagnosis of gonorrhea by finding the diplococci in urethral pus and epithelial cells is a very simple matter. The recognition of the micro-organisms under other conditions is by no means easy. Thus, when gonorrhea becomes chronic and the cocci are no longer taken up by the phagocytes, it raises a little doubt whether Gram-negative cocci may be true gonococci or not, yet it is at precisely this time when a patient getting over gleet and wanting to marry desires to know definitely whether gonococci are any longer present in his urethra or not. Again, when the gonococcus-like organisms occur upon the conjunctiva, in the pus taken from joints, upon the valves of the heart, or in the Fallopian tubes, the same difficulty is met. Probably the greatest perplexity arises when the conjunctiva is called in question, for here there can come about a confusion of the gonococcus, the pneumococcus, and Micrococcus catarrhalis (*q.v.*) which only careful staining and culture experiments can solve. The pneumococcus may be readily separated if its lanceolate form and capsules can be observed, but it is only by seeing that Micrococcus catarrhalis grows readily and luxuriantly upon all the laboratory media, and the gonococcus with difficulty and very sparingly upon any media, that the diagnosis can be made with certainty.

The method of diagnosis by staining and looking for Gram-negative diplococci in the cells is only a "rough and ready" one and is not dependable.

The method of *complement fixation* is probably the court of final resort, but this test is attended with considerable technical difficulty.

Toxic Products.—The toxic metabolic products of the gonococcus appear to be contained within the bodies of the bacteria and disseminated but slightly throughout the culture-media. Christmas,* Nicolaysen,† and Wassermann‡ have studied *gonotoxin*, and have all found that it remains in the bodies of the bacteria. The toxin seems to be quite stable and is not destroyed by temperatures fatal to the cocci. Wassermann obtained some cultures of which 0.1 cc. would kill mice; others, of which 1.0 cc. was required. The poison can be precipitated with absolute alcohol. Small quantities of the toxin introduced into the urethra cause suppuration at the

* "Ann. de l'Inst. Pasteur," 1897.

† "Centralbl. f. Bakt. u. Parasitenk.," 1897, Bd. XXII, Nos. 12 and 13, p. 305.

‡ "Zeitschrift für Hygiene," 1898, and "Berliner klin. Wochenschrift," 1897, No. 32, p. 685.

point of application, fever, swelling of the neighboring lymphatic nodes, and muscular and articular pains.

Pathogenesis.—It is generally believed that gonorrhea cannot be communicated to animals.

There is no doubt but that the gonococcus causes gonorrhea, as it has on several occasions been intentionally and experimentally inoculated into the human urethra with resulting typical disease. It is constantly present in the disease, and very frequently in its sequelæ, though it not infrequently happens that the lesions secondary to gonorrhea are caused by the more common organisms of suppuration that have entered through the surface denudations caused by the gonococcus.

Opinions differ as to whether the gonococci can, with equal facility, penetrate squamous and columnar epithelium. Their attacks are usually made upon surfaces covered with squamous epithelium.

The injection of gonococci into the subcutaneous tissue is not followed by either abscess formation or septic infection.

Gonococci rarely enter the circulation of human beings and occasion a peculiar septic condition with irregular temperature, apt to be followed by invasion of the cardiac valves, joints, or other tissues. P. Kraus* has twice succeeded in cultivating the gonococcus from the blood of patients in the stage of septic infection.

The deep lesions caused by the gonococcus are, however, numerous, and in Young's paper (*loc. cit.*) its widespread powers of pyogenic infection are well shown in a collection of the cases recorded in the literature, and some original observations showing the undoubted occurrence of the gonococcus in gonorrhea, ophthalmia neonatorum, arthritis, tendosynovitis, perichondritis, subcutaneous abscess, intramuscular abscess, salpingitis, pelvic peritonitis, adenitis, pleuritis, endocarditis, septicemia, acute cystitis, chronic cystitis, pyonephrosis, and diffuse peritonitis.

In the beginning of the inflammatory process the cocci grow in the superficial epithelial cells, but soon penetrate between the cells to the deeper layers, where they continue to keep up the irritation as the superficial cells desquamate.

All urethral inflammations, and in gonorrhea all of the inflammatory symptoms, do not depend upon the gonococcus. The periurethral abscess, salpingitis, etc., not infrequently depend upon ordinary pus cocci, and the author has seen a case of gonorrhea with double orchitis, general septic infection, and endocarditis in which the gonococci had no rôle in the sepsis, which was caused by a large coccus that stained beautifully by Gram's method.

In the remote secondary inflammations the gonococci disappear after a time, and the inflammation either subsides or is maintained

* "Berliner klin. Wochenschrift," May 9, 1904, No. 19, p. 494.

by other bacteria. In synovitis, however, the inflammation excited may last for months.

So long as the gonococci persist in his urethra or other superficial tissues the patient may spread the contagion, and after apparent recovery from gonorrhea the cocci may remain latent in the urethra for years, not infrequently causing a relapse if the patient partake of some substance, as alcohol, irritating to the mucous membranes. Bearing this in mind, physicians should be careful that their patients are not too soon discharged as cured and permitted to marry.

Immunization against the gonococcus has not yet been successfully achieved. Wassermann failed altogether; Christmas claims to have immunized goats, but the serum of these animals could not be shown to contain any antitoxin or to be bacteriolytic.

Torrey* prepared an antigonococcus serum by immunizing rabbits with gonotoxin. The culture used was isolated from a case of acute gonorrhea in a medium of rich ascitic fluid and slightly acid beef infusion, peptone broth, equal parts. In speaking about this mixture Dr. Torrey said that the exact reaction was its most important feature, as otherwise the gonococci soon died. Tubes of about 12 cm. of the mixture were heated to about 60°C. for several hours and then tested for sterility. The cocci were cultivated at 36° to 37°C. After eighteen to twenty-four hours' incubation a slight granular growth appears near the surface and on the sides of the tube. This slowly increases until after six days the medium is well clouded on shaking. Large rabbits were used for making the serum, and were intraperitoneally inoculated with 10 cc. of an entire culture. The first inoculation resulted in a loss of weight, sometimes amounting to one-fourth of the body-weight. After an interval of five or six days a second injection is given, then after a similar interval, a third, and so on. The best results were obtained when cultures from six to fifteen days old were employed. The rabbits were bled for the first time after the sixth dose, as if the treatment be pushed they soon fall into a state of cachexia, rapidly emaciate, and die. Each animal furnishes 70 to 90 cm. of the serum, which was inclosed in 2-cm. bulbs, hermetically sealed, and kept without any preservative.

With serum made in this way by Torrey, Rogers† treated a number of obstinate cases of gonorrheal rheumatism, with apparently good results.

Good results in gonorrheal arthritis and in gleet are also claimed for treatment with gonococco-vaccines.

* "Journal Amer. Med. Assoc.," Jan. 27, 1906, XLVI, p. 261.
† "Jour. Amer. Med. Assoc., Jan. 27, 1609," XLVI, p. 261.

CHAPTER IX

CATARRHAL INFLAMMATION

MICROCOCCUS CATARRHALIS (SEIFERT)

General Characteristics.—A small, slightly ovoid, non-motile, non-sporulating, non-flagellated, non-liquefying aërobic and optionally anaërobic, non-chromogenic coccus, pathogenic for man, and not for the lower animals, cultivable upon the ordinary media, staining by the ordinary methods, but not by Gram's method.

This micro-organism, which seems to be closely related to the staphylococci, was first observed, in sections of the lung of a case of influenza, by Seifert.* It was successfully cultivated in 1890 by Kirchner† from 10 cases of an influenza-like affection. It has since been frequently demonstrated in the exudates from various in-

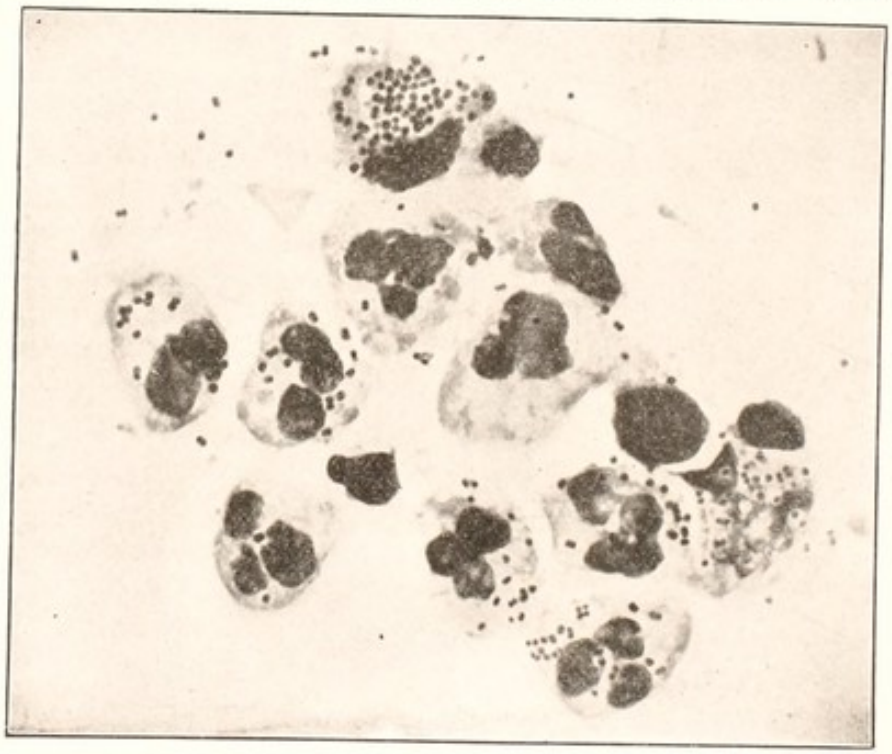


Fig. 144.—Micrococcus catarrhalis in smear from sputum (F. T. Lord; photo by L. S. Brown).

flammatory conditions of the respiratory tract and conjunctiva, and seems to be a not uncommon organism of superficial inflammations. It is a rather troublesome organism, causing some confusion because of its disposition to occur in pairs, which gives it a close resemblance to the pneumococcus except in cases in which the cap-

* "Volkmann's klin. Vortr.," Nr. 240.
† "Zeitschr. f. Hyg.," Bd. 9.

sules of the latter are distinct. It is also readily taken up by the leukocytes, and may so resemble the gonococcus; and it is not always easy, perhaps not always possible, to distinguish it from the Diplococcus intracellularis meningitidis.

Morphology.—The organism is spheric or slightly ovoid, may occur singly, though usually appears in pairs or clusters. Large numbers are enclosed in the leukocytes or other cells. The spheric organisms have a diameter of about 1 μ; the ovoid organisms may measure as much as 1.5 by 2 μ. The relation of the cocci to the cells seems to have something to do with the course of the inflammatory conditions with which they are associated. During the activity of the process large numbers of the cocci may be free; toward its close they may all be enclosed in the leukocytes.

The organisms are not motile and they have no flagella.

Staining.—The cocci stain by ordinary methods, but not by Gram's method.

Cultivation.—The organism can be easily cultivated, and thus differentiates itself from the fastidious gonococcus. The colonies are large, white, irregular in outline, elevated at the center, not viscid, and grow readily at room temperatures upon all the culture media, the best upon blood agar-agar. The vitality of the organism in culture is not great. Very often transplantation made after from four to six days fail to grow; and in the cultures one usually finds many deeply staining, supposedly living cocci, and many poorly staining, supposedly dead organisms.

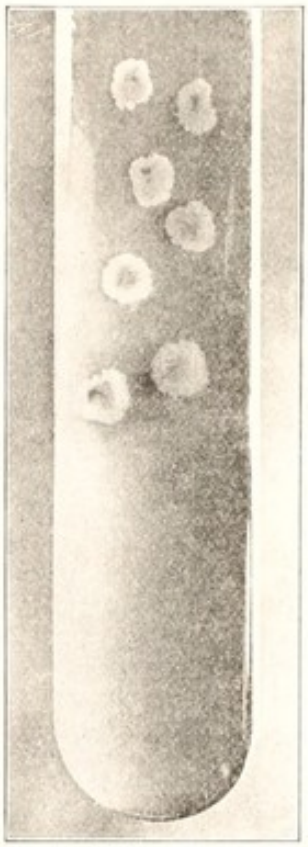


Fig. 145.—Micrococcus catarrhalis colonies on agar (F. T. Lord; photo by L. S. Brown).

Agar-agar.—The culture in general resembles that of Staphylococcus albus. When blood is added to the agar-agar, the growth is more luxuriant, whitish, and usually consists of closely approximated colonies which do not become confluent.

Gelatin.—This medium is not liquefied.

Bouillon.—At the end of the first day no growth seems to have taken place, but at the end of the second day there is a slight clouding and a meager precipitate. The organism seems to maintain its vitality somewhat longer in bouillon than in other culture-media.

Pathogenesis.—The organism seems to be scarcely pathogenic for animals. Kirchner was able to kill a guinea-pig by intrapleural injection, and Neisser, who performed numerous experiments upon mice, guinea-pigs, and rabbits, only once succeeded in producing a

fatal infection, by the intraperitioneal injection of 0.4 cc. of bouillon culture. In this animal the cocci were found in all the internal organs. As has already been said, the organism is found associated with superficial inflammatory conditions of the mucous membrane. It is probably most common in influenza. It has also been found in conjunctivitis, in bronchitis, in whooping-cough, and in pneumonia.

CHAPTER X

CHANCROID

The Bacillus Ducreyi

General Characteristics.—A small, ovoid streptobacillus, with rounded, deeply staining ends, non-motile, non-flagellate, non-sporogenous; aërobic and optionally anaërobic, non-chromogenic, staining by ordinary methods, but not by Gram's method, cultivable on special media only and pathogenic only for man and certain monkeys.

The chancroid, soft chancre, or non-specific sore, as it is called, is a common venereal affection of both sexes, most frequent among those who give little attention to cleanliness. It is characterized by the appearance of a soft reddish papule, which makes its appearance usually upon the genital organs, rarely upon other parts of the body, soon after the infection, and soon becomes transformed to an ugly ulceration whose usual tendency is toward slow and persistent enlargement, though in different cases it may be indolent, active, phagedenic, or serpiginous. The inguinal or other nearby lymph-nodes early enlarge and soon soften and ulcerate. The disease is, therefore, extremely destructive to the tissues invaded, though no constitutional involvement ever takes place.

Specific Organism.—In 1889 Ducrey* described a peculiar organism whose presence he was able to demonstrate with great constancy, sometimes in pure culture, in the lesions of chancroid, and which he believed to be the specific organism of the affection. Unna† later described an organism resembling that of Ducrey, and the later observations of Krefting,‡ Peterson,§ Nicolle,‖ Cheinisse,** and Davis†† have abundantly confirmed the observations of Ducrey and Unna, and proved the identity of the two micro-organisms and their specificity for the disease.

Morphology.—The organism is commonly described as a "streptobacillus." It is very small, short, and ovoid in shape, and occurs habitually in longer or shorter chains. Each organism measures about $1.5 \times 0.5\ \mu$. The ends are rounded and stain deeply. In pure cultures long undivided filaments, at least twenty times as long as the individual bacilli, are not uncommon. There seems to be

* "Congrès. Inter. de Dermatol. et de Syphilog.," Paris, 1889; "Compt. rendu," p. 229.

† "Monatschr. f. praktische Dermatologie," 1892, Bd. XIV, p. 485.

‡ "Archiv. f. Dermatol. u. Syphilol.," 1897, p. 263; 1897, p. 41.

§ "Centralbl. f. Bakt.," etc., 1893, XIII, p. 743.

‖ "Med. Moderne," Paris, 1893, IV, p. 735.

** "Ann. de Dermat. et de Syphil.," Par., 1894, p. 272.

†† "Jour. Med. Research," 1893, IX, p. 401.

no relation between the cells and the bacilli. As a rule, they are free, sometimes they are inclosed in leukocytes. The bacilli are not motile, have no flagella and do not form spores.

Staining.—The organisms are somewhat difficult to stain, as they do not retain the color well, giving it up quickly when washed. They do not stain by Gram's method.

Cultivation.—The first successful isolation and cultivation of the organism seems to have been by Benzançon, Griffon and Le Sours* upon a culture-medium consisting of rabbits' blood 1 part, and agar-agar 2 parts. Davis† has been equally successful in cultivating the organism upon this medium. His method was as follows:

"Tubes of 2 per cent. agar, reaction + 1.5, were melted and mixed with fresh rabbits' blood drawn under aseptic precautions,

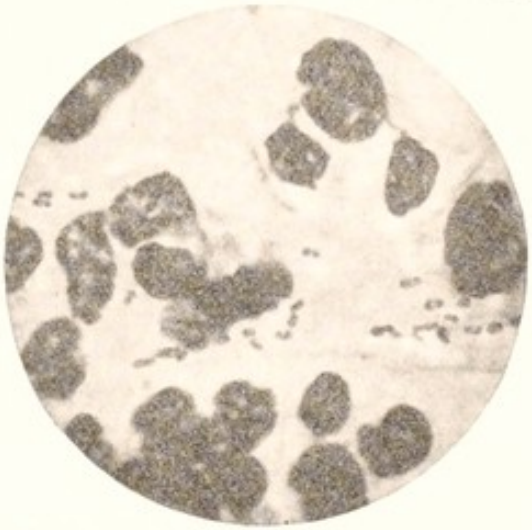


Fig. 146.—Smear of pus of chancroid of penis stained with carbol-fuchsin and briefly decolorized by alcohol. × 1500 (Davis). (Photomicrograph by Mr. L. S. Brown.)

in the proportion of two-thirds agar to one-third blood, and slanted while in a fluid state. At a later period tubes of rabbits' blood-serum uncoagulated, also rabbits' blood bouillon, one-third blood to two-thirds bouillon, were used, and gave equally satisfactory results. By employing small tubes of freshly drawn human blood pure cultures were obtained in several instances from genital lesions, direct, without any special cleansing of the ulcerated surface. This, I believe, is the best medium for obtaining cultures from a source open to contamination, the fresh blood apparently inhibiting to a certain extent the growth of extraneous organisms."

No growth takes place upon ordinary culture-media under either aërobic or anaërobic conditions.

Cultures are best obtained by puncturing an unopened bubo with a sterile needle and planting the pus directly and immediately upon the special medium which should have been warmed in the incubator

* "Ann. de Dermat. et de Syphilog.," 1901, II, p. 1.
† Loc. cit.

so that the pus is not chilled. In this way pure cultures which are difficult to get from the soft sore itself, may be secured.

Colonies.—The colonies appear upon the appropriate media in about twenty-four hours, and attain their complete development in about forty-eight hours. They are at first round bright globules, and later become grayish and opaque. They measure 1 to 2 mm. in diameter and never become confluent. They are difficult to pick up with the platinum wire, tending to slide over the smooth surface of the medium.

Fig. 147.—Culture from ulceration on monkey resulting from inoculation of culture from a case of chancroid of finger, first generation. Stained with carbol-fuchsin and briefly decolorized by alcohol. Culture of twenty-four hours' growth in rabbit's bouillon. × 1500 (Davis). (Photomicrograph by Mr. L. S. Brown.)

Vital Resistance.—The organisms seem to possess little vitality, their life in artificial culture being limited to a few days. Frequent transplantation enabled Davis to carry them on to the eleventh culturai generation.

Pathogenesis.—The organism is pathogenic for man and certain monkeys (macacus), but not for the ordinary laboratory animals. The organism can be found in large numbers in both the genital and extragenital chancroidal lesions, and usually in small numbers in the pus from chancroidal buboes. It has not been encountered elsewhere. Lenglet* isolated the organism in pure culture, and by inoculation with his cultures reproduced the lesions in man.

* "Bull. Med." 1898, p. 1051; "Ann. de Dermatol. et de Syph.," 1901, II, p. 209.

CHAPTER XI

ACUTE CONTAGIOUS CONJUNCTIVITIS

The Koch-Weeks Bacillus

General Characteristics.—A minute, slender bacillus, non-motile, non-flagellated, non-sporogenous, non-liquefying, non-chromogenic, aërobic, and optionally anaërobic, staining by the ordinary methods but not by Gram's method, susceptible of cultivation upon special media only, and specific for acute contagious conjunctivitis.

Acute contagious conjunctivitis is a common and world-wide affection, sometimes called "pink eye," and sometimes erroneously called catarrhal conjunctivitis. All its characteristics, and especially its contagiousness, point to its being a specific disease due to a specific cause, and thus entirely different from ordinary non-specific catarrh.

Specific Micro-organism.—The first bacteriologic investigation of acute contagious conjunctivitis was made by Robert Koch,* when in Egypt investigating a cholera epidemic. While in Alexandria he examined the secretions from 50 cases of conjunctivitis, finding the gonococcus, or an organism closely resembling it. In a less severe form of the disease, however, he found a peculiar small bacillus. He seemed satisfied with this observation, or had no time to pursue the matter farther, for no cultivation or other experiments are mentioned.

The organism was observed from time to time, but no serious consideration seems to have been devoted to it until Weeks† published an account of what seemed to be the identical organism, which he not only observed, but also cultivated, and eventually successfully inoculated into the human conjunctiva. In the same year Kartulis‡ in Alexandria succeeded in cultivating the same organism. In 1894 Morax published a brochure in Paris in which he says that "the disease [which he describes under the name of acute conjunctivitis] is characterized by the constant presence in the conjunctival secretions of a small bacillus seen for the first time by Koch, but studied some years later by Weeks, and now known as the bacillus of Weeks."

Further descriptive and clinical information can be found in a paper by Weeks, "The Status of our Knowledge of the Ætiological Factor in Acute Contagious Conjunctivitis."§

* "Wiener klin. Wochenschrift," 1883, p. 1550.
† "N. Y. Med. Record," May 21, 1887.
‡ "Centralbl. f. Bakt. u. Parasitenk.," 1887, p. 289.
§ "New York Eye and Ear Infirmary Reports," Jan., 1895, vol. III, Part. 1, p. 24.

Morphology.—The organism is very tiny and is said to bear some resemblance to the bacillus of mouse-septicemia. It measures 1 to 2 × 0.25 μ (Weeks). The length is more constant in individuals found in the pus than those taken from cultures. In cultures the organisms are longer and more slender. Involution forms of considerable length and of irregular shape also occur. No spores are observed. The organism has no flagella and is not motile.

Staining.—Weeks found that the organism stained well with watery solutions of methylene blue, basic fuchsin, or gentian violet. The color is fainter than that of the nuclei of the associated pus-corpuscles, and is much less intense in old than in fresh cultures. It is readily given up when treated with alcohol or acids. Morax found that the bacilli did not retain the color in Gram's method.

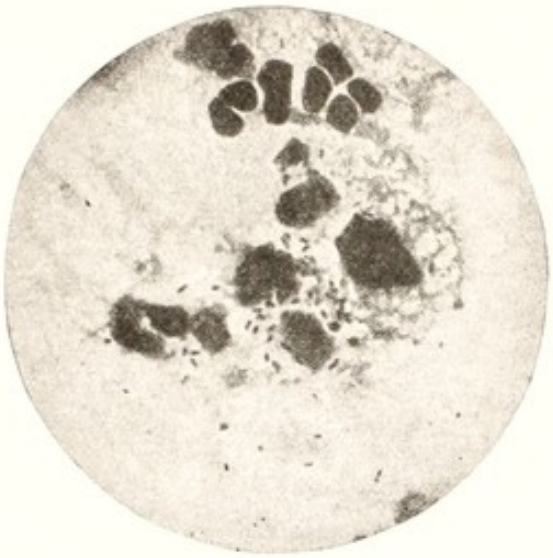


Fig. 148.—The Koch-Weeks bacillus in conjunctival secretion. Magnified 1000 diameters (Rymowitsch and Matschinsky).

Cultivation.—The organism refuses to grow upon any of the ordinary culture-media. Weeks found, however, that if the percentage of agar-agar used was reduced to 0.5 per cent., growths could be secured by incubation at 37°C., and successful transplantations carried on to the sixteenth generation. Abundant moisture was essential. The method of isolation adopted by Weeks was as follows:

"The conjunctival sacs were thoroughly washed with clean water, removing the secretion present by means of absorbent cotton. The patient was then directed to keep the eyes closed. After five or ten minutes had elapsed, the eyes were opened, and the secretion that had formed, lying at the bottom of the cul-de-sac, was removed by means of a sterilized platinum rod and transferred to the surface of the agar. The mass of tenacious secretion was drawn over the surface of the agar and left there, the platinum being thrust into the agar two or three times before removal."

At the end of forty-eight hours a slight haziness appears along the path of the wire, and on the surface of the agar a very small

patch is noticeable; this is of a pearly color and possesses a glistening surface. By the formation of small concentric colonies the growth extends for a short distance. At the end of the fourth or fifth day the growth ceases to advance; it is never abundant. The culture dies in from one to three weeks.

Pathogenesis.—Both Weeks and Morax have tested the organism for pathogenic activity, and in every case in which pure cultures of it were placed upon the human conjunctiva, typical attacks of the acute conjunctivitis resulted. The organism fails to infect any of the lower animals.

Association.—Both Weeks and Morax found the organism in intimate association with a larger club-shaped bacillus, which was regarded as the pseudo-diphtheria bacillus. It seems to be of no pathogenic significance.

The Morax-Axenfeld Bacillus

In 1896 Morax* found a new bacillus in certain cases of epidemic subacute conjunctivitis. Immediately afterward Axenfeld* presented to a congress in Heidelberg cultures of the same bacillus that he had isolated from 51 cases of what he called "Diplobacillen-conjunctivitis" that occurred a few months before as an epidemic in Marburg. De Schweinitz and Veasy,‡ Alt§ and others found the same diplobacillus in America, and many others confirmed the observations in various parts of Europe. It has also been found in Egypt. There is no doubt, therefore, but that this is a widely distributed organism. Morax produced the disease by placing a pure culture of the organism upon the human conjunctiva. He was unable to infect any of the lower animals.

In this subacute form of conjunctivitis there is very little secretion, and to secure the micro-organism either for microscopic examination or for cultivation recourse must be had to minute flakes of grayish mucus that collect upon the caruncle.

Morphology.—The bacillus is small, commonly occurs in pairs or chains. It measures approximately 2 μ in length. It is not motile, has no flagella, and forms no spores. It is somewhat pleomorphous. Involution forms soon appear in artificial cultures.

Staining.—The organism stains by ordinary methods, but does not stain by Gram's method.

Cultivation.—The organism grows only upon alkaline blood-serum or upon culture-media containing blood-serum. Morax made his original observation by using Löffler's blood-serum mixture. The colonies appear in twenty-four hours at 37°C. The blood-

* "Ann. de l'Inst. Pasteur," June, 1896; "Ann. d'Oculist," Jan., 1897.
† "Heidelberg Congress," 1896; "Centralbl. f. Bakt.," etc., 1897, XXI.
‡ "Ophthalmological Record," 1899.
§ "Amer. Jour. of Ophthalmology," 1898, p. 171.

serum is almost immediately liquefied, so that the growing colonies appear to be sinking into the medium after thirty-six hours. The entire tube of medium may eventually be liquefied.

Upon agar-agar containing serum, grayish-white colonies of small size, resembling colonies of gonococci, are formed. Growth is slow. Bouillon is slowly clouded.

Pathogenesis.—The pathogenic and specific nature of the diplobacillus was made clear by Morax, who produced the disease in man by placing a pure culture upon the human conjunctiva.

Zur Nedden's Bacillus

This bacillus was the only organism that Haupf* was able to isolate from a neuroparalytic with confluent peripheral ulcera-

Fig. 149.—The Morax-Axenfeld diplobacillus of conjunctivitis. Magnified 1000 diameters (Rymowitsch and Matschinsky).

tions of the cornea. It seemed to be identical with an organism that zur Nedden had found previously in a case of corneal ulceration in the clinic at Bonn.

Morphology.—It is a tiny bacillus, less than 1 μ in length, slightly curved, generally single, but sometimes in pairs and short chains. It is not motile, has no flagella, forms no spores.

Staining.—It stains ordinarily, but not by Gram's method.

Cultivation.—It is easily cultivated upon the ordinary laboratory media, the cultures being without characteristic peculiarities. Gelatin is not liquefied. Milk is coagulated. Acid but no gas is formed in glucose media. A thick yellowish growth appears upon potato. No indol is formed.

Pathogenesis.—Corneal ulcers were formed in a guinea-pig after artificial implantation in the corneal tissue.

* "Inaugural Dissertation," Bonn, 1902.

Miscellaneous Organisms in Conjunctivitis

In addition to the foregoing organisms, others not infrequently make their appearance as excitants of conjunctivitis. The most frequent of these being the *pneumococcus*, the most dangerous, the *gonococcus*. The former produce a severe conjunctivitis, with the formation of a false membrane, the latter the well-known blenorrhea and ophthalmia neonatorum. *Streptococci, diphtheria bacilli, staphylococci, meningococci, colon bacilli, Bacillus pneumoniæ* (Friedländer), and other organisms have been found and appear to be responsible for conjunctivitis

CHAPTER XII

DIPHTHERIA

Bacillus Diphtheriæ (Klebs-Löffler)

General Characteristics.—A non-motile, non-flagellate, non-sporogenous, non-chromogenic, non-liquefying, aërobic, purely parasitic, pathogenic, toxicogenic bacillus, cultivable upon the ordinary culture media, staining by the ordinary methods and by Gram's method.

In 1883 Klebs* demonstrated the presence of a bacillus in the pseudo-membranes upon the fauces of patients suffering from diphtheria, but it was not until 1884 that Löffler† succeeded in isolating and cultivating it. The organism is now known by both their names, and called the Klebs-Löffler bacillus.

Morphology.—The bacillus is about the length of the tubercle bacillus (1.5–6.5 μ), but about twice its diameter (0.4–1.0 μ), has a slight curve similar to that which characterizes the tubercle bacillus, and has rounded and usually clubbed ends. It does not form chains, though two, three, and rarely four individuals may be found conjoined; usually the individuals are separate from one another. The bacillus has no flagella, it is non-motile, and does not form spores. Distinct polar granules can be defined at the ends of the bacilli. Occasional branched forms are observed, though Abbott and Gildersleeve‡ do not regard branching as a phase of the normal development of the organism and do not find it common upon the standard culture media. The bacillus is peculiar in its pleomorphism, for among the well-formed individuals which abound in fresh cultures a large number of peculiar organisms are to be found, much larger than normal, some with one end enlarged and club shaped, some greatly elongated, with both ends similarly and irregularly expanded. These probably represent an involution form of the organism, for they are present in perfectly fresh cultures.

The involution of the diphtheria bacillus seems to occur in proportion to the rapidity of its growth. Upon Löffler's serum mixture, which seems best adapted for its cultivation, the involution of the organism takes place with great rapidity, so that large clubbed organisms and large organisms with polar granules are very common. On the other hand, upon agar and glycerin agar-agar, where the organism grows very slowly, it usually appears in the form of short spindle and lancet shapes. So different are these forms that

* "Verhandlungen des Congresses für innere Med.," 1883.
† "Mittheilungen aus dem kaiserlichen Gesundheitsamte," 2.
‡ "Centralbl. f. Bakt.," etc., Dec. 18, 1903, Bd. XXXV, No. 3.

Fig. 150.—Bacillus diphtheriæ, five hours at 36° C. This shows only solid staining forms.

Fig. 151.—Bacillus diphtheriæ, same culture, eight hours at 36° C. This also shows solid forms, many of them with parallel arrangement.

Fig. 152.—Bacillus diphtheriæ, same culture, twelve hours at 36° C. The bacilli stain faintly at their ends, and in some small granules are seen at the tip of the faintly stained portions.

Fig. 153.—Bacillus diphtheriæ, same culture, fifteen hours at 36° C. The bacilli stain more unevenly and the granules are larger.

Fig. 154.—Bacillus diphtheriæ, same culture, twenty-four hours at 36° C. This shows clubbed and barred forms as well as granular forms. At the lower part of the field is a paired form which shows the characteristic clubbing of the distal ends.

Fig. 155.—Bacillus diphtheriæ, forty-eight hours at 36° C. This is the same bacillus as in the preceding figures, but from a culture where the colonies were discrete. It shows long filamentous forms.

(Photomicrographs by Mr. Louis Brown. The magnification is the same in all—× 2000. All of the preparations were made from growth on blood-serum.) (Francis P. Denny, in "Jour. of Med. Research.")

a beginner would certainly fail to recognize them as the same species. The small short forms also stain much more uniformly than the large club-shaped bacilli.

Staining.—The bacillus can readily be stained with aqueous solutions of the anilin colors, but more characteristically with Löffler's alkaline methylene blue:

Saturated alcoholic solution of methylene blue	30
1:10,000 aqueous solution of caustic potash	100

Emery prefers Manson's borax methylene blue. A stock solution which keeps well is prepared by dissolving 2 grams of methylene blue and 5 grams of borax in 100 cc. of water. This is diluted with from five to ten times its volume of water for ordinary use. An aqueous solution of dahlia is recommended by Roux.

The Neisser method of staining the diphtheria bacillus, which met with a very cordial reception, is as follows:

The prepared cover-glass is immersed for from two to three seconds in

Alcohol (96 per cent.)	20 parts
Methylene blue	1 part
Distilled water	950 parts
Acetic acid (glacial)	50 parts

Then for three to five seconds in

Bismarck brown	1 part
Boiling distilled water	500 parts

The true diphtheria bacilli appear brown, with a dark blue body at one or both ends; the pseudo-diphtheria bacilli usually exhibit no polar bodies.

Park* found that neither the Neisser nor the Roux stain gave any more information concerning the virulence of the bacilli than the Löffler alkaline methylene blue.

The bacilli stain well by Gram's method, which is excellent for their definition in sections of tissue, though Welch and Abbott found that Weigert's fibrin method and picrocarmin gave the most beautiful results.

Cultivation.—The diphtheria bacillus grows readily upon all the ordinary media, and is very easy to obtain in pure culture, plates not being necessary. It is purely aërobic.

Colonies.—Upon the surface of gelatin plates the colonies attain but a small size and appear to the naked eye as whitish points with smooth contents and regular, though sometimes indented, borders. Under the microscope they appear granular and yellowish-brown, with irregular borders. Upon agar-agar and glycerin agar-agar the colonies are slower to develop, larger, more translucent, without the yellowish-white or china-white color of the blood-serum cultures, and are more or less distinctly divided into a small elevated center

* "Bacteriology in Medicine and Surgery," 1900.

and a flat surrounding zone with indented edges, and a radiated appearance. The colonies that develop upon Löffler's blood-serum mixture are rounded, yellowish-white, good sized and more or less confluent when closely approximated. They are smooth, moist and shining on the surface. They are with difficulty differentiated from those of Bacillus hofmanni, the pseudo-diphtheria bacillus.

Gelatin.—The growth in gelatin puncture is scanty, not characteristic, and consists of small spheric colonies along the line of inoculation. The gelatin is not liquefied.

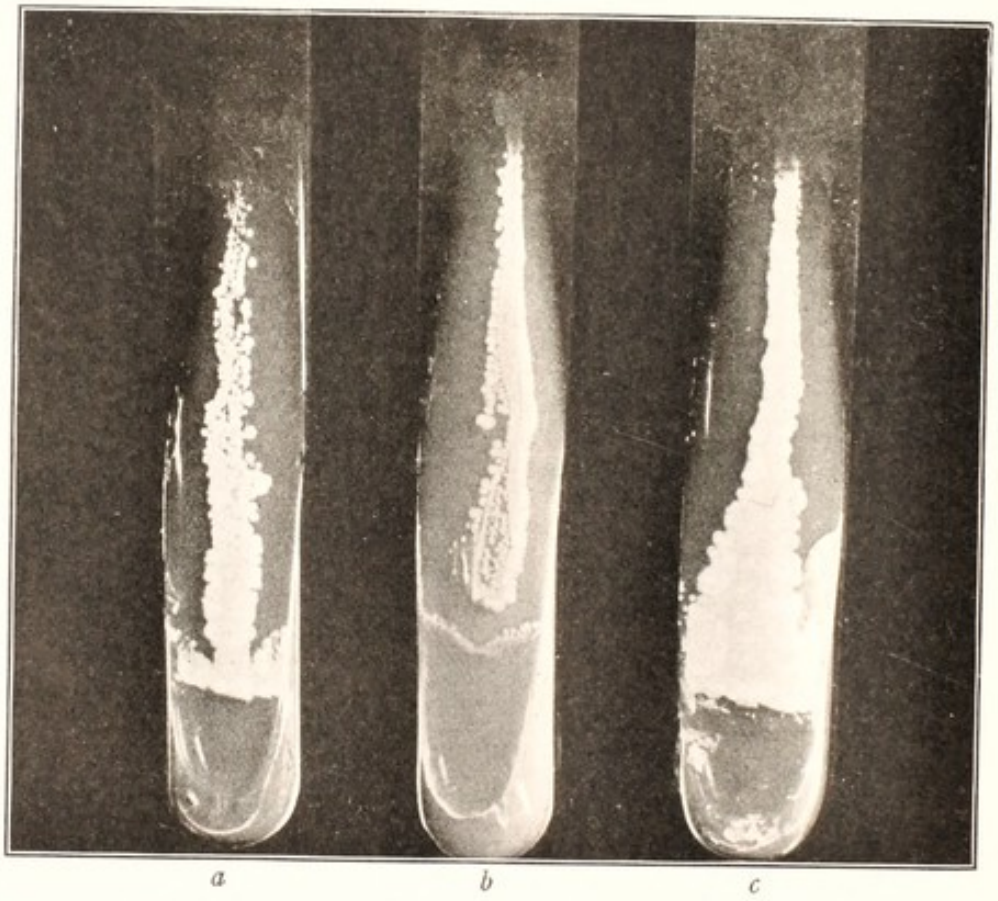


Fig. 156.—Diphtheria bacilli (from photographs taken by Prof. E. K. Dunham, Carnegie Laboratory, New York): *a*, Pseudobacillus; *b*, true bacillus; *c*, pseudobacillus.

Agar-agar.—Cultures upon the surface of agar-agar slants are usually meager when contracted with those upon Löffler's blood-serum mixture, and may be whitish in color. They consist of discrete and confluent whitish colonies devoid of differential qualities. The oftener the organism is transplanted to fresh agar-agar, the more luxuriant its growth becomes. The growth is rapid and luxuriant upon glycerin agar-agar.

Bouillon.—When planted in bouillon a distinct, whitish, granular pellicle forms upon the surface of the clear medium. The pellicle appears quite uniform when the tube or flask is undisturbed, but it is so brittle that it at once falls to pieces if disturbed, the minute

fragments slowly sedimenting and forming a miniature snow-storm in the flask or tube. The organism at times also causes a diffuse cloudiness of the medium, but, not being motile, soon settles to the bottom in the form of a flocculent precipitate which has a tendency to cling to the sides of the glass, and leave the bouillon clear.

No fermentation occurs in bouillon to which sugar is added, though acids are soon formed by which the growth is checked. If, however, the quantity of sugar be too small to check the growth, the acidity gives place to increasing alkalinity at a later period.

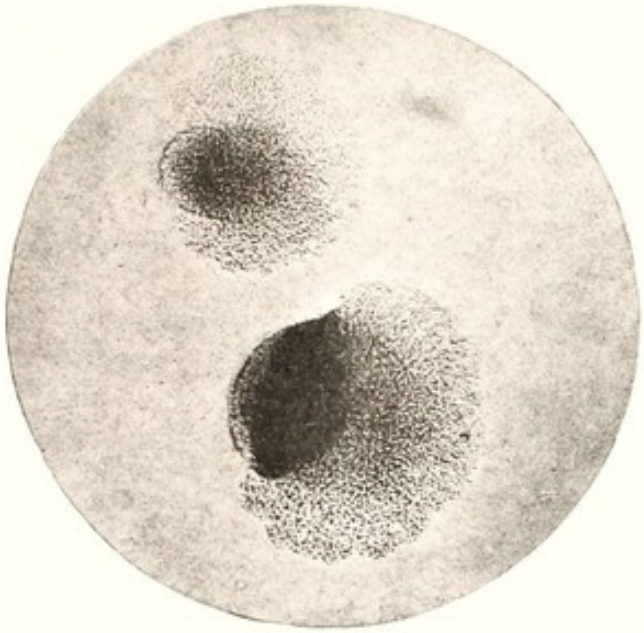


Fig. 157.—Bacillus diphtheriæ; colony twenty-four hours old, upon agar-agar ×100 (Fränkel and Pfeiffer).

Spronck* found that the characteristics of the growth of the diphtheria bacillus in bouillon, as well as the amount of toxin produced, vary according to the amount of glucose in the bouillon.

Zinno† found that digested brain added to the culture bouillon greatly facilitated the growth of diphtheria and tetanus bacilli and increased the toxin-production.

Blood-serum.—The bacillus grows similarly upon blood-serum and Löffler's mixture, but more luxuriously upon the latter, where large, creamy-white, discrete and confluent, moist, shining colonies form. The rapidity of the growth which is abundant in twenty-four hours, and the appearances presented are quite characteristic.

Löffler has shown that the addition of a small amount of glucose to the culture-medium increases the rapidity of growth, and suggests a special medium which bears his name—Löffler's blood-serum mixture:

Blood-serum	3
Ordinary bouillon + 1 per cent. of glucose	1

* "Ann. de l'Inst. Pasteur," Oct. 25, 1895, vol. IX, No. 10, p. 758.

† "Centralbl. f. Bakt.," Jan. 4, 1902, XXXI, No. 2, p. 42.

This mixture is filled into tubes, coagulated, and sterilized like blood-serum, and is one of the best known media to be used in connection with the study of diphtheria.

Material from the infected throat can be taken with a swab or platinum loop and spread upon the surface of several successive tubes of Löffler's blood-serum media. Upon the first a confluent growth of the bacillus usually occurs; but upon the third, scattered cream-white colonies suitable for transplantation can usually be found.

The studies of Michel* have shown that the development of the culture is much more luxuriant and rapid when horses' serum instead of beef or calves' serum is used.

Westbrook suggested that the addition of a small amount of glycerin to the preparation of blood-serum would prevent it from drying so rapidly as usual and would have the added advantage of preventing the growth of certain varieties of bacteria not desired. Dubois† carried out a series of observations upon this question and found that 3 to 5 per cent. of glycerin makes a very valuable addition, as the diphtheria bacilli grow very rapidly and almost in pure culture upon the blood-serum mixture to which it is added. The blood serum is not liquefied or otherwise visibly changed.

Potato.—Upon potato it develops only when the reaction is alkaline. The potato growth is not characteristic.

Milk.—Milk is an excellent medium for the cultivation of Bacillus diphtheriæ. The milk is not coagulated. Litmus milk is useful for detecting the changes of reaction brought about. Alkalinity, which at first favors the development of the bacillus, is soon replaced by acidity that checks it. When the culture becomes old, the reaction may again become strongly alkaline. This variation in reaction seems to depend entirely on the transformation of sugar in the media.

Vital Resistance.—As the diphtheria bacillus does not form spores, it possesses very little vital resistance and is delicate in its thermic sensitivity. It grows slowly at 20°C., rapidly at 37°C., and ceases to grow at about 40°C. It is killed when exposed to 58°C. for a few minutes. Besson states that when dried in fragments of false membrane it resists high temperatures and has been found alive after exposure to 100°C. for an hour. Drying quickly destroys it, but if organic matter be present it may remain alive a long time. Roux and Yersin were able to keep the bacilli alive in a piece of dry pseudo-membrane, kept in the dark, for five months.

Reyes has demonstrated that in absolutely dry air diphtheria bacilli die in a few hours. Under ordinary conditions their vitality,

* "Centralbl. f. Bakt. u. Parasitenk.," Sept. 24, 1897, Bd. XXII, Nos. 10 and 11.

† "Seventeenth Annual Report of the Department of Health and Charities," Indianapolis, Ind., 1907.

when dried on paper, silk, etc., continues for but a few days, though sometimes they can live for several weeks. In sand exposed to a dry atmosphere the bacilli die in five days in the light; in sixteen to eighteen days in the dark. When the sand is exposed to a moist atmosphere, the duration of their vitality is doubled. In fine earth they remained alive seventy-five to one hundred and five days in dry air, and one hundred and twenty days in moist air.

The organism is highly susceptible to disinfectantsexc ept when dried in false membrane.

Metabolic Products.—The diphtheria bacillus forms acids (lactic acid?) in the presence of dextrose, galactose, levulose, maltose, dextrine and glycerin. It also forms acids in meat-infusion bouillon, probably because of the muscle sugars it contains. In the absence of sugars it produces alkalies. It is unable to evolve gas from any carbohydrates. It does not coagulate milk; does not liquefy gelatin or blood-serum.

Palmirski and Orlowski* assert that the bacillus produces indol, but only after the third week. Smith,† however, found that when the diphtheria bacillus grew in dextrose-free bouillon no indol was produced.

Toxin.—The earliest researches upon the nature of the poisonous products of the diphtheria bacillus seem to have been made in 1887 by Löffler,‡ who came to the conclusion that they belonged to the enzymes. The credit of removing the bacteria from the culture by filtration through porcelain and the demonstration of the soluble poison in the filtrate belong to Roux and Yersin.§ Toxic bouillon prepared in this manner was found to cause serous effusions into the pleural cavities, acute inflammation of the kidneys, fatty degeneration of the liver, and edema of the tissue into which the injection was made. In some cases palsy subsequently made its appearance, usually in the hind quarters. The effect of the poison was slow and death took place days or weeks after injection, sometimes being preceded by marked emaciation. Temperatures of 58°C. lessened the activity of the toxin and temperatures of 100°C. destroyed it. It was precipitated by absolute alcohol and mechanically carried down by calcium chlorid. Brieger and Fränkel‖ confirmed the work of Roux and Yersin, and concluded that the poison was a toxalbumin. Tangl** was able to extract the toxin from a fragment of diphtheria pseudo-membrane macerated in water.

The nature of the diphtheria toxin has been studied by Ehrlich††

* "Centralbl. f. Bakt. u. Parasitenk.," March, 1895.
† "Jour. Exp. Med.," Sept., 1897, vol. II, No. 5, p. 546.
‡ "Centralbl. f. Bakt.," etc., 1887, II, p. 105.
§ "Ann. de l'Inst. Pasteur," 1888–1889.
‖ "Berliner klin. Wochenschrift," 1890, 11–12.
** "Centralbl. f. Bakt.," etc., Bd. XI, p. 379.
†† "Klinisches Jahrbuch," 1897.

and found to be extremely complex. As it exists in cultures it is composed of equal parts of toxin and toxoid. Of these, the former is poisonous, the latter harmless for animals—or at least not fatal to them. The toxoids have equal or greater affinity for combining with antitoxin than the toxin and cause confusion in testing the unit value or strength of the antitoxin. In old or heated toxin all of the toxin molecules become changed into toxons or toxoids and the poisonous quality is lost though the power of combining with antitoxin remains.

The toxin is extremely poisonous, and a filtered bouillon containing it may be fatal to a 300-gram guinea-pig in doses of only 0.0005 cc. It is thought not to be an albuminous substance, as it can be elaborated by the bacilli when grown in non-albuminous urine, or, as suggested by Uschinsky, in non-albuminous solutions whose principal ingredient is asparagin. The toxic value of the cultures is greatest in the second week.

This soluble toxin so well known in bouillon cultures is probably only one of the poisonous substances produced by the bacillus. An intracellular, insoluble toxic product seems to have been discovered by Rist,* who found it in the bodies of dried bacilli, and observed that it was not neutralized by the antitoxin.

Pathogenesis.—The Bacillus diphtheriæ is pathogenic for man, monkeys, guinea-pigs, rabbits, dogs, cats, cows, and horses. Sparrows, pigeons and fowls are susceptible to experimental infection; rats and mice are immune. Spontaneous or natural infection is pretty well limited to man. The effects of artificial experimental infection vary with the avenue of infection, the quantity of culture and its virulence.

1. **Subcutaneous inoculation** in rabbits and guinea-pigs is usually fatal in from seventy-two hours to five days. The animal suffers some rise of temperature in twelve to twenty-four hours, soon is depressed, weak, loses flesh, remains quiet and dies. At the seat of infection there is a swelling caused by combined edema, hemorrhage and fibrinous exudation. If the culture be of feeble virulence so that death does not occur, this area sloughs, and then heals slowly.

2. **Intraperitoneal and Intrapleural Infection.**—This is not so serious in its results as might be supposed. Some animals recover from doses that might be fatal under the skin. Death does not occur until after a week or twelve days. Fluid of slightly turbid character with flakes of fibrin is found in the peritoneum.

3. **Mucous Membrane Inoculations.**—When implanted upon the scarified surfaces of the mucous membranes, the bacillus causes the formation of a fibrinous and necrotic pseudo-membrane. Such conditions may recover or death may follow after some days.

In all cases the bacilli remain fairly well-localized at or near the

* "Soc. de Biol. Paris," 1903, No. 25.

seat of inoculation and only rarely invade the blood. Death and illness result from toxemia, not from bacteremia.

When examined post-mortem, the liver is found enlarged and sometimes shows minute whitish points, which upon microscopic examination prove to be necrotic areas in which the cells are completely degenerated, and the chromatin of their nuclei scattered about in granular form. Similar necrotic foci, to which attention was first called by Oertel, are present in nearly all the organs in cases of death from diphtheria intoxication. No bacilli are present in these lesions. Welch and Flexner* have shown these foci to be common to numerous intoxications, and not peculiar to diphtheria.

The lymphatic glands are usually enlarged, and the adrenals enlarged and hemorrhagic. The kidneys show parenchymatous degeneration.

Roux and Yersin found that when the bacilli were introduced into the trachea of animals, a typical pseudo-membrane was formed, and that diphtheritic palsy sometimes followed.

Diphtheria in man is characterized by a pseudo-membranous inflammation of the mucous membranes, particularly of the fauces, though it may occur in the nose, in the mouth, upon the genital organs, or upon wounds. Williams† has reported a case of diphtheria of the vulva, and Nisot and Bumm have reported cases of puerperal diphtheria from which the bacilli were cultivated. It is in nearly all cases a purely local infection, depending upon the presence and development of the bacilli upon the diseased mucous membrane, but is accompanied by a serious intoxication resulting from the absorption from the local lesions of a poisonous metabolic product of the bacilli. The bacilli are found only in the membranous exudation, and are most plentiful in its older portions.

The entrance of the diphtheria bacillus into the internal organs can scarcely be regarded as a frequent occurrence, though metastatic occurrence of the organism with and without associated staphylococci and streptococci, and with and without purulent inflammations have from time to time been reported. Diphtheria bacilli were first found in the heart's blood, liver, spleen, and kidney, by Frosch.‡ Kolisko and Paltauf‖ had already found them in the spleen, and other observers in various lesions of the deeper tissues and occasionally in the organs. In the blood and organs it is commonly associated with Streptococcus pyogenes and sometimes with other bacteria. While present in nearly all of the inflammatory sequelæ of diphtheria, the Klebs-Löffler bacillus probably has very little influence in producing them, the conditions being almost invariably associated with the pyogenic cocci, either the streptococci or

* "Bull. of the Johns Hopkins Hospital," Aug., 1901.
† "Amer. Jour. of Obstet. and Dis. of Women and Children," Aug., 1898.
‡ "Zeitschrift für Hygiene," etc., 1893, XIII, Heft 1.
‖ "Wiener klin. Wochenschrift," 1889.

staphylococci. Howard* studied a case of ulcerative endocarditis caused by the diphtheria bacillus, and Pearce† has observed it in 1 case of malignant endocarditis, 19 out of 24 cases of bronchopneumonia, 1 case of empyema, 16 cases of middle-ear disease, 8 cases of inflammation of the antrum of Highmore, 1 case of inflammation of the sphenoidal sinuses, 1 case of thrombosis of the lateral sinuses, 2 cases of abscesses of the cervical glands, and in esophagitis, gastritis, vulvo-vaginitis, dermatitis, and conjunctivitis following or associated with diphtheria.

A case of septic invasion by the diphtheria bacillus is reported by Ucke,‡ who gives a synopsis of the literature of similar cases.

The disease pursues a variable course. In favorable cases the patient recovers gradually, the pseudo-membrane first disappearing, leaving an inflamed mucous membrane, upon which virulent diphtheria bacilli persist for weeks and sometimes for months. Smith* describes the bacteriologic condition of the throat in diphtheria as follows: "The microscope informs us that during the earliest local manifestations the usual scant miscellaneous bacterial flora of the mucosa is quite suddenly replaced by a rich vegetation of the easily distinguishable diphtheria bacillus. Frequently no other bacteria are found in the culture-tube. This vegetation continues for a few days, then gradually gives way to another flora of cocci and bacilli, and finally the normal condition is reëstablished."

Associated Bacteria.—Streptococcus pyogenes and Staphylococci pyogenes aureus and albus are, in many cases, found in association with the diphtheria bacillus, especially when severe lesions of the throat exist.

In a series of 234 cases carefully and statistically studied by Blasi and Russo-Travali,‖ it was found that in 26 cases of pseudomembranous angina due to streptococci, staphylococci, colon bacilli, and pneumococci, 2 patients died, the mortality being 3.84 per cent. In 102 cases of pure diphtheria, 28 died, a mortality of 27.45 per cent. Seventy-six cases showed diphtheria bacilli and staphylococci; of these, 25, or 32.89 per cent., died. Twenty cases showed the diphtheria bacilli and Streptococcus pyogenes, with 6 deaths—30 per cent. In 7 cases, of which 3, or 43 per cent., were fatal, the diphtheria bacillus was in combination with streptococci and pneumococci. The most dangerous forms met were 3 cases, all fatal, in which the diphtheria bacillus was found in combination with Bacillus coli.

In 157 cases of diphtheria and scarlatina studied at the Boston

* "Amer. Jour. Med. Sci.," Dec., 1894.

† "Jour. Boston Soc. of Med. Sci.," March, 1898.

‡ "Centralbl. f. Bakt. u. Parasitenk.," original, XLVI, Heft 4, March 10, 1908, p. 292.

‖ "Ann. de l'Inst. Pasteur," 1896, p. 387.

City Hospital by Pearce,* there were 94 cases of diphtheria, 46 cases of complicated diphtheria (29 with scarlet fever, 11 with measles, and 5 with measles and scarlet fever), and 17 cases of scarlet fever (in 3 of which measles was also present).

Of the 94 cases of uncomplicated diphtheria, the Klebs-Löffler bacilli were present in the *heart's blood* in 4, twice alone and twice with streptococci. In 9 cases the streptococcus occurred alone; in 1 case the pneumococcus occurred alone. In the *liver* the bacillus was found in 24 cases, alone in 12 and together with the streptococcus in 12; the streptococcus occurred in 27 cases, alone in 14, with the Klebs-Löffler bacillus in 12, and with Staphylococcus pyogenes aureus in 1. Staphylococcus pyogenes aureus occurred in 4 cases, alone in 3 and associated with the streptococcus in 1. The pneumococcus occurred alone in 1 case.

In the *spleen* the Klebs-Löffler bacillus occurred eighteen times, fifteen times alone and three times associated with the streptococcus. The streptococcus occurred in 24 cases, alone in 21, associated with the Klebs-Löffler bacillus twice, and with Staphylococcus pyogenes aureus once. Staphylococcus pyogenes occurred twice, once alone and once with the streptococcus. The pneumococcus occurred twice alone.

In the *kidney* the Klebs-Löffler bacillus occurred in 23 cases, in 15 alone, in 5 associated with the streptococcus, and in 2 with Staphylococcus pyogenes aureus. The streptococcus occurred in 26 cases, in 19 of which it was the only organism present. Staphylococcus pyogenes aureus occurred in 8 cases, in 4 of which it was in pure culture. The pneumococcus occurred four times, three times in pure culture and once with the Klebs-Löffler bacillus.

In the 46 cases of complicated diphtheria, the *heart's blood* showed pure cultures of the streptococcus nine times and the streptococcus associated with the Klebs-Löffler bacillus once. The diphtheria bacillus occurred alone once.

In the *liver*, in 10 cases streptococcus occurred alone, in 7 cases associated with the Klebs-Löffler bacillus, and in 3 cases with Staphylococcus pyogenes aureus. The diphtheria bacillus occurred in pure culture in 5 cases.

The *spleen* contained streptococci only thirteen times and mixed with the diphtheria bacillus twice. The diphtheria bacillus was found in pure culture in 5 cases.

The *kidney* contained pure cultures of streptococci in 10 cases, streptococci associated with diphtheria bacilli five times, and with Staphylococcus pyogenes aureus three times. The diphtheria bacillus occurred alone in 7 cases. Staphylococcus pyogenes aureus and the pneumococcus each alone once, and both together once.

"The clinical significance of this general infection with the Klebs-

* "Jour. Boston Soc. of Med. Sci.," March, 1898.

Löffler bacillus is not apparent. It occurred generally, but not always, in the gravest cases, or those known as 'septic' cases. It is probable that it may be due to a diminished resistance to the tissue-cells, or of the germicidal power of the blood. In this series of fatal cases the number of infections with the streptococcus and with the Klebs-Löffler bacillus was about even, though slightly in favor of the streptococcus."

The mixed infections add to the clinical diphtheria the pathogenic effects of the associated bacteria. The diphtheria bacillus probably begins the process, growing upon the mucous membrane, devitalizing it by its toxin, and producing coagulation-necrosis. Whatever pyogenic germs happen to be present are thus afforded an opportunity to enter the tissues and add suppuration, gangrene, and remote metastatic lesions to the already existing ulceration.

Diphtheritic inflammations of the throat are not always accompanied by the formation of the pseudo-membrane, but in some cases a rapid inflammatory edema in the larynx, without a fibrinous surface coating, may cause fatal suffocation, only a bacteriologic examination revealing the true nature of the disease.

Lesions.—The pseudo-membrane characterizing diphtheria consists of a combined necrosis of the tissues acted upon by the toxin and coagulation of an inflammatory exudate. When examined histologically it is found that the surface of the mucous membrane is chiefly affected. The superficial layers of cells are embedded in coagulated exudate—fibrin—and show a peculiar hyaline degeneration. Sometimes the membrane seems to consist exclusively of hyaline cells; sometimes the fibrin formation is secondary to or subsequent to the hyaline degeneration. Leukocytes caught in the fibrin also become hyaline. From the superficial layer the process may descend to the deepest layers, all of the cells being included in the coagulated fibrin and showing more or less hyaline degeneration. The walls of the neighboring capillaries also become hyaline, and the necrotic mass forms the diphtheritic membrane. The laminated appearance of the membrane probably depends upon the varying depths affected at different periods, or upon differences in the process by which it has been formed. The pseudo-membrane is continuous with the subjacent tissues by a fibrinous reticulum, and is in consequence removed with difficulty, leaving an abraded surface. When the membrane is divulsed during the course of the disease, it immediately forms anew by the coagulation of the inflammatory exudate.

The coagulation-necrosis seems to depend upon the local effect of the toxin. Morax and Elmassian* found that when strong diphtheria toxin is applied to the conjunctiva of rabbits every three minutes for eight or ten hours, typical diphtheritic changes are produced.

* "Ann. de l'Inst. Pasteur," 1898, p. 210.

Flexner* has made a study of the minute lesions caused by bacterial toxins and especially of the diphtheria toxin, and Councilman, Mallory, and Pearce,† of both gross and minute lesions, that the thorough student should read.

Specificity.—Herman Biggs,‡ in an interesting discussion of the occurrence of the diphtheria bacillus and its relation to diphtheria, came to the following conclusions:

1. "When the diphtheria bacillus is found in healthy throats, investigation almost always shows that the individuals have been in contact with cases of diphtheria. The presence of the bacillus in the throat, without any lesion, does not, of course, indicate the existence of the disease."

2. "The simple anginas in which virulent diphtheria bacilli are found are to be regarded from a sanitary standpoint in exactly the same way as the cases of true diphtheria."

3. "Cases of diphtheria present the ordinary clinical features of diphtheria, and show the Klebs-Löffler bacilli."

4. "Cases of angina associated with the production of membrane in which no diphtheria bacilli are found might be regarded from a clinical standpoint as diphtheria, but bacteriological examination shows that some other organism than the Klebs-Löffler bacillus is the cause of the process."

Any skepticism of the specificity of the diphtheria bacillus on my own part was dispelled by a somewhat unique experience. Without having been previously exposed to diphtheria while experimenting in the laboratory the author accidentally drew a living virulent culture of the diphtheria bacillus through a pipet into his mouth. Through carelessness no precautions were taken to prevent serious consequences and two days later the throat was filled with typical pseudo-membrane which private and Health Board bacteriologic examinations showed to contain pure cultures of the Klebs-Löffler bacilli.

Some have been led to doubt the specificity of the diphtheria bacillus because of the existence of what is called the *pseudo-diphtheria bacillus* or bacillus of Hofmann (*q.v.*). Bomstein§ found that though it was possible to modify the activity of virulent bacilli, and bring back the virulence of non-virulent diphtheria bacilli, it was impossible to make the pseudo-diphtheria bacillus virulent. Denny‖ also found that the morphology of the two organisms was continually different when they were grown upon the same medium for the same length of time, and that the short pseudo-diphtheria bacillus never showed any tendency to develop into the

* "Johns Hopkins Hospital Reports," VI, 259.

† "Diphtheria: A Study of the Bacteriology and Pathology of Two Hundred and Twenty Fatal Cases," 1901.

‡ "Amer. Jour. Med. Sci.," Oct., 1896, vol. XXII, No. 4, p. 411.

§ "Archiv Russes de Path.," etc., Aug. 31, 1902.

‖ American Public Health Association, 1902.

large clubbed forms characteristic of the true diphtheria organism. The chief points of difference between the bacilli are that the pseudo-diphtheria bacillus, when grown upon blood-serum, is short and stains uniformly; that cultures grown in bouillon develop more rapidly at a temperature of from 20° to 22°C. than those of the true bacillus; and that the pseudo-bacillus is not pathogenic for animals.

Contagion.—The diphtheria bacilli, being always present in the throats of patients suffering from diphtheria, constitute the element of contagion.

The results obtained by Biggs, Park, and Beebe in New York are of great interest. Bacteriologic examinations conducted in connection with the Health Department of New York City show that virulent diphtheria bacilli may be found in the throats of convalescents from diphtheria as long as five weeks after the discharge of the membrane and the commencement of recovery, and that they exist not only in the throats of the patients themselves, but also in those of their caretakers, who, while not themselves infected, may be the means of conveying the disease germs from the sick-room to the outer world. Still more extraordinary are the observations of Hewlett and Nolen,* that the bacilli remained in the throats of patients seven, nine, and in one case *twenty-three weeks* after convalescence. The hygienic importance of this observation must be apparent to all readers, and serves as further evidence why thorough isolation should be practised in connection with the disease.

Neumann† found that virulent diphtheria bacilli may occur in the nose with the production of what seems to be a simple rhinitis as well as a pseudo-membranous rhinitis. Such cases, not being segregated, may easily serve to spread the contagion of the disease.

Wesbrook, and Wilson and McDaniel‡ have found it convenient to describe three chief types of the diphtheria bacillus as it occurs in twenty-four-hour-old cultures on Löffler's blood-serum, sent to the laboratory for diagnosis. The classification places all types in three general groups: (*a*) granular, (*b*) barred, and (*c*) solid or evenly staining forms. Each group is subdivided into types based on the shape and size of the bacilli. A study of variations in the sequence of types in series of cultures derived from clinical cases of diphtheria shows that (*a*) granular types are usually the most predominant forms at the outset of the disease; (*b*) the granular types usually give place wholly or in part to barred and solid types shortly before the disappearance of diphtheria-like organisms; (*c*) solid types, by many observers called "pseudo-diphtheria bacilli," may cause severe clinical diphtheria. Solid types may sometimes be re-

* "Brit. Med. Jour.," Feb. 1, 1896.
† "Centralbl. f. Bakt. u. Parasitenk.," Jan. 24, 1902, Bd. XXXI, No. 2, p. 41.
‡ "Trans. Assoc. Amer. Phys.," 1900; Trans. Amer. Public Health Asso., 1900.

placed by granular types when convalescence is established and just before the throat is cleared of diphtheria-like bacilli.

From these data the writers conclude that it is not safe to base an opinion regarding the maintenance of quarantine upon the bacterioscopic findings independently of the clinical history of the case.

The occurrence of true diphtheria bacilli in the throats of healthy persons has been a stumbling-block to many practitioners uninformed upon bacteriologic subjects, who fail to account for its presence and also fail to realize how rare its appearance under such circumstances really is.

Park* found virulent diphtheria bacilli in about 1 per cent. of the healthy throats examined in New York city, but diphtheria was prevalent in the city at the time, and no doubt most of the

Fig. 158.—Wesbrook's types of Bacillus diphtheriæ: *a*, *c*, *d*, Granular types; a^1, c^1, d^1, barred types; a^2, c^2, d^2, solid types. × 1500.

persons in whose throats they existed had been in contact with cases of diphtheria. He very properly concludes that the members of a household in which a case of diphtheria exists, though they have not the disease, should be regarded as possible sources of danger, until cultures made from their throats show that the bacilli have disappeared.

Bacteriologic Diagnosis.—It is impossible to make an accurate diagnosis of diphtheria without a bacteriologic examination.

Such an examination is now within the power of every physician. All that is required is a swab made by wrapping a little absorbent cotton about the end of a piece of wire and carefully sterilizing it in a test-tube, and a tube of Löffler's blood-serum-medium, that can

* "Report on Bacteriological Investigations and Diagnosis of Diphtheria, from May 4, 1893, to May 4, 1894." "Scientific Bulletin No. 1," Health Department, city of New York.

be bought from almost any modern druggist. The swab is introduced into the throat and applied to the false membrane, after which it is carefully smeared over the surface of the blood-serum. The tube thus inoculated is stood away in an incubating oven or otherwise kept at the temperature of 37°C. for twelve hours, then examined. If the diphtheria bacillus be present, a smeary, creamy-white layer with outlying colonies will be present. These colonies, *if found by microscopic examination to be made up of diphtheria bacilli*, will confirm the diagnosis of diphtheria. There are very few other bacilli that grow so rapidly upon Löffler's mixture, and scarcely any other is found in the throat.

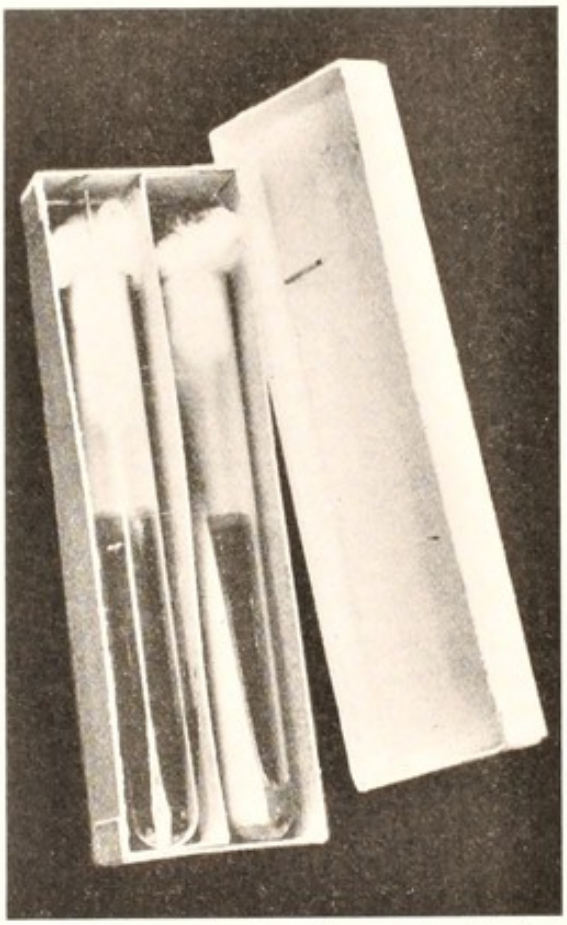


Fig. 159.—The Providence Health Department outfit for diphtheria diagnosis, consisting of a pasteboard box containing a swab-tube and a serum-tube, both with etched surface on which to write the name and address of patients, etc.

When no tubes of the blood-serum mixture are at hand, the swab can be returned to its tube after having been wiped over the throat of the patient, and can be shipped to the nearest laboratory.

When an early diagnosis is required, Ohlmacher recommends that the microscopic examination of the still invisible growth be made in five hours. A platinum loop is rubbed over the inoculated surface; the small amount of material thus secured is mixed with distilled water, spread on a cover-glass, dried, fixed, stained with methylene blue, and examined. An abundance of the organisms is usually found and valuable time is saved preparatory to the use of the antitoxin.

Diphtheria Antitoxin.—Behring* discovered that the blood of animals rendered immune against diphtheria by inoculation, first with attenuated and then with virulent organisms, contained a neutralizing substance (*Anti-körper*) capable of annulling the effects of the bacilli or the toxin when simultaneously or subsequently inoculated into susceptible animals. This substance, held in solu-

* "Deutsche med. Wochenschrift," 1890, Nos. 49 and 50; "Zeitschrift für Hygiene," 1892, XII, 1.

tion in the blood-serum of the immunized animals, is the *diphtheria antitoxin*. For the method of preparing see Antitoxins. The serum may be employed for purposes of *prophylaxis* or for *treatment*.

Prophylaxis.—The serum can be relied upon for prophylaxis in cases of exposure to diphtheria infection. In most cases a single dose of 1000 units is sufficient for the purpose. The protection thus afforded does not continue longer than about six weeks. The transitory nature of the immunity afforded by prophylactic injections of antitoxin is probably dependent upon the fact that the antitoxin is slowly eliminated.

Fig. 160.—Sterilized test-tube and swab for collecting pus and fluids for bacteriologic examination (Warren).

Treatment.—Diphtheria antitoxin is always to be administered by the hypodermic method at some point where the skin is loose. Some clinicians prefer to inject into the abdominal wall; some, into the tissues of the back. A slightly painful swelling is formed, which usually disappears in a short time. In a few cases sudden death, with symptoms suggesting *anaphylaxis* (*q.v.*), has followed the injection.

Ehrlich asserts that a dose of 500 units is valueless for the treatment of diphtheria, 2000 units being probably an average dose for an adult and 1000 units for a child. It is far better to err on the side of administering too much than on that of not enough. Forty thousand units have been administered to a moribund child with resulting cure. The administration of the remedy should be repeated in twelve hours if the disease is one or two days old, in six hours if three or four days old, in four hours if still older. The serum may have to be given two, three, four, or even more times, according to the case. Occasionally there is an outbreak of local urticaria—rarely general urticaria. Sometimes considerable local erythema results. Fever and pain in the joints (serum disease of von Pirquet) also occur, especially if the patients have been previously treated with horse-serum.

Diphtheria paralysis is said to be more frequent after the use of antitoxin than in cases treated without it. McFarland* has shown that this is to be expected, as the palsies usually occur after bad cases of the disease, of which a far greater number recover when antitoxin

* "Medical Record," New York, 1897.

is used for treatment. The subject has been worked over in an interesting manner, from the experimental side, by Rosenau.*

An interesting collection of statistics upon the antitoxic treatment of diphtheria in the hospitals of the world has been published by Professor Welch,† who, excluding every possible error in the calculations, "shows an apparent reduction of case-mortality of 55.8 per cent."

Nothing should so impress the clinician as the necessity of beginning the antitoxin treatment *early in the disease*. Welch's statistics show that 1115 cases of diphtheria treated in the first three days of the disease yielded a fatality of 8.5 per cent., whereas 546 cases in which the antitoxin was first injected after the third day of the disease yielded a fatality of 27.8 per cent.

On the other hand, it can scarcely be said that any time is *too late* to begin the serum treatment, for the experiences of Burroughs and McCollum in the Boston City Hospital show that by immediate and repeated administration of very large doses of the serum, apparently hopeless cases far advanced in the disease, may often be saved.

After the toxin has occasioned destructive organic lesions of the nervous system and in the various organs and tissues of the body, no amount of neutralization can restore the integrity of the parts, and in such cases antitoxin must fail.

One disadvantage under which the diphtheria antitoxic serum is administered both for purposes of prophylaxis and treatment, is the inability of the operator to find out what may be the already existing antitoxin content of the patient's blood. Though it is certain that existing diphtheria is proof that the patient needs the remedy, it is by no means certain that all normal persons exposed to diphtheria in institutions, etc., require it for prophylactic purposes. Some may already possess enough to defend them and the promiscuous administration of the serum to every child in an asylum, may result in sensitizing some to the allergizing effect of the horse-serum without just reason. A means by which some knowledge of the normal diphtheria-toxin neutralizing quality of the blood of a healthy individual can be arrived at, has been devised by Schick,‡ and is now known as *Schick's reaction*. It consists in the intracutaneous administration of a minute dose of diphtheria toxin. If the patient's blood contains the neutralizing substance, no reaction takes place; if it contain none, a reddened and tumefied circumscribed area appears. W. H. Park uses one-fiftieth of the L+ dose of diphtheria toxin, injecting it into the skin with a very fine hypodermic needle. Kolmer prefers to use one-fortieth of the L+ dose. The presence of one-thirtieth of a unit of antitoxin in 1 cc. of the patient's blood pre-

* "Bulletin No. 38 of the Hygienic Laboratory, U. S. Public Health and Marine Hospital Service," Washington, D. C., 1907.

† "Bull. of the Johns Hopkins Hospital," July and Aug., 1895.

‡ "Münchener. med. Wochenschrift, 1913, p. 2605.

vents the reaction. Kolmer* has also made use of the Schick reaction for the important purpose of determining how long the antitoxin serum injected into the patient remains and confers immunity. When the reaction reappears, the immunity can be supposed to have disappeared, and the patient again becomes susceptible to the infection.

A very interesting paper by Park† shows the effect of the introduction of antitoxin upon the death-rate from diphtheria and the advantages of its employment. From it the following table is taken:

"Combined statistics of deaths and death-rates from diphtheria and croup in New York, Brooklyn, Boston, Pittsburgh, Philadelphia, Berlin, Cologne, Breslau, Dresden, Hamburg, Königsberg, Munich, Vienna, London, Liverpool, Glasgow, Paris, and Frankfort:

Year	Population	Deaths from diphtheria and croup	Deaths per 100,000
1890	16,526,135	11,059	66.9
1891	17,689,146	12,389	70.0
1892	18,330,787	14,200	77.5
1893	18,467,970	15,726	80.4
1894	19,033,902	15,125	79.9
1895‡	19,143,188	10,657	55.6
1896	19,489,682	9,651	49.5
1897	19,800,629	8,942	45.2
1898	20,037,918	7,170	35.7
1899	20,358,837	7,256	35.6
1900	20,764,614	6,791	32.7
1901	20,874,572	6,104	29.2
1902	21,552,398	5,630	26.1
1903	21,865,299	5,117	23.4
1904	22,532,848	4,917	21.8
1905	22,790,000	4,323	19.0

Bacilli Resembling the Diphtheria Bacillus

Bacillus Hofmanni

The *pseudo-diphtheria bacillus* (bacillus of Hofmann-Wellenhof§)—Bacillus pseudo-diphthericus—was first found by Löffler‖ in diphtheria pseudo-membranes and in the healthy mouth and pharynx. Ohlmacher has found it with other bacteria in pneumonia; Babes, in gangrene of the lung; and Howard,** in a case of ulcerative endocarditis not secondary to diphtheria.

Park†† found that all bacilli with the typical morphology of the diphtheria bacillus, found in the human throat, are virulent Klebs-Löffler bacilli, while forms closely resembling them, but more uniform in size and shape, shorter in length, and of more homogeneous staining properties with Löffler's alkaline methylene-blue solution, can with reasonable safety be regarded as pseudo-diphtheria bacilli, especially if it be found that they produce an alka-

* "Phila. Pathological Society," Feb. 11, 1915.
† "Journal of the Amer. Med. Assoc.," Feb. 17, 1912, LVIII, No. 7, p. 453.
‡ Introduction of antitoxin treatment.
§ "Wiener klin. Woch.," 1888, No. 3.
‖ "Centralbl. f. Bakt. u. Parasitenk.," II, 105.
** "Bull. of the Johns Hopkins Hospital," 1893, 30.
†† "Scientific Bulletin No. 1," Health Department, city of New York, 1895.

line rather than an acid reaction by their growth in bouillon. The pseudo-diphtheria bacilli were found in about 1 per cent. of throats examined in New York; they seem to have no relationship to diphtheria, and are never virulent.

Morphology.—This micro-organism bears a more or less marked resemblance to Bacillus diphtheriæ, but differs in certain particulars that usually make it possible to recognize and identify it. It is shorter and stouter than its relative, is straight, usually slightly clubbed. It usually stains intensely, and commonly shows but one unstained transverse band. When the bacilli are short and have a single band, they may resemble cocci. When longer they may show two transverse bands.

There are no flagella and no spores.

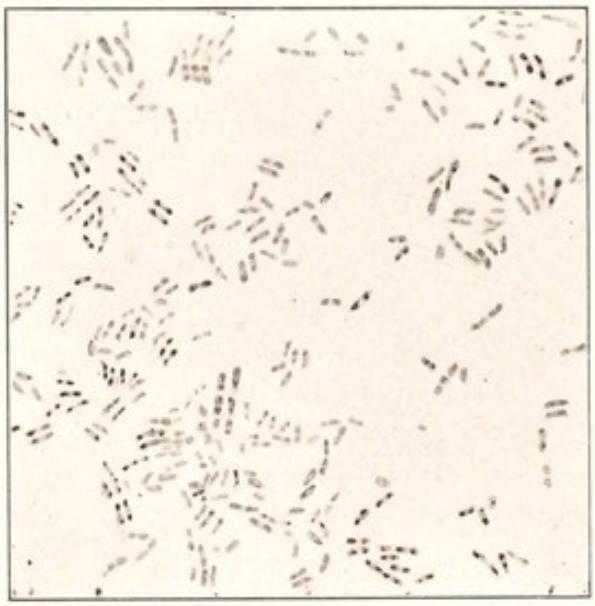


Fig. 161.—Pseudo-diphtheria bacilli.

Staining.—The organism stains intensely and more uniformly than Bacillus diphtheriæ. When colored by Neisser's or Roux's method, no metachromatic end bodies can be defined.

Cultivation.—The organism is usually discovered in smears made for the diagnosis of diphtheria, and sometimes occasions considerable confusion through its cultural similarities and morphologic resemblances to Bacillus diphtheriæ. It grows more luxuriantly upon the ordinary culture-media than B. diphtheriæ. The colonies are larger, less transparent and whiter, as seen upon agar-agar. In bouillon there is more marked clouding and less marked pellicle formation. Upon Löffler's blood-serum the cultures are too much alike to be easily differentiated.

G. F. Petri* found no substances in filtrates of cultures of Hofmann's bacillus capable of neutralizing diphtheria antitoxin; he also found that horses immunized with large quantities of filtrates of the

* "Jour. of Hygiene," April, 1905, vol. v, No. 2, p. 134.

Hofmann bacillus did not produce any antitoxin to diphtheria toxin. Eleven different cultures were studied and the results are very important.

Cobbett* and Knapp† show that there is a chemicobiologic difference between the true and pseudo-diphtheria bacilli, in that the latter does not ferment dextrin or any of the sugars as the true bacillus does.

Chemistry.—The chemical peculiarities of the culture serve to make certain that Bacillus hofmanni is an independent micro-organism. Under no circumstances does it produce or can it be made to produce toxin. Under no circumstances can it be made to produce acid through the decomposition of sugars.

Pathogenesis.—Dr. Alice Hamilton‡ carefully studied 29 organisms, of which 26 corresponded fully with the pseudo-diphtheria bacilli. They were divisible into three groups: I, Those non-pathogenic for guinea-pigs; II, those that produce general bacteremia in guinea-pigs, and are neutralized by treatment with the serum of a rabbit immunized against a member of the group; III, organisms which form gas in glucose media, produce bacteremia in guinea-pigs, and are neutralized neither by diphtheria nor by pseudo-diphtheria antitoxin. Some of the organisms of the second group are also pathogenic for man. Instead of regarding the pseudo-diphtheria bacillus as a harmless saprophyte, Dr. Hamilton believes it an important organism explaining some of the paradoxes that we find at hand. Thus, cases of supposed diphtheria irremediable by or deleteriously affected by antitoxic serum may depend upon one of these organisms. It is also probably one of them that Councilman found in his case of "general infection by Bacillus diphtheriæ," and that Howard encountered in his case of acute ulcerative endocarditis without diphtheria, from the valves of whose heart cultures of a diphtheria-like organism not pathogenic for guinea-pigs was isolated.

The still more recent and comprehensive work of Clark§ shows that no kind of manipulation is capable of so modifying Bacillus hofmanni as to make its identity with B. diphtheriæ in the least likely. Clark is, however, willing to admit the probability that the organisms may have descended from a common stock.

Bacillus Xerosis

This bacillus was first described in 1884 by Kutschbert and Neisser,‖ who regarded it as the cause of xerosis conjunctivæ, having found it upon the conjunctiva in that disease. It has, however, been so frequently found upon the normal conjunctiva that it can no longer be looked upon as pathogenic. It is also found upon other

* "Centralbl. f. Bakt. u. Parasitenk.," 1898, XXIII, 395.
† "Jour. of Med. Research," 1904, XII (N. S., vol. VII), p. 475
‡ "Jour. Infectious Diseases," 1904, I, p. 690.
§ "Journal of Infectious Diseases," VII, 1910, 335.
‖ "Deutsche med. Wochenschrift," 1884, Nos. 21, 24.

mucous membranes than the conjunctiva; thus, Leber found it in the mouth, the pelvis of the kidney, and in intestinal ulcers. From the investigations of Sattler, Fränkel and Franke, Schleich, Weeks, Fick, Baumgarten, and others it appears that Bacillus xerosis is a harmless saprophyte that is occasionally found upon the conjunctiva. Happening to be found in xerosis it was accorded undue distinction.

Morphology.—It resembles Bacillus diphtheriæ very closely, but is probably a little shorter. The ends are clubbed, and in them metachromatic bodies are stained by Neisser's and Roux's methods.

There is no motility; there are no flagella and no spores.

Cultivation.—Upon Löffler's medium and other media commonly used for the diagnosis of diphtheria, the organism grows with so close resemblance to the Bacillus diphtheriæ as to make the differentiation difficult. Transplanted to other media, it continues to resemble B. diphtheriæ.

Chemistry.—The organism is incapable of forming any toxin. It ferments sugars like Bacillus diphtheriæ, with the exception of saccharose, which B. xerosis ferments, but which B. diphtheriæ cannot ferment. B. xerosis also fails to ferment dextrin, which B. diphtheria ferments.

These sugar-decomposing properties form the most reliable methods of differentiating Bacillus diphtheriæ, B. hofmanni, and B. xerosis.

Pathogenesis.—The organism is not pathogenic for man and is certainly not the cause of xerosis. It is not toxicogenic and is not known to be pathogenic for any animal.

CHAPTER XIII

VINCENT'S ANGINA

VINCENT'S angina is an acute, specific, infectious, pseudo-membranous form of pharyngitis or tonsillitis characterized by the formation of a soft yellowish-green exudate upon the mucous membranes, which, when removed, leaves a bleeding surface which becomes an ulcer. Sometimes these ulcers are superficial, sometimes they are deep, necrotic, and fetid. There is considerable pain on swallowing, some fever, and some prostration. The patient not infrequently keeps up and about, though feeling very badly. The ulcerations sometimes persist for several months. As there is considerable swelling of the glands of the neck and as the pseudo-membrane is sometimes quite distinct, the disease is apt to be mistaken for diphtheria, and may be differentiated from it only by a bacteriologic examination. When such an examination is made two apparently different micro-organisms may be found. The first is the Bacillus fusiformis; the second, Spirochæta vincenti.

BACILLUS FUSIFORMIS (BABES (?))

In 1882 Miller* described a fusiform bacillus that occurred in small numbers between the gums and the teeth and in cavities in carious teeth in the human mouth. In 1884 Cornil and Babes† also described a fusiform bacillus which seems to be somewhat different, that occurred in a necrotic exudation from a pseudo-membranous—diphtheritic—pharyngitis in school children. Lammershirt, Vincent, Nicolle, Plaut, and others observed similar cases. Later Lichtowitz and Sabrazes observed great numbers of fusiform bacilli in the pus of a maxillary empyema. Elders and Matzenauer observed similar organisms in *noma*. Fusiform bacilli are, therefore, not infrequently associated with necrotic processes of various kinds. Similar but not identical bacilli were found by Babes in the gums of scorbutic patients.

SPIROCHÆTA VINCENTI (PLAUT-VINCENT)

Plaut‡ and Vincent§ observed that in the ulcerative and necrotic pharyngitis described, together with the fusiform bacilli, there were varying numbers of spiral organisms. These were difficult to stain,

* "Micro-organisms of the Human Mouth." Philadelphia, 1890.
† "Les Bactéries," 1884.
‡ "Deutsche med. Wochenschrift," 1894, XLIX.
§ "Ann. de l'Inst. Pasteur," 1896, 488.

always took faint but uniform coloring, varied in length, and showed such irregular and non-uniform undulations as to appear more serpentine than "corkscrew-like." They seem never to occur without associated fusiform bacilli. The writers believe these organisms and not the bacilli to be the cause of the angina, but the relation of the organisms to one another and to the morbid conditions with which they were associated was a point long under debate, since none of those studying either organism succeeded in artificially cultivating it.

Relation of the Organisms to One Another

We have, in Vincent's angina, to do with two micro-organisms that occur in habitual association. Neither was found to be cultivable by the earlier writers. The spirochæta could not be cultivated by Vincent, and of the various fusiform bacilli, one found by Babes in scurvy, which was obviously different from the others, was alone susceptible of cultivation. Later, however, reports were made of the growth of the organisms in mixed cultures. Still later, Veillon and Zuber, Ellermann, Weaver, and Tunnicliff were able to secure pure cultures of the fusiform bacillus. Quite a number of writers reached the conclusion that the organisms were not different, but were different stages of the same organism. Tunnicliff* found that in pure cultures of Bacillus fusiformis, after forty-eight hours, spiral organisms resembling those seen in smear preparations from the original source were found. From Tunnicliff's results it would seem as though Bacillus fusiformis and Spirochæta vincenti are identical organisms in different stages of their life-history. But the matter is not yet settled for Krumweide and Pratt* by a different method of cultivation have apparently obtained B. fusiformis pure—*i.e.*, free from the spirochæta—have not found any apparent transformation of the bacilli into spirochæta, and insist that the two are essentially different organisms.

Cultivation.—The organisms were cultivated by Tunnicliff upon the surface of ascitic fluid agar-agar (1 : 3) under strictly anaërobic conditions at 37°C. After two or three days the fusiform bacillus appeared in the form of delicately whitish colonies, 0.5 to 2 mm. in diameter, resembling colonies of streptococci. By transplanting these, pure cultures of Bacillus fusiformis were obtained. In the transplantation tubes the organism again grew in the form of similar whitish colonies, a flocculent deposit accumulating at the bottom of the water of condensation.

Löffler's Blood-serum Mixture.—After twenty-four to forty-eight hours similar colonies appear and a similar flocculent deposit collects in the condensation water.

Rabbit's Blood Agar-agar.—The growth is similar, but brownish in color.

*"Jour. of Infectious Diseases," 1913, XIII, 199; 438.

Glycerin Agar-agar.—No growth.

Glucose Agar-agar Stab.—A delicate whitish growth with small

Fig. 162.—Bacillus fusiformis. Pure culture grown forty-eight hours anaërobically on Löffler's blood-serum. (Ruth Tunnicliff in "Journal of Infectious Diseases.")

lateral prolongations develops along the path of the wire in twenty-four to forty-eight hours. Some gas is formed.

Litmus Milk.—In forty-eight hours there is a moderate growth.

Fig. 163.—Bacillus fusiformis. Pure culture grown forty-eight hours anaërobically in the fluid of condensation of Löffler's blood-serum. (Ruth Tunnicliff in "Journal of Infectious Diseases.")

The litmus becomes decolorized. There is no coagulation. When oxygen is admitted the medium regains its lost color.

Potato.—No growth.

Bouillon and Dextrin-free Bouillon.—No growth.

Glucose-bouillon.—No growth when more than 1 per cent. of glucose is present. The medium is clouded with some sediment.

From all of the cultures a somewhat offensive odor is given off.

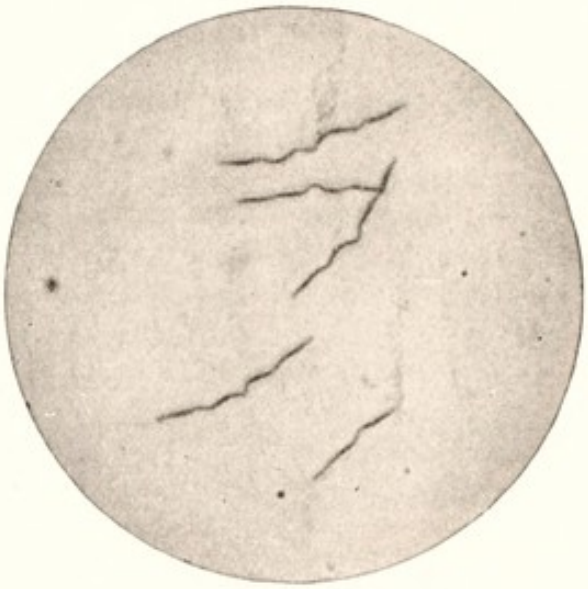


Fig. 164.—Bacillus fusiformis. Pure culture grown four days in ascites broth (Ruth Tunnicliff in "Journal of Infectious Diseases.")

Morphology.—The Bacillus fusiformis presents the same appearances, no matter what medium it grows upon. It measures 3 to 10 μ in length, 0.3 to 0.8 μ in thickness. The greatest diameter is at the

Fig. 165.—Bacillus fusiformis. Smear from gum in normal mouth. (Ruth Tunnicliff in "Journal of Infectious Diseases.")

center, from which the organisms gradually taper to blunt or pointed extremities.

The organisms stain with Löffler's alkaline methylene blue, with diluted carbol-fuchsin, by Gram's method, and by Giemsa's method.

The staining is intense, but is rarely uniform, the substance usually being interrupted by vacuoles or fractures, reminding one of those seen in the diphtheria and tubercle bacilli. The organism forms endospores sometimes situated at the center, but more frequently toward one end. In twenty-four to forty-eight hours filaments are seen. These are of the same diameter throughout, and usually contain deeply staining bodies, sometimes round, oftener in bands. Most of the filaments are made up of strings of bacilli, but some stain uniformly. Tunnicliff found that about the fourth or fifth day the spirals made their appearance, sometimes in enormous numbers. As a rule, they stained uniformly, some showed the dark bodies seen in the bacilli and filaments. They had from one to twenty turns, which were not uniform. The spirals were flexible, the ends pointed. The spirals persisted in the cultures, at times for fifty-five days.

Neither the bacilli nor the spirals showed any progressive movement, though with the dark-field illuminator they showed a slight vibratile and rotary movement. No flagella were observed.

Pathogenesis.—Pure cultures of the organisms were inoculated into guinea-pigs without result. As in Vincent's angina the throat always contains staphylococci and streptococci, and not infrequently diphtheria bacilli, it is thought by many that Bacillus fusiformis does not initiate the morbid process, but is a secondary invader, by which simpler inflammations are intensified and made necrotic.

This seems to be particularly true of diphtheria, and may account for the occurrence of noma, in which gangrenous condition of the mouth and genitals the organisms have been found in great numbers.

Bacillus fusiformis, with the associated spirals are not confined to Vincent's angina, but are found in a variety of other necrotic and gangrenous affections. Vincent* himself found them in all cases of hospital gangrene; Veillon and Zuber,† found them in certain cases of appendicitis; Bernheim and Popischell‡ in gangrenous laryngitis; Silberschmidt§ in fetid brochitis; Freejmuth and Petruschky,‖ Seiner** and others in noma; Wolbach†† in certain chronic ulcers of the legs in Gambia.

The complete literature of the subject collected by Beitzke, is published in the Centralbl. für Bakt. u. Parasitenk. (Referata) 1904, XXXV, p. 1.

* "Ann. de l'Inst. Pasteur, 1896, X, 488.
† "Archiv. de med. Exp.," 1898, p. 517.
‡ "Jahresb. für Kinderheilkunde," 1898, XLV.
§ "Centralbl. f. Bakt., etc.," 1901, Orig., XXX, 159.
‖ "Deutsche med. Wochenschrift," 1898, p. 232.
** "Wiener klin. Wochenschrift," 1899, No 2.
†† "Journal of Medical Research," 1912–13 XXVII, 27.

CHAPTER XIV

THRUSH

Oïdium Albicans (Robin)

Thrush, Soor (German), Muguet (French), or parasite stomatitis is an affection of marasmatic infants and adults characterized by the occurrence of peculiar whitish patches upon an inflamed oral mucous membrane. The white of the patches consists of material that is not easily removed, but which when detached leaves a bleeding surface upon which it forms again. Upon microscopic examination the white substance proves to be composed of masses of mycelia with enlarged epithelial cells and leukocytes. The affection is far more frequent in children than in adults. It seems not to occur among healthy children, but among those suffering from marasmus, and particularly among those whose mouths have already become sore through neglect. It is usually confined to the mouth, but may spread to the pharynx, to the larynx, in rare cases to the esophagus, in very rare cases to the stomach and intestines, and in exceptional cases, both in adults and children, may become a generalized disease through hematogenous distribution, and be attended by mycotic inflammatory lesions in the kidneys, the liver, and the brain.

The specific micro-organism seems to have been discovered in 1839 by Langenbeck* and Berg.† Langenbeck missed the significance of the organism altogether, for, finding it in a case of typhoid fever, he conceived it to be the cause of that disease. Berg, on the other hand, regarded it as the cause of the thrush. Robin‡ furnished the first correct description of the organism and gave it its name, Oïdium albicans. Many systematic writers have exercised themselves concerning the exact place in the botanical system in which the organisms should be placed. Thus, Gruby and Heim regarded it as a sporotrichum; Robin, as an oïdium; Quinquaud, as a syringospora; Hallein called it Stemphylium polymorpha; Grawitz, as Mycoderma vini; Plaut, as Monilia candida; Guidi, Ress, Brebeck-Fischer, as a saccharomyces; Laurent, as Dematium albicans; Linossier and Roux, as a mucor, and Alav, Olsen, and Vuillemin, as Endomyces albicans. The matter is still undecided and until it is finally agreed upon it seems best to resort to the original name, Oïdium albicans.

Morphology.—The organism consists of elements that bear a close resemblance to yeast cells and multiply by budding, of hyphæ and

* See Kehrer, "Ueber den Soorpilz," etc., Heidelberg, 1883.

† See Behrend, "Deutsche med. Wochenschrift," 1890.

‡ "Histoire naturelle des vegetaux parasites qui croissent sur l'homme et sur les animaux vivants," Paris, 1853.

mycelial threads into which these grow, and of chlamydospores and conidia.

The yeast-like elements measure 5 to 6 μ in length and 4 μ in breadth. They have an oval form and cannot be distinguished from yeast cells. The mycelia are formed by elongation of these elements, some of which appear slightly elongate, some greatly elongate and slender and more or less septate, like those of the true molds. They are refractile, doubly contoured, and contain droplets, vacuoles, and granules. In the interior of the hyphæ conidia-like organs often appear, and chalmydospores are found. The latter are large, oval, doubly contoured, highly refracting, and have been seen by Plaut to germinate.

The morphology is, however, extremely varied, and the greatest differences of interpretation have been expressed regarding the different elements.

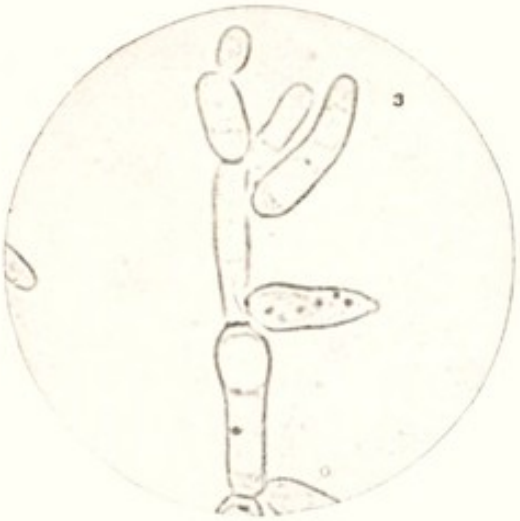


Fig. 166.—Oïdium. (Kolle and Wassermann.)

Cultivation.—The organism grows readily in artificial media, both with and without free access of oxygen. An acid reaction is most appropriate.

Colonies.—The superficial colonies upon gelatin plates are rounded, waxy, and coarsely granular. The deep colonies are irregular in shape and show feathery processes extending into the medium. The color varies according to the composition of the medium, from snow white on ordinary gelatin to meat-red on beet-root gelatin. A sour odor is given off from the cultures.

Gelatin Punctures.—Along the line of puncture there is a slow formation of rounded, feathery, colorless colonies, not unlike those shown by many molds. The gelatin is slowly liquefied only when it contains sugar. In such cultures chlamydospores are abundant.

Agar-agar.—Cultures are similar to those in gelatin.

Bouillon.—The organism grows only at the bottom of the tube in the form of yellowish-white flocculi.

Potato.—Various in different cases. Often floury.

Milk.—The organism grows very poorly in milk, which is not coagulated or fermented.

Fermentation.—The organism utilizes dextrin, mannite, alcohol, lactose, and glycerin without fermentation. Saccharose is destroyed without invertin formation. Glucose, levulose, and maltose are fermented very slowly.

Fig. 167.—Oïdium albicans. Culture in gelatin (Hansen).

Metabolic Products.—In addition to the ferments that act upon the sugars, etc., and soften the gelatin, the organism forms alcohol, aldehyd, and acetic acid.

Pathogenesis.—Animals are not known to suffer from spontaneous infection. Grawitz was able to induce thrush in puppies. Stooss inoculated the scarified vaginas of rabbits with mixed cultures of pyogenic cocci and oïdium and obtained thrush plaques. The oïdium alone was unable to secure a foothold. Döderlein, Grosset, and Stooss all succeeded in producing abscesses, sometimes by subcutaneous injection of the oïdium, but usually only when it was combined with pus cocci. In such abscesses the cocci are killed off by phagocytes, and when cultures are made only the oïdium grows. Plaut points out that this is exactly the reverse of what happens in artificial cultures of the two organisms where the cocci outgrow and kill off the oïdium.

Intravenous injection sometimes causes generalized oïdium infection, with colonies of the micro-organism in the kidneys, heart-muscle, peritoneum, liver, spleen, stomach, and intestines. The central nervous system may also show small foci of the infection.

Immunity.—Roger* and Noissette† were able to immunize animals against oïdium.

* "Compt.-rendu de la Société de Biologie," Paris, 1896.
† "Thèse de Paris," 1898.

CHAPTER XV

WHOOPING-COUGH

The Bordet-Gengou Bacillus

The subacute, contagious, undoubtedly infectious disease of childhood, characterized by periodic attacks of spasmodic cough and laryngeal spasm, terminating in a prolonged crowing inspiration and frequently followed by vomiting and prostration, known as pertussis, or whooping-cough, "Keuchhusten" (German) and "coqueleuch" (French), has long been subject to bacteriologic investigation. Deichler, Kurloff, Szemetzchenko, Cohn, Neumann, Ritter, and Afanassiew have all written upon bacteria which they supposed to be the causal factors of the disease, but which time has consigned to oblivion. Koplik* and Czaplewski and Hensel† described micro-organisms that for some years attracted attention and caused more or less discussion as to which might be the real excitant of the disease or whether they were identical organisms. As time passed, both observations lacked sufficient confirmation to carry conviction of their importance, and they, too, fell into oblivion. A still different organism was described by Vincenzi,‡ but also failed to meet sufficient confirmatory evidence to prevent it from meeting the fate of its predecessors.

Spengler,§ Kraus and Jochmann,‖ and Davis** showed the frequent presence of minute bacilli in the sputum and also in the lesions of the disease. They were, almost beyond doubt, influenza bacilli.

In 1906 Bordet and Gengou†† described a new organism whose importance was supported by such weighty evidence as the formation of an endotoxin sufficiently active to explain the symptoms, and the fixation of complement by the serum of the infected animal. This organism, therefore, presents itself as sufficiently meritorious to maintain the field for the present.

Morphology.—The organisms, as found in the sputum, occur as very minute ovoid rods of about the same size as the influenza bacillus. They measure approximately 1.5 μ in length by 0.3 μ in breadth. They do not remain united as chains or rods, but separate

* "Centralbl. f. Bakt.," etc., Sept. 15, 1897, XXII, 8 and 9, p. 222.

† "Deutsch. med. Wochenschrift," 1897, No. 57, p. 586; "Centralbl. f. Bakt.," etc., Dec. 22, 1897, XXII, Nos. 22 and 23, p. 641.

‡ "Atti della Accademia di Medicina in Torino," LXI, 5–7; "Centralbl. f. Bakt.," etc., Jan. 19, 1898, XXIII, p. 273.

§ "Deutsch. med. Wochenschrift," 1897, 830.

‖ "Zeitschrift für Hygiene," etc., 1901, XXXVI, 193.

** "Jour. Infectious Diseases," 1906, III, 1.

†† "Ann. de l'Inst. Pasteur," 1906, XX, 731.

as individuals. They are somewhat pleomorphous, yet the variations are not considerable. Involution forms are not common. There are no spores, no flagella, no motility.

Staining.—The organisms do not hold the stain well. Most of the bacilli are pale, some contain uncolored areas or vacuoles. In some cases the ends of the bacilli appear more deeply stained than the middle. They do not stain by Gram's method. The discoverers recommend that the organism be stained with—

Toluidin blue	5	Dissolve and add 500 of 5 per cent. aqueous carbolic acid. After two days filter.
Alcohol	100	
Water	500	

Isolation.—The organisms occur in almost pure cultures in the whitish expectoration which escapes from the bronchi in the beginning of the disease. Later they become few and may disappear, though the symptoms of the disease persist.

Fig. 168.—The Bordet-Gengou bacillus of whooping-cough. Twenty-four-hour-old culture upon solid media containing blood (Bordet-Gengou).

Cultivation.—The cultures were secured upon a special medium made as follows:

I.	Potato chips	1	Boil, pour off the fluid.
	4 per cent. aqueous glycerin	2	
II.	Potato extract (made as above)	50 cc.	Boil, dissolve, filter, and tube; 2 to 3 cc. to a tube.
	0.6 per cent. aqueous NaCl	150 cc.	
	Agar-agar	5 gm.	

III. To each tube add an equal volume of defibrinated rabbits' (or, better, human) blood before cooling to the point of coagulation. Permit the tubes to solidify in the oblique position.

At first the growth is scant, but upon transplantation grows better and better, until finally it may be made to grow upon other media, such as blood-agar, ascitic agar, or broth to which blood or ascitic fluid has been added. The organism is a strict aërobe. It grows best at 37°C., but also grows at temperatures as low as 5° to 10°C.

On appropriate culture-media Wollstein found it might remain alive for two months.

Metabolic Products.—An endotoxin was found by Bordet and Gengou, the method of preparing which was improved by Besredka* as follows: The growth upon agar-agar is removed with a small quantity of salt solution, dried *in vacuo*, and ground in a mortar with a small measured quantity of salt. Enough distilled water is then added to make a 0.75 per cent. solution, after which the mixture is centrifugalized and decanted. Of this preparation 1 to 2 cc. usually killed a rabbit about twenty-four hours after intravenous injection. Subcutaneous injection caused a necrosis without suppuration and without constitutional symptoms. Small quantities of the toxin placed in the rabbit's eye caused local necrosis, with little inflammatory reaction. The introduction of dead or living cultures into the peritoneal cavity of guinea-pigs caused death with great effusion and hemorrhage in the peritoneal tissues.

Pathogenesis.—Inoculation of monkeys with cultures of the bacillus failed to produce the disease. Klimenko,† however, succeeded in infecting monkeys and pups by intratracheal introduction of pure cultures. After a period of incubation an illness came on, the most marked symptoms being pyrexia and pulmonary irritation. After two or three weeks the dogs died. Postmortem examination showed catarrh of the respiratory tissues with patches of bronchopneumonia. Healthy dogs contracted the disease by contact with those suffering from the infection. Fränkel‡ obtained similar results.

The differences between the Bordet-Gengou bacillus and the influenza bacillus are not great. In size, mode of occurrence, grouping and staining there is much resemblance between the two. Culturally, however, they differ because the influenza bacillus grows best upon hemoglobin or blood agar-agar, which is less adapted for the isolation of the Bordet-Gengou bacillus than the culture-medium recommended for its cultivation, upon which the influenza bacillus does not grow well. Further, we have as differential features the peculiar endotoxin of the Bordet-Gengou bacillus, the successful infection of dogs and monkeys with the disease resembling whooping-cough, and the transmission of this infection from animal to animal by natural means.

The subject of complement deviation as a proof of the specific nature of the organism is still under consideration. Bordet and Gengou found that the serum of convalescent patients fixed complement when applied to the bacilli; Fränkel and Wollstein,§ that it did not. It is claimed by Bordet and Gengou that the difference in results came about through the employment of different culture-media in performing the complement fixation tests.

* Bordet, "Bull. de la Soc. Roy. de Bruxelles," 1907.
† "Centralbl. f. Bakt.," etc. (Orig.), XLVIII, 64.
‡ "Münchener med. Wochenschrift," 1908, p. 1683.
§ "Journal of Exp. Med.," 1909, XI, 41.

CHAPTER XVI

PNEUMONIA

LOBAR OR CROUPOUS PNEUMONIA

Diplococcus Pneumoniæ (Weichselbaum)

General Characteristics.—A minute, spheric, slightly elongate or lancet-shaped, non-motile, non-flagellate, non-sporogenous, aërobic and optionally anaërobic, non-chromogenic, non-liquefying diplococcus, pathogenic for man and he low er animals, staining by ordinary methods and by Gram's method.

"Pneumonia," while generally understood to refer to the lobar form of the disease particularly designated as *croupous pneumonia*, is a vague term, comprehending a number of quite dissimilar inflammatory conditions of the lung. This being true, no single micro-organism can be "specific" for all. Indeed, pneumonia must be conceived of as a group of diseases, and the various micro-organisms associated with it must be separately considered in connection with the particular varieties of the disease in which they occur.

The micro-organism, that can be demonstrated in at least 75 per cent. of cases of lobar pneumonia, which is almost universally accepted to be the cause of the disease, and about whose specificity very few doubts can now be raised, is the Diplococcus pneumoniæ or *pneumococcus*, of Fränkel and Weichselbaum.

Priority of discovery of the pneumococcus seems to be in favor of Sternberg,* who as early as 1880 described an apparently identical organism which he secured from his own saliva. Pasteur† seems to have cultivated the same micro-organism, also from saliva, in the same year. The researches of the observers whose names are now attached to the organism were not completed until five years later. It is to Telamon,‡ Fränkel,§ and particularly to Weichselbaum,‖ however, that we are indebted for the discovery of the relation which the organism bears to pneumonia.

Distribution.—The pneumococcus is one of a group of widely disseminated organisms of the respiratory tract. It is characterized by certain peculiarities of morphology, certain metabolic peculiarities, a definite pathogenesis, and a distinct agglutinative reaction

* "National Board of Health Bulletin," 1881, vol. II.
† "Compte-rendus Acad. des Sciences," 1881, XCII, p. 159.
‡ "Compte-rendus de la Société d' anatom. de Paris," Nov. 30, 1883.
§ "Deutsche med. Wochenschrift," 1885, 31.
‖ "Wiener med. Jahrbuch," 1886, p. 483.

with immune serum. Recent researches make it certain that some of the organisms formerly looked upon as pneumococci are different and perhaps harmless. The pneumococcus is a purely parasitic, pathogenic organism, best known to us in croupous pneumonia, where it is present in the lungs, sputum, and blood. It may be found in the saliva of a large number of healthy persons (Parke and Williams*), especially during the winter months (Longcope and Fox†), and the inoculation of human saliva into rabbits frequently causes septicemia in which the pneumococci are abundant in the blood and tissues. Its frequent occurrence in the saliva led Flügge to describe it as Bacillus septicus sputigenus. It is occasionally found in inflammatory lesions other than pneumonia, as will be pointed out below.

Morphology.—The organism is variable in morphology. When grown in bouillon it appears oval, has a pronounced disposition to occur in pairs, and not infrequently forms chains of five or six members, so that some have been disposed to look upon it as a streptococcus (Gamaléia). In the fibrinous exudate from croupous pneumonia, in the rusty sputum, and in the blood of rabbits and mice, the organisms occur in pairs, have a lanceolate shape, the pointed ends usually being approximated, and are usually surrounded by a distinct halo or capsule of clear, colorless, homogeneous material, thought by some to be a swollen cell-wall, by others a mucus-like secretion given off by the cells. When grown in culture-media, especially upon solid media, the capsules are not apparent. The elongate form has led Migula‡ to describe it under the name Bacterium pneumoniæ.

The organism measures about 1 μ in greatest diameter, is without motility, has no flagella and forms no spores.

Staining.—It stains well with the ordinary solutions of the anilin dyes, and gives most beautiful pictures in blood and tissues when stained by Gram's and Weigert's methods.

To demonstrate the capsules, the glacial acetic acid method of Welch§ may be used. The cover-glass is spread with a thin film of the material to be examined, which is dried and fixed as usual. Glacial acetic acid is dropped upon it for an instant, poured (not washed) off, and at once followed by anilin-water gentian violet, in which the staining continues several minutes, the stain being poured off and replaced several times until the acid has all been removed.

Finally, the preparation is washed in water containing 1 or 2 per cent. of sodium chlorid, and may be examined at once in the salt solution, or mounted in balsam after drying. The capsules are more distinct when the examination is made in water.

* "Jour. Exp. Med.," Aug. 7, 1905, VII, p. 403.
† Ibid., p. 430.
‡ "System der Bakterien," Jena, 1900, p. 347.
§ "Bull. of the Johns Hopkins Hospital," Dec., 1892, p. 128.

Hiss* recommends the following as an excellent method of staining the capsules of the pneumococcus: The organism is first cultivated upon ascites serum-agar to which 1 per cent. of glucose is added. The drop containing the bacteria to be stained is spread upon a cover-glass mixed with a drop of serum or a drop of the fluid culture-medium, and dried and fixed. A half-saturated aqueous solution of gentian violet is applied for a few seconds and then washed off in a 25 per cent. solution of carbonate of magnesium. The preparation is then mounted in a drop of the latter solution and examined.

If it is desired to stain the capsules and preserve the specimens permanently in balsam, Hiss employs a 5 or 10 per cent. solution of fuchsin or gentian violet (5 cc. saturated alcoholic solution of dye in 95 cc. of distilled water). The stain is applied to the fixed

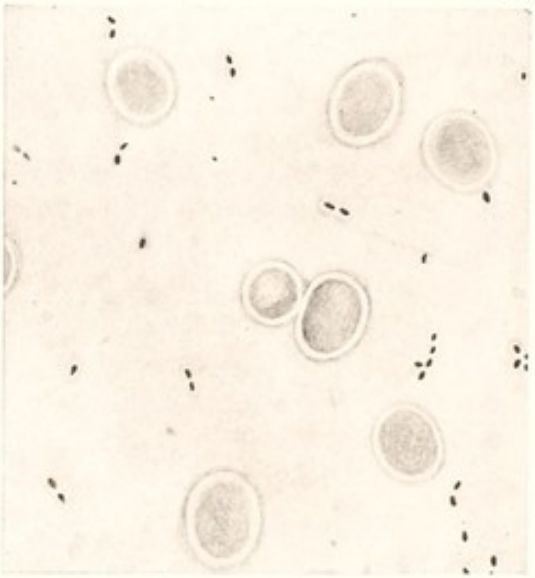


Fig. 169.—Capsulated pneumococci in blood from the heart of a rabbit; carbol-fuchsin, partly decolorized. × 1000.

specimen and heated until it begins to steam, when the stain is washed off in a 20 per cent. solution of crystals of sulphate of copper. The preparation is then dried and mounted in balsam.

Hiss finds this stain a useful aid in differentiating the pneumococcus from the streptococcus, with which it is easily confounded if the capsules are not distinct, and to which it is probably closely related.

Isolation.—When desired for purposes of study, the pneumococcus may be obtained by inoculating white mice with pneumonic sputum and recovering the organisms from the heart's blood, or it may be obtained from the rusty sputum of pneumonia by the method employed by Kitasato for securing tubercle bacilli from sputum: A mouthful of fresh sputum is washed in several changes of sterile

* Abstract, "Centralbl. f. Bakt. u. Parasitenk.," Bd. XXXI, No. 10, p. 302, March 24, 1902. More complete details appear in a later paper in the "Journal of Experimental Medicine," VI, p. 338.

water to free it from the bacteria of the mouth and pharynx, carefully separated, and a minute portion from the center transferred to an appropriate culture-medium.

Buerger,* in conducting a research upon pneumococcus and allied organisms with reference to their occurrence in the human mouth, under the auspices of the Rockefeller Institute, used a 2 per cent. glucose-agar of a *neutral*, or, at most, 0.5 per cent. phenolphthalein acid titer.

"The medium was usually made from meat infusion and contained 1.5 to 2 per cent. peptone and 2.4 per cent. agar. Stock plates of these media (serum-agar and 2 per cent. glucose-serum-agar) were poured. The agar or glucose-agar was melted in large tubes and allowed to cool down to a temperature below the coagulation point of the serum. One-third volume of rich albuminous ascitic fluid was added, and the resulting media poured into Petri plates. These were tested by incubation and stored in the ice-chest ready for use. . . .

"The plan finally adopted [for inoculating the plates] was as follows: A swab taken from the mouth was thoroughly shaken in a tube of neutral bouillon. From this primary tube, dilutions in bouillon with four, six, and eight loops may be made. A small portion of the dilute mixture was poured at a point near the periphery of the prepared plates. By a slight tilting motion the fluid was carefully distributed over the whole surface of the plates. Care must be taken to avoid an excess of fluid. It was found that plates made in this way gave a sufficiently thick and discrete distribution of surface colonies."

Cultivation.—The organism grows upon all the culture-media except potato, but only between the temperature extremes of 24° and 42°C., the best development being at about 37°C. The growth is always meager, probably because of the metabolic formation of formic acid. The addition of alkali to the culture-medium favors the growth of the pneumococcus by neutralizing this acid. Hiss and Zinsser† advise that the culture-media used for the pneumococcus be made with 3 to 4 per cent. of peptone.

Colonies.—The colonies which develop at 24°C. upon gelatin plates (15 per cent. of gelatin should be used to prevent melting at the temperature required) are described as small, round, circumscribed, finely granular white points which grow slowly, never attain any considerable size, and do not liquefy the gelatin.

If agar-agar be used instead of gelatin, and the plates kept at the temperature of the body, the colonies appear transparent, delicate, and dewdrop-like, scarcely visible to the naked eye, but under the microscope appear distinctly granular, a dark center being surrounded by a paler marginal zone.

Upon the medium recommended by Buerger for isolating the pneumococcus, the colonies appear in from eighteen to twenty-four hours, the surface colonies being circular and disk-like. When viewed from above, the surface appears glassy with a depressed center. When viewed from the side or by transmitted light, they appear as distinct milky rings with a transparent center. This

* "Jour. Exp. Med.," Aug. 25, 1905, VII, No. 5.
† "Text-book of Bacteriology," 1910, p. 356.

"ring type" is regarded as characteristic and enables the organism to be separated without difficulty from the streptococcus.

Gelatin Punctures.—In gelatin puncture cultures, made with 15 instead of the usual 10 per cent. of gelatin, the growth takes place along the entire puncture in the form of minute whitish granules distinctly separated from one another. The growth in gelatin is always meager. The medium is not liquefied.

Agar-agar and Blood-serum.—Upon agar-agar and blood-serum the growth consists of minute, transparent, semi-confluent, colorless, dewdrop-like colonies. The medium is not liquefied. Upon glycerin agar-agar the growth is more luxuriant. The addition of a very small percentage of blood-serum facilitates growth.

Bouillon.—In bouillon the organisms grow well, slightly clouding the medium. With the death of the organisms and their sedimentation, the medium clears again after a few days.

Milk.—Milk is an appropriate culture-medium, its casein being coagulated. Alkaline litmus milk is slowly acidified.

Potato.—The pneumococcus does not grow upon potato.*

Vital Resistance.—The organism usually dies after a few days of artificial cultivation, and so must be transplanted every three or four days. In rabbit's blood, in sealed tubes kept cold, it can sometimes be kept alive for several weeks. Hiss and Zinsser† find that when the organism is planted in "calcium-carbonate-infusion broth" and kept in the ice-chest, the cultures often remain alive for several months. Bordoni-Uffreduzzi‡ found that when pneumococci were dried in sputum attached to clothing, and were exposed freely to the light and air, they retained their virulence for rabbits for from nineteen to ninety-five days. Direct sunlight destroyed their virulence in twelve hours. Guarniere§ found that dried blood containing pneumococci remained virulent for months.

The pneumococcus is destroyed in ten minutes by a temperature of 52°C. It is highly sensitive to all disinfectants, weak solutions quickly killing it.

Metabolic Products.—Hiss‖ found that the pneumococcus produces acid from monosaccharids, disaccharids, and such complex saccharids as dextrin, glycogen, starch, and inulin. The fermentation of inulin by pneumococci is a most important means of differentiating it from streptococci.

Toxic Products.—Nothing definite is known about the metabolic toxic products of the pneumococcus.

Auld** found that if a thin layer of prepared chalk were placed

* Ortmann asserts that the pneumococcus can be grown on potato at 37°C., but this is not generally admitted. The usual acid reaction of potato makes it an unsuitable culture-medium.

† *Loc. cit.*

‡ "Arch. p. l. Sc. Med.," 1891, XV.

§ "Atti della R. Acad. Med. di Roma," 1888, IV.

‖ "Jour. Exp. Med.," VII, No. 5, Aug. 25, 1905.

** "Brit. Med. Jour.," Jan. 20, 1900.

upon the bottom of the culture-glass, it neutralized the lactic acid produced by the pneumococcus, and enabled it to grow better and produce much stronger toxin. Macfadyen* found that by freezing cultures of the pneumococcus with liquid air, destroying them by trituration in the frozen state and then extracting the fragments with 1 : 1000 caustic potash solution, a toxin whose activity corresponded fairly well with the virulence of the culture could be secured. This toxin killed rabbits and guinea-pigs in doses varying from 0.5 to 1 cc.

It is undoubtedly an endotoxin that is liberated from the bodies of the pneumococci as they undergo autolysis or are dissolved by the enzymic action of the body juices or the cells. The toxin liberated by autolysis has been carefully studied by Rosenow,† who "finds it soluble in ether. It is formed during retrogressive changes in the pneumococci. Heating the clear autolysate to 60°C. for twenty minutes destroys it, while toxic pneumococcus suspensions remain toxic even after boiling. Hydrochloric acid in weak solutions destroys the toxicity of pneumococcus autolysates. The toxic substance is absorbed by blood charcoal from which it can again be obtained by shaking with ether. Autolyzed virulent pneumococci and non-virulent pneumonia diminish the toxicity slightly while unautolyzed virulent pneumococci increase it. The toxic substance is probably a base which contains amino groups of nitrogen. Indications have been obtained showing that during pneumococcus infections toxic substances are produced that do not call forth any immunizing response." Rosenow‡ found that the autolysate contained a proteolytic enzyme. He also found§ that it was capable of producing, in dogs, symptoms strikingly like anaphylaxis, with a striking drop in the blood pressure, pronounced hemorrhages, marked depression of respiration, extreme cyanosis and the presence of CO_2 in the stomach.

Pathogenesis.—If a small quantity of a pure culture of the virulent organism be introduced into a mouse, rabbit, or guinea-pig, the animal dies in one or two days. Exactly the same result can be obtained by the introduction of a piece of the lung-tissue from croupous pneumonia, by the introduction of some of the rusty sputum, and frequently by the introduction of human saliva. Postmortem examination of infected animals shows an inflammatory change at the point of subcutaneous inoculation, with a fibrinous exudate similar to that succeeding subcutaneous inoculation with the diphtheria bacillus. At times, and especially in dogs, a little pus may be found. The spleen is enlarged, firm, and red-brown. The blood with which the cavities of the heart are filled is firmly coagulated, and, like that in other organs of the body, contains large numbers of the bacteria,

* Ibid., 1906, II.
† "Journal of Infectious Diseases," 1912, X. 94, 235.
‡ "Journal of Infectious Diseases," 1912, X. 287.
§ Ibid., p. 480.

most of which exhibit a lanceolate form and have distinct capsules. The disease is thus shown to be a bacteremia unassociated with conspicuous tissue changes.

In such cases the lungs show no consolidation. Even if the inoculation be made by a hypodermic needle plunged through the breast-wall into the pulmonary tissue, pneumonia rarely results. Gamaléia* reported that pneumonic consolidation of the lungs of

Fig. 170.—Lung of a child, showing the appearance of the organ in the stage of red hepatization of croupous pneumonia. The pneumonia has been preceded by chronic pleuritis, which accounts for the thickened fibrous trabeculæ extending into the tissue, and which may have had something to do with the peculiarly prominent appearance of the bronchioles throughout the lung.

dogs and sheep could be brought about by injecting the pneumococcus through the chest-wall into the lung. Tchistowitsch† stated that by intratracheal injections of cultures into dogs he succeeded in producing in 7 out of 19 experiments typical pneumonic lesions. Monti‡ claimed to have found that a characteristic croupous pneu-

* "Ann. de l'Inst. Pasteur," 1888, II, 440.
† Ibid., 1890, III, 285.
‡ "Zeitschrift für Hygiene," etc., 1892, XI, 387.

monia results from the injection of cultures into the trachea of susceptible animals. A very interesting review of the literature of the experimental aspects of the subject, embracing 198 references, will be found in Wadsworth's paper upon "Experimental Studies on the Etiology of Acute Pneumonitis."*

The final proof that true pulmonary consolidation, *i.e.*, pneumonia, can be produced experimentally by cultures of the pneumococcus is to be found in a paper by Lamar and Meltzer.† These investigators etherized dogs, kept the mouth open by means of a large wooden gag, drew the tongue forward by means of hemostatic forceps, and then, seizing the median glosso-epiglottic fold, pulled it forward so that the posterior aspect of the epiglottis presented an inclined plane. Into this concavity one end of a tube is placed. Under the protection of the left index-finger the tube was directed into the larynx and pushed down slowly and gently through the trachea until a resistance was met with. The inner end of the tube was then found to engage in a bronchus—usually the right bronchus. A pipette containing a liquid culture of the pneumococcus was next attached to the external end of the tube, and by means of a syringe the culture (about 6 cc.) was injected into the bronchus. The syringe was then removed, the piston withdrawn, and the syringe again attached to the pipette. By the injection of air the culture was driven deeper into the bronchi. The tube was then clamped and withdrawn and the animal released. By these means experimental pneumonia, with the typical consolidation and lobar distribution, was produced in 42 successive cases. The course of the inflammatory disturbance thus produced was rapid, and in one case nearly complete consolidation had occurred in seven hours.

Lesions.—The lesions of croupous pneumonia of man are almost too well known to need description. The distribution of the disease conforms more or less perfectly to the divisions of the lung into lobes, one or more lobes being affected. An entire lung may be affected, though, as a rule, the apex escapes consolidation and is simply congested. The invaded portion of the lung is supposed to pass through a succession of stages clinically described as (1) congestion, (2) red hepatization, (3) gray hepatization, and (4) resolution. In the first stage bloody serum is poured out into the air-cells, filling them with a viscid reddish exudate. In the second stage this coagulates so that the tissue becomes solid, airless, and approximately like liver tissue in appearance. The third stage is characterized by dissolution of the erythrocytes and invasion of the diseased air-cells by leukocytes, so that the color of the tissue changes from red to gray. At the same time the coagulated exudate begins to soften and leave the air-cells by the natural passages, and the stage of resolution begins.

* "Jour. Amer. Med. Sciences," 1904, CXXVII, p. 851.
† "Jour. Exp. Med.," 1912, XV, No. 2, p. 133.

The pneumococci, though present in enormous numbers in the pulmonary lesions, are not confined to them. In practically all cases pneumonia is a blood infection (bacteremia) as well as a pulmonary infection. It is through the blood infection that many of the complications and sequelæ of the disease are brought about.

The pneumococcus is not infrequently discovered in diseased conditions other than croupous pneumonia; thus, Foa, Bordoni-Uffreduzzi, and others found it in cerebro-spinal meningitis; Fränkel, in pleuritis; Weichselbaum, in peritonitis; Banti, in pericarditis; numerous observers, in acute abscesses; Gabbi isolated it from a case of suppurative tonsillitis; Axenfeld observed an epidemic of conjunctivitis caused by it; Zaufal, Levy, and Schröder and Netter have been able to demonstrate it in the pus of otitis media, and Foulerton and Bonney* isolated it from a case of primary infection of the puerperal uterus. It has also been found in arthritis following pneumonia, and in primary arthritis without previous pneumonia by Howard.†

Interesting statistics concerning the relative frequency of pneumococcus infections in adults given by Netter‡ are as follows:

Pneumonia	65.95
Broncho-pneumonia	15.85
Meningitis	13.00
Empyema	8.53
Otitis media	2.44
Endocarditis	1.22
Hepatic abscess	1.22

In 46 consecutive pneumococcus infections of children he found:

Otitis media	29
Broncho-pneumonia	12
Meningitis	2
Pneumonia	1
Pleurisy	1
Pericarditis	1

Susceptibility.—Not all animals are equally susceptible to the action of the pneumococcus. Mice and rabbits are highly sensitive; dogs, guinea-pigs, cats, and rats are much less susceptible, though they may also succumb to the inoculation of large doses.

Specificity.—The etiologic relationship of the pneumococcus to pneumonia is based chiefly upon the frequency of its presence in croupous pneumonia. Netter§ found it 82 times in 82 autopsies upon such cases; Klemperer, 21 times out of 21 cases studied by puncturing the lung with a hypodermic syringe. Weichselbaum obtained it in 94 out of 129 cases; Wolf, in 66 out of 70; and Pierce, in 110 out of 121 cases. In about 5 per cent. of the cases it remains localized in the respiratory apparatus; in 95 per cent., it invades the

* "Trans. Obstet. Soc. of London," 1903, part II, p. 128.
† "Johns Hopkins Hospital Bulletin," Nov., 1903.
‡ "Compte-rendu," 1889.
§ "Compte-rendu," 1889.

blood. An interesting paper upon this subject has been written by E. C. Rosenow.*

The conditions under which it enters the lung to produce pneumonia are not known. It is probable that some systemic depravity is necessary to establish susceptibility, and in support of this view we may point out that pneumonia is very frequent, and exceptionally severe and fatal, among drunkards, and that it is the most frequent cause of death among the aged. Whether, however, any particular form of vital depression is necessary to predispose to the disease, further study will be required to tell.

Virulence.—Pneumococci vary greatly in virulence, and rapidly lose this quality in artificial culture. When it is desired to maintain or increase the virulence, a culture must be frequently passed through animals. Washbourn found, however, that a pneumococcus isolated

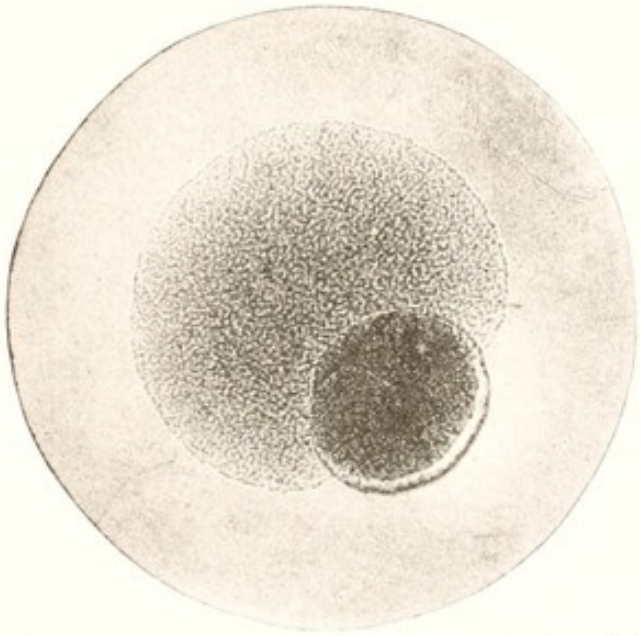


Fig. 171.—Diplococcus pneumoniæ. Colony twenty-four hours old upon gelatin. × 100 (Fränkel and Pfeiffer).

from pneumonic sputum and passed through one mouse and nine rabbits developed a permanent virulence when kept on agar-agar so made that it was not heated beyond 100°C., and alkalinized 4 cc. of normal caustic soda solution to each liter beyond the neutral point determined with rosolic acid. The agar-agar is first streaked with sterile rabbit's blood, then inoculated. The cultures are kept at 37.5°C. Ordinarily pneumococci seem unable to accommodate themselves to a purely saprophytic life, and unless continually transplanted to new media die in a week or two, sometimes sooner. Lambert found, however, that in Marmorek's mixture (bouillon 2 parts and ascitic or pleuritic fluid 1 part) the organisms would sometimes remain alive as long as eight months, preserving their virulence during the entire time.

* "Jour. Infectious Diseases," 1904, 1, p. 280.

Virulence can also be retained for a considerable time by keeping the organisms in the blood from an infected rabbit, hermetically sealed in a glass tube, on ice.

Bacteriologic Diagnosis.—It is usually unnecessary to call upon the bacteriologist to assist in making the diagnosis of pneumonia. If, for any reason it be considered necessary, three means are available: 1, the blood culture; 2, the inoculation of animals with the expectoration; 3, the cultivation of the organism from the expectoration.

1. To make the blood culture, the elbow is encircled with a band, the skin washed and after an application of iodine has been made, a hollow needle is introduced into one of the distended veins, and the blood permitted to drop into a small flask or tube of appropriate media.

2. To inoculate an animal with the sputum, or with fluid drawn from the lung or pleura. A white mouse or a rabbit can be selected as suitable. Both animals are so susceptible that the introduction of one drop beneath the skin is usually fatal in twenty-four to forty-eight hours.

Caution must be exercised in using this means of diagnosis, however, as the pneumococcus sometimes occurs in normal saliva, and is a common associated organism in tuberculosis and other respiratory diseases.

3. The recovery of the organism from the sputum can be accomplished by stroking appropriate media with a platinum wire dipped in the sputum. The characteristic colonies can be picked up and transplanted as soon as they appear.

Identification of the Organism.—Wadsworth* has been able to show that agglutination reactions can be obtained by concentrating the pneumococci in isotonic solution and adding the serum. The method does not seem easily applicable for diagnosis. Neufeld† and Wadsworth‡ have also found that when rabbit's bile is added to a pneumococcus culture so as to produce lysis of the organisms, the addition of pneumococcus-immune serum to the clear fluid so obtained results in a specific precipitation. This seems to have little practical importance, however, for purposes of diagnosis. It is, however, of some importance in assisting in the recognition of the pneumococcus and differentiating it from the streptococcus, for when the latter organisms are similarly treated no precipitate takes place.

Buerger§ found that all pneumococci, irrespective of source, were agglutinated by pneumococcus-immune serum, that such serum was capable of agglutinating various pyogenic streptococci, certain atypical organisms, and certain strains of Streptococcus mucosus capsulatus. The sera of pneumonia patients varies in its power to

* "Jour. Med. Research," 1904, X, p. 228.
† "Zetschrift für Hygiene," 1902, XI.
‡ *Loc. cit.*
§ "Jour. Exp. Med.," Aug. 25, 1905, VII, No. 5.

agglutinate different pneumococci; some strains were agglutinated, others not. The sera of normal individuals and of normal rabbits possess no agglutinating power for pneumococci, the atypical organisms, certain streptococci, or Streptococcus mucosus capsulatus.

As pneumococci sometimes grow in chains instead of in pairs, and as the capsules are not always more distinct than the capsules that sometimes surround streptococci, it may be necessary to resort to special methods of cultivation for the final identification of the organism. One of the first to be recommended is the use of the blood-agar plate, to which reference has been made in the section upon Streptococcus pyogenes.

A second important method, and one that not only differentiates the pneumococcus from the streptococcus, but from the common organisms of similar morphology that infect the mouth, is the *inulin-serum water* fermentation test of Hiss.* In using this medium, Ruediger† found it best prepared as follows: Dissolve 5 gm. of NaCl, 20 gm. of Witte's peptone, and 20 gm. of pure inulin in 1000 cc. of distilled water. Add 20 cc. of a 5 per cent. solution of pure litmus, and tube, putting 2 cc. of the mixture into each tube, and sterilize in the autoclave. After sterilization add (with a sterile pipet) 2 cc. of sterile, heated ascitic fluid, or, preferably, heated beef-serum, to each tube, and incubate twenty-four hours before using. Great care must be taken not to use ascitic fluid that contains fermentable carbohydrates. Each lot must be tested with some strongly fermentative bacterium, and the absence of fermentable carbohydrates proved. Ruediger prefers this preparation to the original solution of Hiss because he found that some pneumococci would not grow on the latter. Fermentation of the inulin is regarded as characteristic of the pneumococcus.

The pneumococcus produces red colonies upon litmus-inulin-agar plates, which makes their use desirable when pneumococci are to be isolated from saliva, throat secretions, or other material in which similar appearing organisms are apt to occur. Ruediger found no other mouth bacteria that produced red colonies on these plates.

Immunity.—Pneumonia is peculiar in that the disease in human beings terminates by crisis as though from some source a supply of antitoxin or other immunizing agent was suddenly liberated, but unfortunately also in that recovery is followed by immunity of such brief duration as to permit the occurrence of frequent relapses. It is also well known that many cases show a subsequent predisposition to fresh attacks of the disease.

Immune Serum.—G. and F. Klemperer‡ have shown that the serum of rabbits immunized against the pneumococcus protects

* "Jour. Amer. Med. Assoc.," 1906, vol. XLVII, p. 1171.
† "Jour. of Exp. Med.," 1905, vol. VI, p. 317.
‡ "Berliner klin. Wochenschrift," 1891, Nos. 34 and 35.

animals infected with virulent cultures. When applied to human medicine, the serum failed to do good.

The treatment of pneumonia by the injection of blood-serum from convalescent patients, tried by Hughes and Carter,* has been abandoned as useless and dangerous.

Antipneumococcic sera have been experimentally investigated by De Renzi,† Washbourn,‡ and Pane.§

Washbourn prepared an *antipneumococcus serum* that protected rabbits, against ten times the fatal dose of live pneumococci, in doses of 0.3 cc. In general, the lines upon which he operated were those of Behring, Marmorek's work with the streptococcus furnishing most of the details. Two cases of human pneumonia seem to have derived some benefit from large doses of this serum. The sera of Pane and De Renzi were not so powerful as those of Washbourn, requiring about 1 cc. to protect a rabbit.

McFarland and Lincoln‖ succeeded in immunizing a horse against large doses of a virulent culture of the pneumococcus, and obtained a serum of which 0.5 to 0.25 cc. protected rabbits from many times the fatal dose.

The experiments by Pässler** showed some gain over the earlier work.

The antipneumococcic sera thus far produced have given disappointing results in clinical application.

A leukocytic extract prepared by Hiss and Zinsser†† from an aleuronat exudation in the rabbit's pleura has led to results sufficiently encouraging in the treatment of pneumonia in man to warrant further investigation along similar lines.

Rosenow‡‡ found that pneumococci suspended in sodium chlorid solutions autolyse rapidly. By means of this autolysis it is possible to separate, at least to a large degree, the toxic from the antigenic parts of the pneumococcus, as the toxic part goes into solution. The injection of the non-toxic and, as it appears, antigenic portion—autolyzed pneumococci—causes a marked increase in the immunity curve as measured by the specific increase in pneumococcus opsonin. The injection of such autolyzed pneumococci into 25 patients with lobar pneumonia seemed to have a marked beneficial effect.

Sanitation.—Pneumonia is undoubtedly a transmissible disease. Exactly how infection takes place is not known, but seeing that the infectious agent is in the respiratory tract, from which it is easily discharged into the atmosphere during cough, etc., and the facility

* "Therapeutic Gazette," Oct. 15, 1892.
† "Il Policlinico," Oct. 31, 1896, Supplement.
‡ "Brit. Med. Jour.," Feb. 27, 1897, p. 510.
§ "Centralbl. f. Bakt. u. Parasitenk.," May 29, 1897, XXI, 17 and 18, p. 664.
‖ "Jour. Amer. Med. Assoc.," Dec. 16, 1899, p. 1534.
** "Deutsches Archiv für klin. Med.," Bd. 1905; LXXXII, Nos. 3, 4, "Jour. Amer. Med. Assoc.," May 13, 1905, p. 1538.
†† "Jour. Med. Research," 1908, XIX, 323.
‡‡ "Jour. Amer. Med. Assoc.," June 10, LIV, No. 24, p. 1943.

with which it can then be inhaled by those nearby, it seems justifiable to conclude that the primary entrance of the organism into the body is through the respiratory tract. Wood* has shown that "the organisms in the sputum do not remain long in suspension and die off rapidly under the action of light and desiccation. In sunlight or diffuse daylight the bacteria in such powder die within an hour, and in about four hours if kept in the dark. The danger of infection from powdered sputum may, therefore, be avoided by ample illumination and ventilation of the sick-room in order to destroy or dilute the bacteria, and by the avoidance of dry sweeping or dusting. Articles which may be contaminated and which cannot be cleaned by cloths dampened in a suitable disinfectant should be removed from the patient's vicinity.

Pneumococcus (Friedländer)—Bacterium Pneumoniæ (Zopf†)—Bacillus Capsulatus Mucosus (Fasching‡)

General Characteristics.—An encapsulated, non-motile, non-flagellated, non-sporogenous, non-liquefying, aërobic and optionally anaërobic, non-chromogenic, aërogenic and pathogenic organism, staining by ordinary methods but not by Gram's method.

This organism was discovered by Friedländer§ in 1883 in the pulmonary exudate from a case of croupous pneumonia, and, being thought by its discoverer to be the cause of that disease, was called the pneumococcus, and later the *pneumobacillus*. The grounds upon which the specificity of the organism was supposed to depend were soon found to be insufficient, and the organism of Friedländer is at present looked upon as one whose presence in the lung is, in most cases, unimportant, though it is sometimes associated with and is probably the cause of a special form of pneumonia, which, according to Stuhlern,‖ is clinically atypical and commonly fatal. Fränkel points out that Friedländer's error in supposing his organism to be the chief parasite in pneumonia depended upon the fact that his studies were made by the plate method, which permitted the discovery of this bacillus to be made more easily than that of the slowly growing and more delicate pneumococcus. In the light of present knowledge Friedländer's bacillus must be looked upon as *the type of a group* of organisms varying among themselves in many minor particulars.

Distribution.—The organism is sometimes found in normal saliva; it is a common parasite of the respiratory apparatus; not infrequently occurs in purulent accumulations; is occasionally found in feces, and sometimes occurs under external saprophytic conditions. Thus it is probably identical with the "capsulated canal-water bacillus" by

* "Jour. Exp. Med.," Aug. 25, 1905, VII, No. 5, p. 624.
† "Spaltpilze," 1885, p. 66.
‡ "Centralbl. f. Bakt.," 1892, etc., XII, p. 304.
§ "Fortshritte der Medizin," 1883, 22, 715.
‖ "Centralbl. f. Bakt.," etc. (Originale), July 21, 1904, Bd. XXXVI, No. 4, p. 493.

Mori,* and may belong to the same group in which we find Bacillus aërogenes capsulatus.

Morphology.—Though usually distinctly bacillary in form, the organism is of variable length and when paired sometimes bears a close resemblance to the pneumococcus of Fränkel and Weichselbaum. It measures 0.5 to 1.5 μ in breadth and 0.6 to 0.5 μ in length. It frequently occurs in chains of four or more elements and occasionally appears elongated. It is these variations in form that have led to the description of the organism by different writers as a coccus, a bacterium, and a bacillus. It is commonly surrounded by a distinct transparent capsule, hence its name "capsule bacillus" and Bacillus capsulatus mucosus. The organism is non-motile, has no spores, and no flagella. It stains well with the ordinary anilin dyes, but does not retain the color when stained by Gram's method.

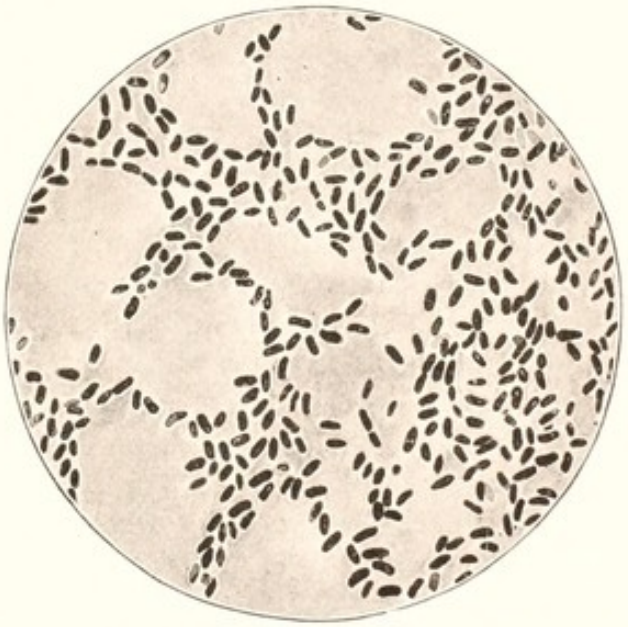


Fig. 172.—Bacterium pneumoniæ (modified after Migula).

Cultivation.—Colonies.—If pneumonic exudate be mixed with gelatin and poured upon plates, small white spheric colonies appear at the end of twenty-four hours, and spread out upon the surface of the gelatin to form whitish masses of a considerable size. Under the microscope these colonies appear irregular in outline and somewhat granular. The gelatin is not liquefied.

Bouillon.—There is nothing characteristic about the bouillon cultures of Friedländer's bacillus. The medium is diffusely clouded. A pellicle usually forms on the surface and a viscid sediment soon accumulates.

Gelatin Puncture.—When a colony is transferred to a gelatin puncture culture, a luxuriant growth occurs. Upon the surface a somewhat elevated, rounded white mass is formed, and in the track

* "Zeitschrift für Hygiene," 1888, IV, p. 53.

of the wire innumerable little colonies spring up and become confluent, so that a "nail-growth" results. No liquefaction of the gelatin occurs. Gas bubbles not infrequently appear in the wire track. The cultures sometimes become brown in color when old.

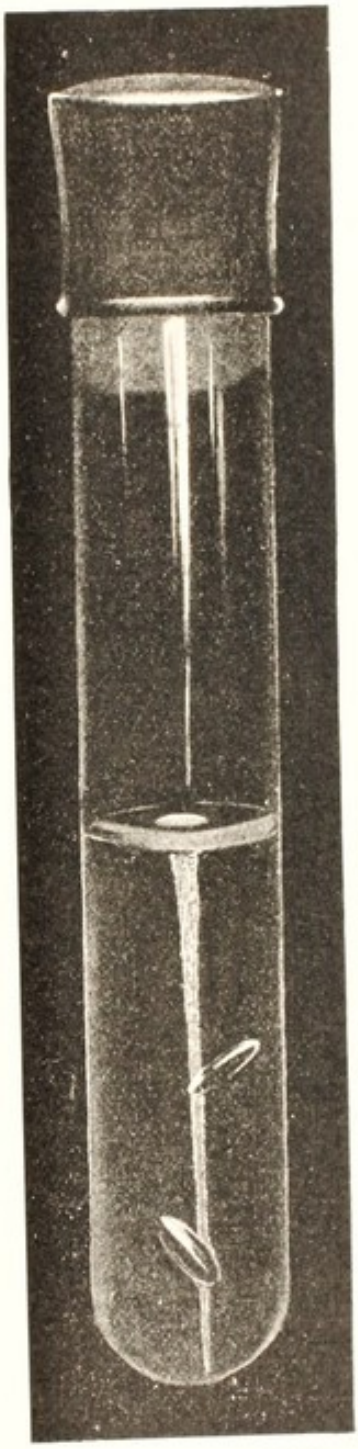


Fig. 173.—Friedländer's pneumobacillus; gelatin stab culture, showing the typical nail-head appearance and the formation of gas bubbles, not always present (Curtis).

Agar-agar.—Upon the surface of agar-agar at ordinary temperatures a luxuriant white or brownish-yellow, smeary, viscid, circumscribed growth occurs.

Blood-serum.—The blood-serum growth is similar to that upon agar.

Potato.—Upon potato the growth is luxuriant, quickly covering the entire surface with a thick yellowish-white layer, which sometimes contains bubbles of gas.

Milk is not coagulated as a rule. Litmus milk is reddened.

Vital Resistance.—The bacillus grows at a temperature as low as 16°C., and, according to Sternberg, has a thermal death-point of 56°C.

Metabolic Products.—Friedländer's bacillus ferments nearly all the sugars, with the evolution of much gas. It generates alcohol, acetic and other acids, and both CO_2 and H. According to the best authorities the organism does not form indol. There is, however, some difference of opinion upon the subject.

Perkins* divides the organisms of this group into three chief types according to their reactions toward carbohydrates:

I. Bacillus aërogenes type which ferment all carbohydrates, with the formation of gas.

II. Bacillus pneumoniæ (Friedländer) type which ferment all carbohydrates *except lactose*, with formation of gas.

III. Bacillus lactis aërogenes type which ferment all carbohydrates *except saccharose*, with formation of gas.

Pathogenesis.—Friedländer found considerable difficulty in producing pathogenic changes by the injection of his bacillus into the lower animals.

* "Jour. of Infect. Dis.," 1904, 1, No. 2, p. 241.

Rabbits and guinea-pigs were immune to its action, and the only important pathogenic effects that Friedländer observed occurred in mice, into whose lungs and pleura he injected the cultures, with resulting inflammation.

That Friedländer's bacillus may be the cause of true lobar pneumonia there can be no room for doubt after the demonstrations of Lamar and Meltzer,* who found that its experimental introduction into the bronchi of dogs was followed by true lobar pneumonia. The lesions in these dogs, like those in human beings, were paler in color, the lung tissue less friable, and the exudate more viscid than those caused by the pneumococcus.

Pneumonia in man, caused by Bacillus mucosus capsulatus, is atypical clinically, very severe, and often fatal.

Curry† found Friedländer's bacillus in association with the pneumococcus in acute lobar pneumonia; in association with the diphtheria bacillus in otitis media associated with croupous pneumonia; and in the throat in diphtheria. In pure culture it was obtained from vegetations upon the valves of the heart in a case of acute endocarditis with gangrene of the lung; from the middle ear, in a case of fracture of the skull with otitis media; and from the throat in a case of tonsillitis. Zinsser has twice cultivated Friedländer's bacillus from inflamed tonsils in children.

Abel‡ cultivated it from the discharges of fetid ozena, and supposed it to be the specific cause.

Occasionally Friedländer's bacillus bears an important relationship to lobular or catarrhal pneumonia, an interesting case having been studied by Smith.§ The histologic changes in the lung were remarkable in that the "alveolar spaces of the consolidated areas were dilated and for the most part filled with the capsule bacilli." In some alveoli there seemed to be pure cultures of the bacilli; others contained red and white blood-corpuscles; in some there was a little fibrin. The bacillus obtained from this case, when injected into the peritoneal cavity of guinea-pigs, produced death in eleven hours. The peritoneal cavity after death contained a large amount of thick, slimy fluid; the intestines were injected and showed a thin fibrinous exudate upon the surface; the spleen was enlarged and softened, and the adrenals much reddened. Cover-glass preparations from the heart, blood, spleen, and peritoneal cavity showed large numbers of the capsule bacilli.

Howard‖ has also called attention to the importance of this bacillus in connection with numerous acute and chronic infectious processes, among which may be mentioned croupous pneumonia, suppura-

* "Jour. Exp. Med.," 1912, XV, 133.
† "Jour. Boston Soc. of Med. Sci.," March, 1898, vol. II, No. 8, p. 137.
‡ "Zeitschrift für Hygiene," XXI.
§ "Jour. Boston Soc. of Med. Sci.," May, 1898, vol. II, No. 10, p. 174.
‖ "Phila. Med. Jour.," Feb. 19, 1898, vol. I, No. 8, p. 336.

tion of the antrum of Highmore and frontal sinuses, endometritis, perirenal abscesses, and peritonitis.

Virulence.—The virulence of the organism seems to vary under different conditions. It is sometimes harmless for the experiment animals, but when injected into mice and guinea-pigs usually produces local inflammatory lesions, and sometimes death from septic invasion.

CATARRHAL PNEUMONIA OR BRONCHO-PNEUMONIA

This form of pulmonary inflammation occurs in local areas, commonly situated about the distribution of a bronchiole. It cannot be said to have a specific micro-organism, as almost any irritating foreign matter accidentally inhaled may cause it. The majority of the cases, however, are infectious in nature and result from the inspiration, from higher parts of the respiratory apparatus, of the staphylococci and streptococci of suppuration, Friedländer's bacillus, the bacillus of influenza, and other well-known organisms.

TUBERCULOUS PNEUMONIA

The progress of pulmonary tuberculosis is at times so rapid that the tubercle bacilli are distributed with the softened infectious matter throughout the entire lung or to large parts of it, and a distinct pneumonic inflammation occurs. Such a pneumonia may be caused by the tubercle bacillus, or the tubercle bacillus together with staphylococci, streptococci, tetragenococci, pneumococci, pneumobacilli, and other organisms accidentally present in a lung in which ulceration and cavity formation are advanced.

PLAGUE PNEUMONIA

The pneumonic form of plague is characterized by consolidation of the lung histologically and anatomically, indistinguishable from pneumococcic and other extensive pulmonary infections.

MIXED PNEUMONIAS

It frequently happens that pneumonia occurs in the course of influenza or shortly after convalescence from it. In these cases a mixed infection by the influenza bacilli and pneumococci is commonly found. Sometimes pneumococci and staphylococci simultaneously affect the lung, purulent pneumonia with abscess formation being the conspicuous feature. Almost any combination of bacteria may occur in the lungs, so that it must be left for the student to work out what the particular effects of each may be.

Among the mixed forms of pneumonia may be mentioned those called by Klemperer and Levy "complicating pneumonias," occurring in the course of typhoid fever, etc.

CHAPTER XVII

INFLUENZA

Bacillus Influenzæ (R. Pfeiffer)

General Characteristics.—A minute, non-motile, non-flagellated, non-sporogenous, non-liquefying, non-chromogenic, aërobic, pathogenic bacillus, staining by the ordinary methods, but not by Gram's method, and susceptible of artificial cultivation, chiefly through the addition of hemoglobin to the culture-media.

Notwithstanding the number of examinations conducted to determine the cause of influenza, it was not until 1892, after the great epidemic, that Pfeiffer* found, in the blood and purulent bronchial discharges, a bacillus that conformed, in large part, to the requirements of specificity.

Morphology.—The bacilli are very small, having about the same diameter as the bacillus of mouse septicemia, but only half its length (0.2 by 0.5 μ). They are usually solitary, but may be united in chains of three or four.

They are non-motile, have no flagella, and, so far as is known, do not form spores.

Staining.—They stain rather poorly except with such concentrated and penetrating stains as carbol-fuchsin and Löffler's alkaline methylene blue, and even with these more deeply at the ends than in the middle, so that they appear not a little like diplococci. They do not stain by Gram's method.

Canon† recommends a rather complicated method for the demonstration of the bacilli in the blood. The blood is spread upon clean cover-glasses in the usual way, thoroughly dried, and then fixed by immersion in absolute alcohol for five minutes. The best stain is Czenzynke's:

Concentrated aqueous solution of methylene blue	40
0.5 per cent. solution of eosin in 70 per cent. alcohol	20
Distilled water	40

The cover-glasses are immersed in the solution, and kept in the incubator for from three to six hours, after which they are washed in water, dried, and mounted in Canada balsam. By this method the erythrocytes are stained red, the leukocytes blue; and the bacilli, also blue, appear as short rods or as dumb-bells.

Large numbers of bacilli may be present, though sometimes only a few can be found after prolonged search, as they are prone to occur

* "Deutsche med. Wochenschrift," 1892, 2; "Zeitschrift für Hygiene," 1893, xiii, 357.

† "Centralbl. f. Bakt.," etc., Bd. xiv, p. 860.

in widely scattered but dense clusters. They are frequently inclosed within the leukocytes. It is scarcely necessary to pursue so tedious a staining method for demonstrating the bacilli, for they stain well enough for recognition by ordinary methods.

Isolation.—The influenza bacillus grows poorly upon artificial culture-media, and is not easy to isolate, because the associated bacteria tend to outgrow it. When isolated it is difficult to keep, as it soon dies in artificial cultures.

Pfeiffer found that the organism grew when he spread pus from the bronchial secretions upon serum-agar. Subcultures made from the original colonies did not "take." By a series of experiments he was able to make the organism grow when he transferred it to agar-agar, the surface of which was coated with a film of blood taken,

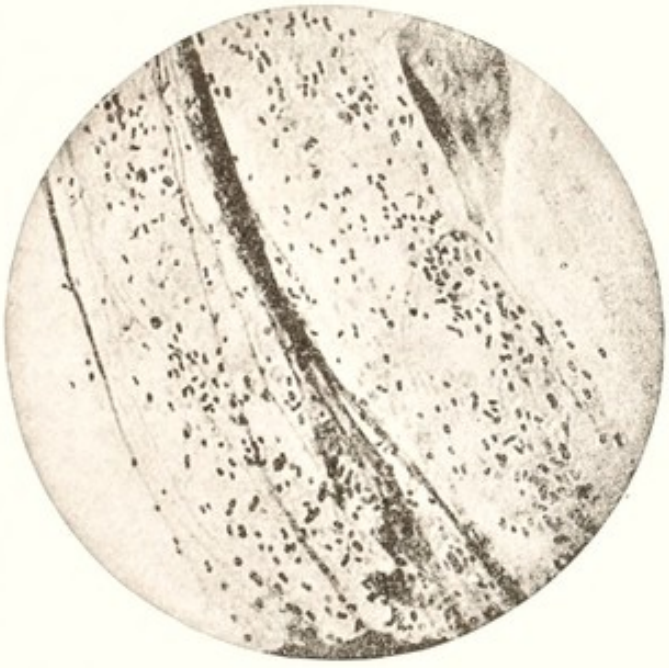


Fig. 174.—Bacillus of influenza. Smear from sputum (after Heim).

with precautions as to sterilty, from the finger-tip. Later it was found that the addition of hemoglobin to the culture-medium was equally efficacious. By the use of such blood-smeared agar and glycerin-agar the organism can now be successfully cultivated. The isolation is best achieved through the use of bronchial secretions, carefully washed in sterile water or salt solution to remove contaminating organisms from the mouth.

Cultivation.—Upon blood-spread glycerin agar-agar, after twenty-four hours in the incubator, minute colorless, transparent, dewdrop-like colonies may be seen along the line of inoculation. They look like condensed moisture, and Kitasato makes a special point of the fact that they never become confluent. The colonies may at times be so small as to require a lens for their detection.

No growth takes place at room temperature. The organisms die

quickly and must be transplanted every three or four days if they are to be kept alive.

The organism is aërobic and scarcely grows at all where the supply of oxygen is not free.

In bouillon a scant development occurs, small whitish particles appearing upon the surface, subsequently sinking to the bottom and causing a "wooly" deposit there. The bacillus grows more luxuriantly upon culture-media containing hemoglobin or blood, and can be transferred from culture to culture many times before losing vitality.

Vital Resistance.—Its resisting powers are very restricted, as it speedily succumbs to drying, and is certainly killed by an exposure to a temperature of 60°C. for five minutes. It will not grow at any temperature below 28°C.

Fig. 175.—Bacillus of influenza; colonies on blood agar-agar. Low magnifying power (Pfeiffer).

Specificity.—From the fact that the bacillus is found chiefly in cases of influenza, that it is present as long as the purulent secretions of the disease last, and then disappears, and that Pfeiffer was able to demonstrate its presence in all cases of uncomplicated influenza, it seems that his conclusion that the bacillus is specific is justifiable. It is also found in the secondary morbid processes following influenza, such as pneumonia, endocarditis, middle-ear disease, meningitis, etc. Horder* has cultivated it from the valvular vegetations of 2 cases of endocarditis following influenza.

Davis† found the influenza bacillus in the respiratory passage of a large number of patients suffering from whooping-cough.

* "Path. Soc. of London," "Brit. Med. Jour.," April 22, 1905.

† "Jour. Infectious Diseases," 1906, III, 1.

Pathogenesis.—The bacillus is pathogenic for very few of the laboratory animals. The guinea-pig is susceptible of fatal infection, the dose required to cause death varying considerably.

Pfeiffer and Beck* produced what may have been influenza in monkeys by rubbing their nasal mucous membranes with pure cultures.

Immunity.—As influenza is a disease that commonly relapses, and from which one rarely seems to acquire protection against future attacks, there must be scarcely any immunity induced through ordinary infection. Moreover, the organism once finding its way into the body seems to remain almost indefinitely, especially when, as in

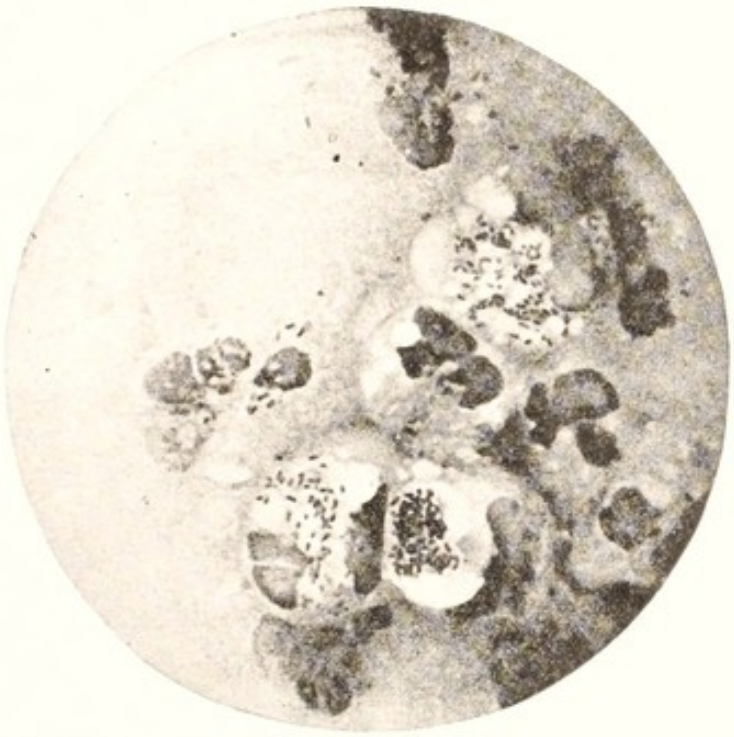


Fig. 176.—Bacillus of influenza; cover-glass preparation of sputum from a case of influenza, showing the bacilli in leukocytes. Highly magnified (Pfeiffer).

pulmonary tuberculosis, there is already present an abnormal condition furnishing discharges or exudates in which it can thrive.

Delius and Kolle† found that the toxicity of the culture does not depend upon a soluble toxin, but upon an intracellular toxin. The outcome of the researches, which were made most painstakingly, was total failure to produce experimental immunity.

Increasing doses of the cultures, injected into the peritoneal cavity, enabled the animals to resist more than a fatal dose, but never enabled them to recover when large doses of living cultures were administered.

A. Catanni, Jr.,‡ trephined rabbits and injected influenza toxin

* "Deutsche med. Wochenschrift," 1893, XXI.
† "Zeitschrift für Hygiene," etc., Bd. 1897, XXIV, Heft 2.
‡ Ibid., Bd., 1896, XXIII.

into their brains, at the same time trephining control animals, into some of whose brains he injected water. The animals receiving 0.5 to 1 mg. of the living culture died in twenty-four hours with all the nervous symptoms of the disease, dyspnea, paralysis beginning in the posterior extremities and extending over the whole body, clonic convulsions, stiffness of the neck, etc. Control animals injected in the same manner with water, and with a variety of other pathogenic bacteria never manifested similar symptoms. The virulence of the bacillus increased rapidly when transplanted from brain to brain.

Diagnosis of Influenza.—Wynekoop* employs for diagnosticating influenza and isolating the bacillus, a culture outfit similar to that used for diphtheria diagnosis, except that the serum contains more hemoglobin. The swab is used to secure secretions from the pharynx and tonsils, and from the bronchial secretions of patients with influenza, then rubbed over the blood-serum. In many such cultures minute colonies corresponding to those of the influenza bacillus were found. Those most isolated were picked up with a wire and transplanted to bouillon, from which fresh blood-serum was inoculated and pure cultures secured.

Carbol-fuchsin was found most useful for staining the bacilli. Wynekoop observed that influenza and diphtheria bacilli sometimes coexist in the throat, and that influenza bacilli are present in the sore eyes of those in the midst of household epidemics of influenza.

THE PSEUDO-INFLUENZA BACILLUS

Pfeiffer† has also described a pseudo-influenza bacillus—a small, non-motile, non-flagellated, non-sporogenous, Gram-negative bacillus—that he found in certain cases of broncho-pneumonia in children. It differed from the influenza bacillus by a slightly greater size, a tendency to grow in chains, and to undergo involution. Martha Wollstein‡ believes that they are influenza bacilli.

* "Bureau and Division Reports," Department of Health, city of Chicago, Jan., 1899.

† "Zeitschrift für Hygiene," etc., 1892, XIII.

‡ "Jour. Exp. Med.," 1906, VIII.

CHAPTER XVIII

MALTA OR MEDITERRANEAN FEVER

Micrococcus Melitensis (Bruce); Bacillus Melitensis (Babes)

General Characteristics.—A non-motile, non-flagellate, non-sporogenous non-chromogenic, non-liquefying, pathogenic coccus, staining by the ordinary methods, but not by Gram's method; characterized by remarkably slow growth and by pathogenic action upon monkeys.

In 1877, while working in Malta, Bruce* succeeded in finding in every fatal case of Malta fever a micrococcus which could be isolated in pure cultures from the spleen, liver, and kidney, which grew readily on artificial media, and which, when injected into monkeys, produced the disease.

Morphology.—Micrococcus melitensis, as Bruce called it, is a round or slightly oval organism measuring about 0.3 μ in diameter. It is usually single, sometimes in pairs, but never in chains. When viewed in the hanging drop it is said to exhibit active "molecular" movements, but is not motile and has no flagella. Babes† declares it to be a bacillus.

Staining.—It stains well with aqueous solutions of the anilin dyes, but not by Gram's method.

Thermal Death Point.—This has been fixed by Dalton and Eyre‡ at 57.5°C.

Cultivation.—The best medium for its cultivation is said to be ordinary *agar-agar*. After inoculating, by a puncture, from the spleen of a fatal case of Malta fever, the tubes should be kept at 37°C. The growth first appears after several days, in the form of minute pearly white spots scattered around the point of puncture and along the needle path. After some weeks the colonies grow larger and join to form a rosette-like aggregation, while the needle tract becomes a solid rod of yellowish-brown color. After a lapse of months the growth still remains restricted to the same area and its color deepens to buff.

When the sloping surface of inoculated agar-agar is examined by transmitted light, the appearance of the colonies is somewhat different. At the end of nine or ten days, if kept at 37°C., some of the colonies have a diameter of 2 to 3 mm. They are round in form, have an even contour, are slightly raised above the surface of the agar-

* "Practitioner," XXXIV, p. 161.
† Kolle and Wassermann, "Die Pathogene Mikroörganismen," III, p. 443.
‡ "Jour. of Hygiene," 1904, IV, p. 157.

agar, and are smooth and shining in appearance. On examining the colonies by transmitted light, the center of each is seen to be yellowish, while the periphery is bluish-white in color. The same colonies by reflected light appear milky-white. Colonies on the surface of the agar-agar are found to be no larger than hemp-seed after a couple of months of cultivation.

When kept at 25°C., no colonies become visible to the naked eye before the seventh day; at 37°C., before the third or fourth day.

In *bouillon* culture kept at 37°C., diffuse clouding of the medium occurs in three or four days. There is no scum on the surface. No indol is formed. In sugar bouillon there is no fermentation.

In *milk* the organism grows slowly without coagulation and without acid production.

The growth in *gelatin* takes place at room temperature with great slowness, first appearing in about a month, and no liquefaction of the medium occurs.

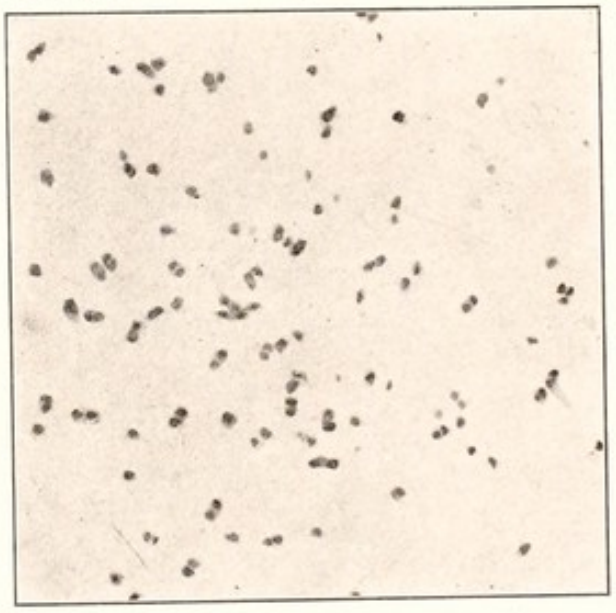


Fig. 177.—Micrococcus melitensis.

No growth takes place on boiled *potato*.

Plate cultures are not adapted to the study of the organism because of its extreme slowness of growth.

Bacteriologic Diagnosis.—The specific agglutinative effect of the serum can be made use of for the purpose of diagnosis. This has been studied by Wright,* Birt and Lamb,† and later by Bassett-Smith.‡

All of the observers have shown that the agglutinative reaction takes place both with living and dead cultures of the Micrococcus melitensis, but that to make the diagnosis dilutions of serum equal to about 1 : 30, never greater than 1 : 50, must be used. Birt and

* "Lancet," 1897, March 6; "Brit. Med. Jour.," 1897, May 15.

† Ibid., 1899, II, p. 701.

‡ "British Med. Jour.," 1902, II, p. 861.

Lamb also arrive at certain conclusions regarding the prognosis based upon a study of the agglutinative phenomena. Their conclusions are:

1. Prognosis is unfavorable if the agglutinating reaction is persistently low.
2. Also if the agglutinating reaction rapidly fall from a high figure to almost zero.
3. A persistently high and rising agglutinating reaction sustained into convalescence is favorable.
4. A long illness may be anticipated if the agglutination figure, at first high, decreases considerably.

The agglutination reaction appears early, is available by the end of the first week, and often persists for years after convalescence.

The organisms may sometimes be cultivated from the blood taken from a vein, but are more certainly to be secured by splenic puncture.

Pathogenesis.—The micro-organism is not pathogenic for mice, guinea-pigs, or rabbits, but is fatal to monkeys, goats, dogs, horses, asses, and mules, when agar-agar cultures are injected beneath the skin.

The micro-organism usually seems to be absent from the circulating blood, though Hughes has cultivated it from the heart's blood of a dead monkey.

Bruce not only succeeded in securing the micro-organism from the cadavers of Malta fever, but has also obtained it during life by splenic puncture.

Accidental inoculation with Micrococcus melitensis, as by the prick of a hypodermic needle, is almost invariably followed by an attack of the disease. Six cases of this kind in human beings have occurred in connection with bacteriologic work on Malta fever at Netley and two additional at the Royal Naval Hospital at Haslar and in the Philippines.*

Treatment.—The treatment of Mediterranean fever by means of bacterio-vaccines has been attempted with what seems to be glittering results by Bassett-Smith.†

The report of "British Government Commission for the Investigation of Mediterranean Fever," published by the Royal Society, April, 1907, has greatly elucidated our knowledge of the pathogeny of the disease by showing that the Micrococcus melitensis leaves the body of the patient in the urine and in the milk. It has not been found in the saliva, sweat, breath, or feces. The discovery of the organism in the milk suggested that it might be through milk that the specific organisms were disseminated, and an investigation of the goats at Malta, where the disease is most prevalent, and their milk most generally used, showed that a large percentage of the animals were infected with the specific cocci. The commission has, therefore, concluded that it is by goats' milk that the disease is commonly disseminated, though they point out that fly-transmission is also

* See Wright and Windsor, "Jour. of Hygiene," 1902, II, p. 413.
† "Journal of Hygiene," 1907, VII, p. 115.

possible. In the Colonial Office Report on Malta in 1907 it was shown that over 40 per cent. of the goats of Malta gave the serum reaction, showing that they had had the disease, while 10 per cent. of them were actually secreting the cocci in their milk. The authorities permit no milk to be used in the garrison unless it is boiled, and notice that by this simple measure the incidence of the disease, which was 9.6 in 1905, had fallen to 2 in the corresponding month of 1906. In Report VII. of the Mediterranean Fever Commission (1906–07) we read:

"The epidermologists are led to believe that quite 70 per cent. of the cases are due to the ingestion of goat's milk." In their opinion ordinary contact with the sick, conveyance of infection by biting insects, house-flies, dust, drain emanations, food (other than milk), and water, play a very subordinate part, if any, in setting up Mediterranean fever in man. The excellent results following the preventive measures directed against goat's milk in barracks and hospitals also point to goat's milk as being the chief factor. Among the soldiers this resulted in a diminution of about 90 per cent.

"For example, in the second half of 1905 there were 363 cases of Mediterranean fever, whereas in the corresponding part of 1906 there were only 35 cases. Among the sailors there was also as marked a fall in the number of cases. The Naval Hospital had a bad reputation, as about one-third of the cases of fever occurring in the fleet at Malta could be traced to residence in this hospital, either as patients suffering from other diseases or among the nursing staff. The goats supplying the hospital were found to be infected, and since their milk was absolutely forbidden, not a single case of Malta fever has occurred in or been traced to residence in this hospital."

CHAPTER XIX

MALARIA

PLASMODIUM MALARIÆ (LAVERAN); PLASMODIUM VIVAX (GRASSI AND FELETTI); PLASMODIUM FALCIPARUM (WELCH)

MALARIA, or paludism, has been known since the days of ancient medicine, and has always been regarded as the typical miasmatic disease. Its name, *mala aria*, means "bad air," and is Italian derived from the Latin, *malus* and *aer*, coming from the Greek ἀήρ, air, from ἄειν, to blow. The other name, paludism, from the Latin *palus*, a "marsh," refers the disease to the bad air coming from marshes.

It is a disease of extremely wide geographic distribution, and since the supposed requirement, marshy ground, is found in nearly all countries, and the disease is particularly prevalent in the marshy districts of those countries in which it occurs, the connection between the marshes and the disease seemed clear. Indeed, the two are intimately connected, but not in the original sense as will be shown below.

Both hemispheres, all of the continents, and most of the islands of the sea suffer more or less from malaria, and in many places, especially in the tropics, it is so pestilential as to make the country uninhabitable. Probably no better idea of the wide distribution and severity of the disease can be obtained than by reference to Davidson's "Geographical Pathology."*

The disease assumes the form of a fever of intermittent or remittent type, characterized by certain peculiar paroxysms. When typical, as in well-marked intermittent fever, these are ushered in by depression, headache, and chilly sensations, which are soon followed by pronounced rigors in which the patient shivers violently, his teeth chattering. The temperature soon begins to rise and attains a height of 102°, 104°, or even 106°F., according to the severity of the case. As the temperature rises the sense of chilliness disappears and gives place to burning sensations. The skin is flushed, hot, and dry. After a period varying in length the skin begins to break out into perspiration, which is soon profuse, the fever and headache disappear and the patient commonly sinks into a refreshing sleep. The frequency of the paroxysms varies with the type of the disease, which, in its turn, can be referred to the kind of infection by which it is caused. The paroxysms exhaust the patient and incapacitate him and may eventually prove fatal, though in by far the greater number of cases the disease gradually expends itself and a partial or complete recovery ensues. Some cases, known as pernicious, are rapidly fatal,

* D. Appleton & Co., New York, 1892.

others develop into a chronic cachexia, with profound anemia and complete incapacitation for physical or mental effort. The discovery of Peruvian or Jesuits' bark, and its introduction into Europe by the Countess del Cinchón, the wife of the Viceroy of Peru, about 1639, marked an important epoch in the study of malarial fever. The isolation of its alkaloids, quinin and cinchona, begun in 1810 by Gomez and perfected in 1820 by Pelletier and Coventou, a second great epoch. But the most important epoch began in 1880, when Charles Louis Alphonse Laveran,* a French physician engaged in the study of malarial fever in Algeria, announced the discovery of a parasite, to which he gave the name Plasmodium malariæ, in the blood of patients suffering from the disease. His observations were immediately confirmed, Bütschli recognizing the parasitic nature of the bodies observed. For the discovery he was awarded the Bréant prize.

Laveran, however, threw no light upon the source of infection, and malaria continued to be described as a miasmatic disease.

It was, however, recognized that there were different types of parasites corresponding to the different clinical forms of the disease, and Golgi† succeeded in correlating the various appearances of the parasites so as to express their life cycles. But in spite of the interesting and important work of Golgi, Celli, Bignami and Marchiafava, and many others, no progress was made in accounting for the entrance of the parasites into the human body.

This problem had long interested Sir Patrick Manson, who had devised a theory which, though wrong in detail, proved in the end to open the door to the next important discovery. Finding that the malarial parasites could not be shown to leave the body in any of its eliminations, and remembering that the same was true of the filarial worms and their embryos, Manson came to the conclusion that they must be taken out of the blood by some suctorial insect. The one naturally first considered was the mosquito, which was known to abound wherever malaria prevailed. Examining mosquitoes that had been permitted to distend themselves with the blood containing the parasites, Manson found that in the stomach of the insect the peculiar phenomenon known as "flagellation," long before observed by Laveran, took place in the parasites, giving rise to long, slender, lashing, and, finally, free-swimming filaments. These, he conjectured, might be the form in which the parasites left the mosquito to infect the swamp water, with which human infection eventually was brought about. Here Manson failed, but while he was investigating he explained the whole matter to Major Ronald Ross, who was soon to go to India, and whom he advised to make the matter a subject for study when he arrived at his destination. Ross‡ accepted the opportunity that soon presented itself, and, after a most

* "Accad. d. Méd.," Paris, Nov. 28 and Dec. 28, 1880.

† "R. Accad. di Medicina di Torino," 1885, XI, 20.

‡ "Indian Medical Gazette," XXXIII, 14, 133, 401, 448.

painstaking investigation, the details of which are given in a paper which can be found in the International Medical Annual,* 1890, made the second great discovery in the parasitology of malarial fever. He found that, as Manson thought, the mosquito is the definitive host of the parasite, but that the matter is much less simple than was imagined, for the organisms taken up by the mosquito undergo a complicated life cycle requiring about a fortnight for completion, after which, not the water into which the mosquito might fall and into which its contained organisms might escape, but the mosquito itself becomes the agent of infection. In other words, the parasites taken up by the mosquito, after the completion of the necessary developmental cycle, are returned by the mosquito to new human beings, who thus become infected. Thus it was shown that malaria is not a miasmatic disease at all, but that it is an infectious disease whose parasites divide their life cycle between man and the mosquito, each becoming infected by the other. The only rôle of the swamp is to furnish the mosquitoes, and since these are only more numerous where swamps are numerous, but may occur without swamps, the not infrequent occurrence of malarial fevers apart from swamps is also explained. Ross further discovered that all mosquitoes are not equally susceptible of infection, and, therefore, not all able to spread the infection. Indeed, he so carefully studied the mosquitoes as to narrow the infectability and infectivity of mosquitoes down to one single family, the Anophelinæ, and to one single genus, Anopheles.

There remained, however, one more important fact to be elucidated, and one more mysterious body to be accounted for, viz., the "flagellated" body that had misled Manson. This was found by MacCallum† to be but the spermatozoit of the male parasite. While observing one of the malarial parasites of birds—Plasmodium danliewskyi—he saw one of these "flagella" swimming away from its parent parasite, and followed it carefully, moving the slide upon the stage of the microscope. It, and others of its kind, approached a large globular parasite, to which one effected an attachment and into which it entered. MacCallum realized that he had observed the sexual fertilization of the organism. In 1900 two demonstrations of momentous importance were made. First, Sambon and Low went to Italy, to one of the most pestilential parts of the Campagna Romana, and lived there during three months of the most malarious time of the year in a mosquito-proof house, taking every precaution to avoid mosquitoes, and escaped infection; second, anopheles mosquitoes infected in Italy, by biting malarial patients, were taken to England, where they were permitted to bite Dr. P. J. Manson and Mr. George Warren, both of whom, after a period of incubation suffered from malarial paroxysms and showed plasmodia in their bloods. What may perhaps be regarded as the final step in the per-

* E. B. Treat & Co., New York.
† "Journal of Exper. Med.," 1898, III, 117.

fection of the knowledge of the parasite was reached in 1911, when C. C. Bass* devised a method of cultivating the parasite in its asexual stage, *in vitro*.

Thus from its time-honored place as the typical miasmatic disease, full of mystery and obscurity, malarial fever suddenly had a flood of light thrown upon it by which every peculiarity was fully illuminated.

In summarizing the knowledge thus set forth we find the following facts:

1880—Discovery of the Plasmodium malariæ by Laveran.

1890—Discovery of its human developmental cycle by Golgi.

1895—Discovery of the mosquito cycle and mode of transmission by Ross.

1898—Discovery of the sexual fertilization of the parasite by MacCallum.

1911—Discovery of the method of cultivating the parasites *in vitro* by C. C. Bass.

The interest aroused by Laveran's original discovery gave a great impetus to the study of hematology with special reference to parasites, and it soon became evident that the plasmodium was but one of a group of similar parasites. Of these we have now become acquainted with the following:

Parasite	Disease	Host	Insect host
Plasmodium malariæ.	Quartan fever.	Man.	Anopheles, Myzorrhynchus, Myzomyia, Cellia.
Plasmodium vivax.	Tertian fever.	Man.	Anopheles, Myzorrhynchus, Myzomyia, Cellia.
Plasmodium falciparum.	Aestivo-autumnal fever.	Man.	Anopheles, Myzorrhynchus, Myzomyia, Cellia.
Plasmodium kochi.		Cercopithicus.	Unknown.
Plasmodium inui.		Macacus (Inuus cynomolgus).	Unknown.
Plasmodium pitheci.		Orang - outang (Pithecus satyrus).	Unknown.
Plasmodium brazilianum.		Brachyrus calores.	Unknown.
Plasmodium cynomolgi.		Inuus cynomolgus and Inuus nemistrinus.	Unknown.
Plasmodium grassii (Proteosoma grassi).		Sparrows, canary birds, and other small birds.	Culex pipens.
Plasmodium danliewskyi (Halteridium danliewskyi).		Owls, hawks, crows, and other large birds.	Unknown.

* "Journal of the American Medical Association," 1911, XLVII, 1534.

These micro-organisms correspond in all essentials. They are protozoan parasites belonging to the sporozoa and live in the blood (hematozoa) as parasites of the red corpuscles. They all have two life cycles, one which is asexual in the intermediate warm-blooded host, and one that is sexual in the definitive cold-blooded (insect) host. Though the intermediate hosts vary and may be birds or

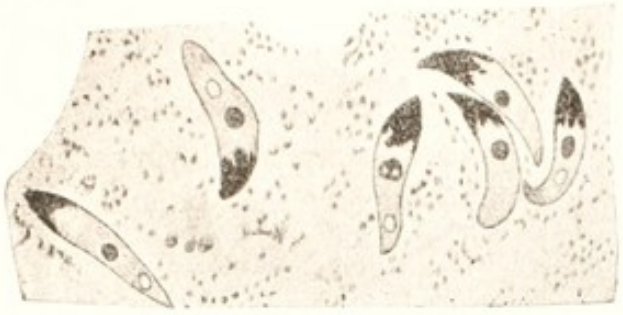


Fig. 178.—Plasmodium falciparum. Oökinetes in the stomach of Anopheles (Grassi).

mammals, the insect hosts, so far as known, are always mosquitoes. The mosquitoes become infected by biting and sucking the blood of infected animals; the warm-blooded animals become infected by being bitten by infected mosquitoes, and so on, in endless cycles.

The parasites differ but little in the details of structure and development, so that the following description may serve as a type for all:

From the proboscis of the mosquito, with its saliva, from cells in the salivary glands where they have been harbored, tiny elongate spindles, measuring about 1.5 μ in length and 0.2 μ in breadth, and known as *sporozoits*, enter the blood of the individual bitten. These sporozoits attach themselves to the red blood-corpuscles, gradually lose their elongate form, and become irregularly spherical. There is some difference of opinion as whether the little bodies are simply upon the corpuscles, as Koch believed, or in the corpuscles, as the majority of writers believe, but it is an immaterial difference, for the parasite soon makes clear that it is consuming the corpuscle. This little body is known as a *schizont*. When stained with polychrome methylene-blue, and examined under a high power of the microscope, it appears as a little ring with a dark chromatin dot upon one side. It grows steadily, feeding upon the hemoglobin, which seems to be chemically transformed into fine or coarse granules of a bacillary or rounded form, presumably melanin. In a length of time that

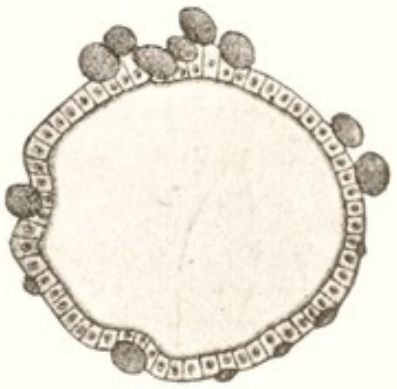


Fig. 179.—Plasmodium falciparum. Transverse section of the stomach of Anopheles, showing the oökinetes of the parasite in various stages of development attached to the outer surface (Grassi).

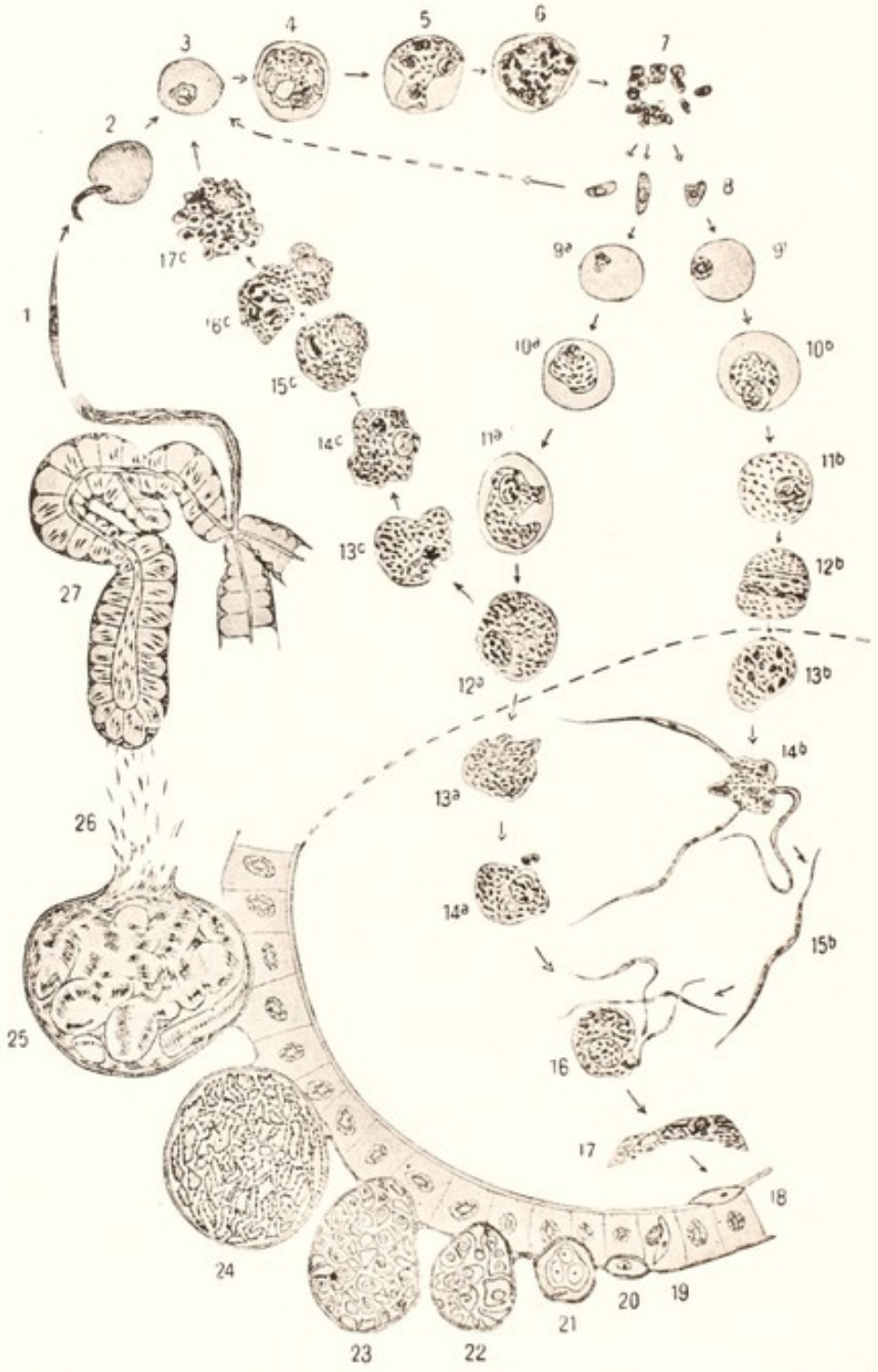


Fig. 180.—Developmental cycle of plasmodium vivax, the tertian malarial parasite. Figures 1 to 17 are magnified 1200 diameters; 18 to 27, only 600 diameters: 1, Sporozoit; 2, penetration of a sporozoit into a red blood-corpuscle; 3 and 4, schizont developing in the red blood-corpuscles; 5 and 6, nuclear division of the schizont; 7, free merozoits; 8 (following the arrows to the left to 3), merozoits entering red blood-corpuscles, and multiplying by schizogony 3 to 7; after longer continuance of the disease the sexual forms arise; 9a to 12a, macrogametocytes; 9b to 12b, microgametocytes still in the circulatory blood of man. If the macrogametocytes (12a) are not taken into the alimentary canal of the mosquito, they multiply parthenogenetically (12a, 13c to 17c) and the resulting merozoits (17c) become schizonts (3 to 7). The figures below the dotted line represent what takes place in the alimentary canal of anopheles (13 to 17); 13b and 14b the formation of microgametocytes; 13a and 13b, maturation of the macrogametes; 15b, a microgamete: 16, fertilization; 17, oökinete;

varies—twenty-four to forty-eight hours (Plasmodium falciparum), forty-eight hours (Plasmodium vivax), seventy-two hours (Plasmodium malariæ)—the schizonts mature, becoming nearly as large or quite as large as the corpuscles. The pigment granules now collect at the center and the substance of the parasite divides into a group of equal-sized *merozoits*, commonly known as *spores*. Of these there are usually eight in the meroblasts of Plasmodium malariæ, from fifteen to twenty-five in those of Plasmodium vivax, and from eight to twenty-five in Plasmodium falciparum. As the spores become fully formed and ready to separate, the paroxysm of the disease begins. It ends as the spores are freed and enter new corpuscles to begin the cycle over again. After a good many paroxysms have occurred it may be observed that not all of the schizonts change to meroblasts and form spores. Some remain large spheroidal bodies or, as in Plasmodium falciparum, assume a peculiar crescentic form and remain unchanged in the blood. These are the sexual parasites. The female is usually the larger and is known as the *makrogametocyte*, the male, the smaller, the *microgametocyte*. These are the bodies which, when removed by the mosquito, lay the foundation of its infection. When they are withdrawn for microscopic examination or exposed to the intestinal juices of the mosquito, the microgametocyte becomes tumultuous, its granules are observed to be in a state of active cytoplasmic streaming, and suddenly there burst forth long slender filaments, the *microgametes* or *spermatozoits*. These correspond with the *flagella* of Laveran and others, and are the same bodies that Manson thought might be the form in which the parasite leaves the insect's body. The microgametes lash vigorously for a time, then, breaking loose, swim away, and, as MacCallum observed, conjugate with *macrogametes*, sexually perfect cells formed from the macrogametocytes by "reduction division" and polar body formation, thus fertilizing them. As the result of this fertilization a *zygote* or *oökinete* is formed. It assumes a somewhat elongate pointed form and attaches itself to the wall of the mosquito's stomach. In the course of time it penetrates and appears upon the outside, projecting into the body cavity. It grows larger and rounder, divides into several segments, and eventually forms an *oöcyst* with many small cells, which break up into myriads of tiny elongate fusiform bodies, the *sporozoits*. These, in the course of time, seem to find their way to the salivary glands, entering into the epithelial cells and taking radial positions about the

18, oökinete on the wall of the mosquito's stomach; 19, penetration of the gastric epithelium by the oökinetes; 20 to 25, stages of sporogenesis on the outer wall of the mosquito's stomach; 26, migration of the sporozoits to the salivary glands of the mosquito; 27, salivary gland with sporozoits in the epithelial cells, and escape of the sporozoits from the salivary glands through the insect's proboscis at the time a human host is bitten; 1, free sporozoit from the mosquito's saliva in the human blood; 2, penetration of the sporozoit into a red blood-corpuscle, beginning the human cycle again (Lühe).

nuclei, where they remain for a time. Later, they leave the cells with the saliva, and when the mosquito again bites, enter the warm-blooded host to infect it, if of the appropriate species.

The whole cycle in the mosquito varies, according to the external temperature, from ten days to a fortnight. The mosquito may remain alive for more than one hundred days, and must bite frequently to satisfy its needs. It remains infective so long as the sporozoits remain in the saliva, which is usually as long as the insect is alive. Here it may be remarked that as it is only the female mosquitoes that bite, it is only by them that the infection can be spread. It is an interesting question, not yet solved, whether any of the sporozoits entering into the mosquito's ovaries can infect its eggs so that a new generation of mosquitoes may be born infective.

The longer the human infection persists, the greater the number of gametocytes formed, until sometimes in æstivo-autumnal malaria, no schizonts are any longer found, though the blood contains large numbers of gametocytes. In such cases the gametocytes, especially the crescents of æstivo-autumnal fever, but sometimes also those of tertian and quartan fever undergo *regressive schizogony*, by *parthenogenesis*, in the patient's blood, and without fertilization suddenly break up into spores which enter the red blood-corpuscles and occasion a relapse of the infection that had apparently spent itself.

The Human Malarial Parasites

There are three known forms of human malarial parasites: Plasmodium malariæ, Plasmodium vivax, and Plasmodium falciparum.

I. Plasmodium Malariæ (Laveran,* 1880).—This is the smallest of the human malarial parasites. Its occurrence is relatively infrequent, as is that of the quartan fever that it occasions. The schizogonic period is seventy-two hours long, and as each is completed, a paroxysm of the disease occurs.

Synonyms.—Oscillaria malariæ pro parte, Laveran, 1881. Plasmodium var. quartana, Golgi, 1890. Hæmamœba malariæ. Grassi et Feletti, 1892. Hæmamœba laverani var. quartana, Labbé, 1894. Plasmodium malariæ quartanum, Labbé, 1899. Hæmomenas malariæ, Ross, 1900. Plasmodium golgii, Sambon, 1902. Plasmodium quartanæ, Billet, 1904; Celli, 1904.

The parasite, in the red blood-corpuscles, first appears as a tiny ring, at one side of which there is a chromatin dot. At this time the organism cannot be differentiated from Plasmodium vivax. At the end of twenty-four hours the organism seems to extend itself more or less linearly, and sometimes appears as a long drawn band which crosses the substance of the unchanged corpuscle. In another twenty-four hours the breadth of the parasite is two or three times as great, and it has become pigmented. The corpuscle itself is still unchanged. In the last twenty-four hours the parasite enlarges, becomes more or less quadrilateral, finally rounds up, shows depressions upon the sur-

* "Acad. de Med.," Nov. 23, Dec. 28, 1880.

face, corresponding to the divisions into which it is to segment, the pigment gathers at the center, and the substance undergoes cleavage resulting in the formation of from six to fourteen, but usually eight, spores. It is to be noticed that it is not until a few hours before segmentation that the parasite becomes as large as the corpuscle, and that the corpuscle is never enlarged nor bleached by the presence of the parasite. The meroblasts form regular rosettes, or "daisy-heads," within the corpuscles.

In single infections the parasites are all of the same age and all mature at the same time, so that in any examination of the blood they will all appear uniform. It is, however, sometimes true that the patient may have been infected one day by one mosquito bite, and again infected the next day or the third day by a second mosquito bite, so that his blood contains two crops of the microparasites, arriving at maturity at different times. This perplexes the clinician

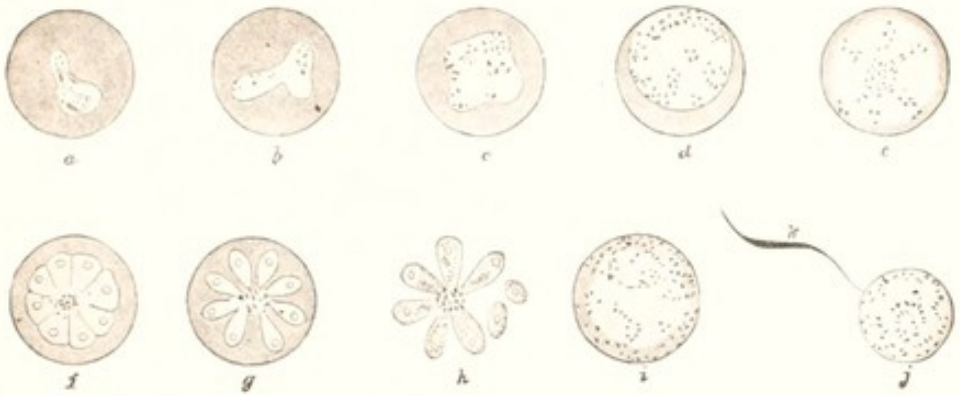


Fig. 181.—Parasite of quartan malarial fever: *a*, *b*, *c*, *d*, enlarging intracellular parasites; *e*, *f*, *g*, *h*, segmentating parasites forming a distinct rosette from which the spores separate; *i*, macrogametocyte; j, microgametocyte; k, sporozoit.

through the variety of parasitic forms in the blood and the abnormal frequency of the paroxysms.

The gametocytes of the parasite remain for some time in the red corpuscles without division, but, finally, become free spherical bodies. Two sizes can be made out, the larger, the macrogametocyte or female, the other, the microgametocyte or male. Each has protoplasm, with a tendency to take a blue-gray color and appear uniformly granular, except that at some part of the periphery of each there is a circular or semicircular area that is free from granules. This area is larger in the microgametocyte.

II. Plasmodium Vivax (Grassi and Feletti,* 1890).—This is the most common of the malarial parasites of man, and occasions the "benign" tertian fever. It is a large parasite, the full-grown schizont

Synonyms.—Oscillaria malariæ pro parte, Laveran, 1881. Plasmodium var. tertiana, Golgi, 1889. Hæmamœba vivax, Grassi et Feletti, 1890. Hæmamœba laverani var. tertiana, Labbé, 1894. Plasmodium malariæ tertianum, Labbé, 1899. Hæmamœba malariæ var. magna, Laveran, 1900. Hæmamœba malariæ var. tertianæ, Laveran, 1904. Plasmodium tertianæ pro parte, Billet, 1904.

* "Centralbl. f. Bakt. u. Parasitenk.," 1890, VII, 396; 1891, X, 449, 481, 517.

(meroblast), ready to form merozoits, and the gametocytes all exceeding the size of the red blood-corpuscles. It matures in forty-eight hours, but not with mathematic precision. In single infections the greater number of the parasites are of the same age and present the same appearance, but various shapes and ages may be found together. In double infections, with paroxysms every day, parasites of different ages may be found.

The youngest form in which the parasite can be observed is that of a tiny ring in a red blood-corpuscle. The periphery of this ring (when the blood is stained with polychrome methylene blue) is outlined with blue, at one side there is a distinct blue dot, and the center appears colorless and like a vacuole. The dot is usually on the side of the vacuole that has the thinner protoplasmic outline. The smallest such rings usually have a diameter equal to about ⅓ the diameter of the blood-corpuscle. The tiny ring-form, or, as it might better be called, the "seal-ring form," continues until the

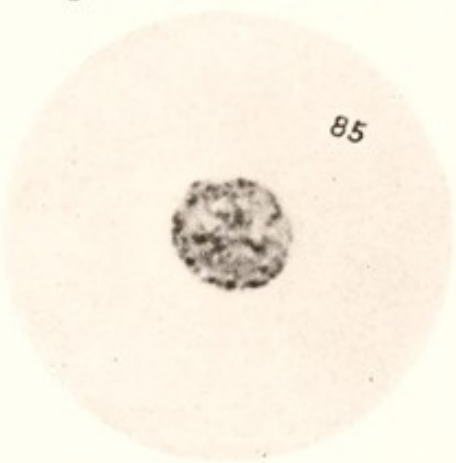


Fig. 182.

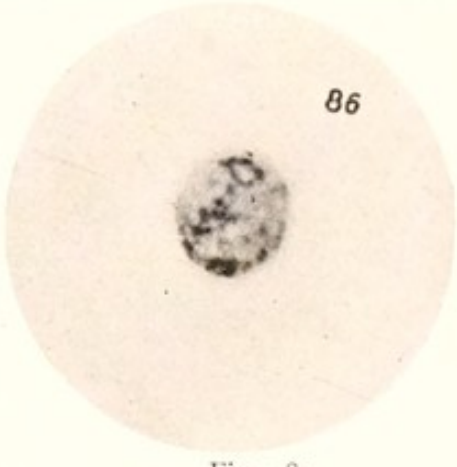


Fig. 183.

Figs. 182, 183.—Gametocytes of plasmodium malariæ: 85, The macrogametocyte; 86, the microgametocyte (Kolle and Wassermann).

schizont becomes half the diameter of the blood-corpuscle, when its protoplasm has begun to increase so rapidly that the vacuole no longer appears to be so conspicuous. The organism also becomes irregular in shape and is actively ameboid, its protoplasm streaming this way and that when examined in fresh blood. At this time it may be noticed that the infected blood-corpuscle is increasing in volume, sometimes becoming twice the normal size, and also becoming pale in color. It seems also as though the disk shape of the corpuscle was lost, and it had become swollen into a more spherical—sometimes irregular—form. The parasite, which may still show a relic of its original ring-form, now shows plentifully throughout its protoplasm exceedingly fine granules of yellow-brown pigment. When from thirty-six to forty hours old, all trace of the "seal-ring" form disappears, the ameboid action becomes less marked, and the parasites (now three-quarters the size of the enlarged pale and misshapen corpuscles in which they are contained)

DESCRIPTION OF PLATES II AND III.

Various forms of malarial parasites: Figs. 1 to 10 inclusive, tertian parasites; Figs. 11 to 19 inclusive, quartan parasites; Figs. 20 to 26 inclusive, estivo-autumnal parasites.

1.—Normal red blood-cell. 2.—Young tertian ring. 3.—Large tertian ring. 4.—Half-grown tertian parasite. 5.—Infected cell showing Schüffner's dots. 6.—Adult tertian parasite. 7.—Beginning sporulation. 8.—Sporulation completed. 9.—Tertian microgametocyte. 10.—Tertian macrogamete. 11.—Young quartan ring. 12.—Older quartan ring. 13.—Quartan band. 14.—Older quartan band. 15.—Full-grown quartan parasite. 16.—Mature parasite with divided chromatin. 17.—Sporulation completed. 18.—Quartan microgametocyte. 19.—Quartan macrocyte. 20.—Young estivo-autumnal ring. 21.—Large estivo-autumnal ring. 22.—Mature parasite. 23.—Sporulation completed. 24.—Estivo-autumnal microgametocyte. 25.—Estivo-autumnal macrogamete. 26.—Estivo-autumnal ovoid.

(From Deaderick, "A Practical Study of Malaria.")

PLATE II

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PLATE III

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appear as irregular, ragged, protoplasmic bodies filled with fine pigment granules. In about forty-five hours they completely fill the enlarged corpuscles, and begin to gather their protoplasm into rounded formations in which the pigment is no longer distributed, but occurs in irregular stripes or gathers together into a rounded clump. In a couple of hours the blood-corpuscle has disappeared and the rounded parasite, larger than normal red corpuscles, with a lobulated surface, and with its pigment granules collected to form one or two rounded masses, is seen to have reached the stage of the meroblast. This does not form the rosette or "daisy-head" shown by the quartan parasite, but might better be compared to a mulberry, and eventuates in the formation of from fifteen to twenty-five small, rounded or ovoid, pale, unpigmented bodies, the mero-

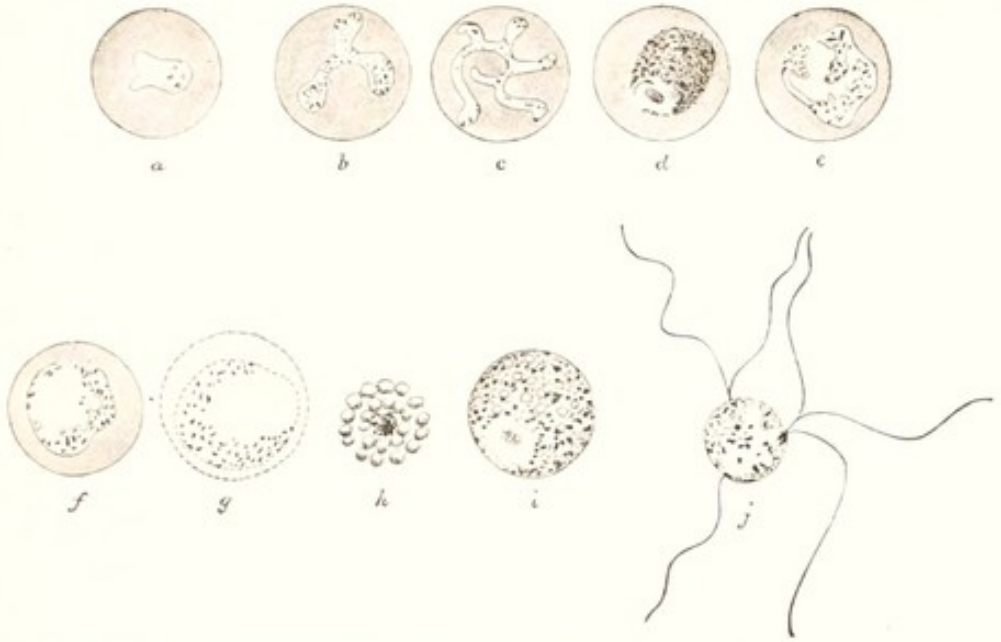


Fig. 184.—Parasite of tertian malarial fever: *a*, *b*, *c*, *d*, *e*, *f*, *g*, Growing pigmented parasite in the red blood-corpuscles; *h*, spores formed by segmentation of the parasite—no rosette is formed, but concentric rings of the cytoplasm divide; *i*, macrogametocyte; *j*, microgametocyte with spermatozoits.

zoits or spores. These become freed from the pigment and attached to new red corpuscles, in which they are easily recognized as the "tiny-rings" that begin the schizogonic cycle. The gametocytes of the tertian parasite, the "free spheres," as they are sometimes called, are large, rounded or slightly ovoid bodies, with a uniformly dull bluish-gray or grayish-green protoplasm, in the interior of which there is always a circular or semicircular area peripherally or centrally situated, and colorless. Except in this area the pigment is distributed throughout the parasite. The larger or macrogametocyte, the female parasite, measures 10 to 14 μ in diameter. It has a greenish or grayish-green or almost colorless protoplasm, containing an oval or bean-shaped colorless area almost half as large as the organism itself. Yellowish-brown pig-

ment in short, broad rods is sparingly scattered throughout the substance elsewhere.

The microgametocyte or male form is approximately the size of a red blood-corpuscle—8 to 9 μ in diameter. It stains more deeply than its mate and contains more and coarser pigment granules.

III. Plasmodium Falciparum (Welch,* 1897).—This is the

Synonyms.—Oscillaria malariæ pro parte, Laveran, 1881. Hæmamœba præcox, Grassi et Feletti, 1890. Laverania malariæ, Grassi et Feletti, 1890. Hæmamœba malariæ præcox, Grassi et Feletti, 1892. Hæmomenas præcox, Ross, 1899. Plasmodium malariæ præcox, Labbé, 1899. Plasmodium præcox, R. Blanchard, 1900. Hæmamœba malariæ var. parva, Laveran, 1900. Plasmodium immaculatum, Schaudinn, 1902. Laverania præcox, Nocard et Leclainche, 1903.

parasite of estivo-autumnal or malignant tertian malarial fever. It is a very small parasite, whose occurrence, even multiple occurrence, in the corpuscles does not change their size or shape. It does, however, quickly change the appearance of the corpuscles, which become polychromatophilic, and frequently show numerous small dots—the granulations of Schüffner—in the corpuscular substance.

The first appearance of the schizont is in the form of tiny rings, which appear to lie upon rather than in the corpuscles, and are first seen at the edges. The rings are outlined by extremely fine lines

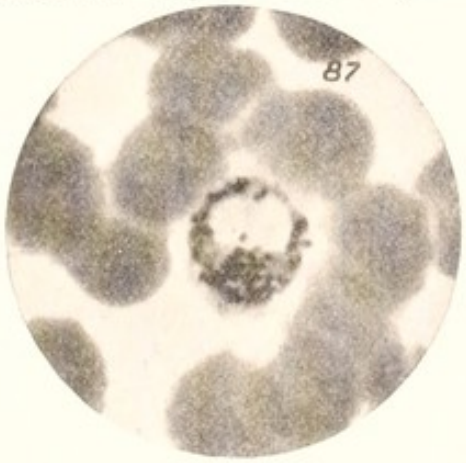


Fig. 185.

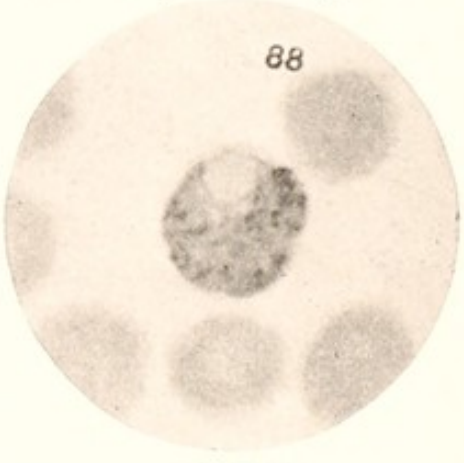


Fig. 186.

Figs. 185, 186.—Gametocytes of plasmodium vivax: 87, The microgametocyte; 88, the macrogametocyte (Kolle and Wassermann).

and sometimes seem to be incompletely closed, so that they are like horseshoes rather than circles. They increase to several times the original size without losing the ring shape, and are variously known as "middle-sized rings" and "large rings." They are with difficulty differentiated from the "tiny rings" of the tertian parasite. As the "large ring" stage is reached the parasites begin to disappear from the peripheral blood to complete their growth and undergo meroblast formation in the capillaries of the spleen, the brain, and the bone-marrow. Here the full-grown parasites—meroblasts—appear as irregular disks, resembling those of the quartan parasite,

* Article "Malaria" in "A System of Practical Medicine by American Authors," 1897, p. 138.

but smaller in size. The pigment is gathered toward the center in a little mass, and eight to twenty-five merozoits are formed in a morula or mulberry-like mass similar to those of the tertian parasite. Two or three parasites to the corpuscle are frequent. They are actively ameboid, do not mature simultaneously, and hence

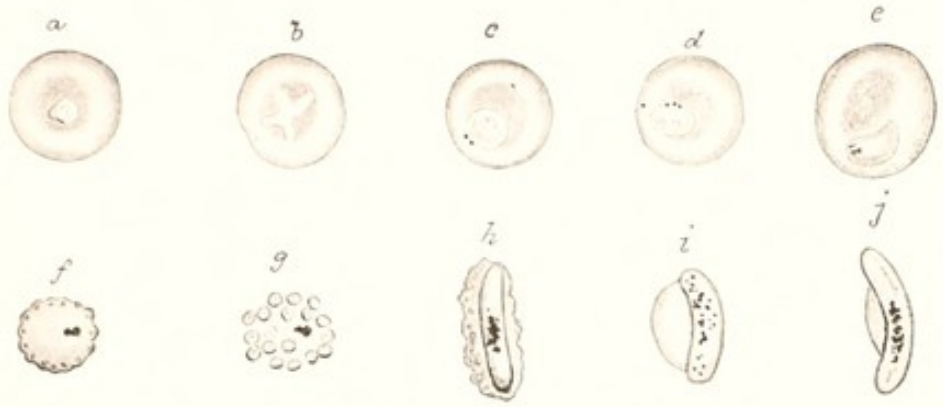


Fig. 187.—Parasite of estivo-autumnal fever: *a*, *b*, *c*, Ring-like and cross-like hyaline forms; *d*, *e*, pigmented forms; *f*, *g*, segmentary forms; *h*, *i*, *j*, crescents.

there are no regularly occurring paroxysms. The duration of the asexual cycle is from twenty-four to forty-eight hours.

The gametocytes are striking and characteristic ovoid and crescentic bodies—*crescents*—1½ times the diameter of a red blood-corpuscle in length, and about half the diameter of the corpuscle in breadth. The ends color more intensely with methylene blue

Fig. 188.

Fig. 189.

Figs. 188, 189.—Gametocytes of plasmodium falciparum: 91, The microgametocyte; 92, the macrogametocyte (Kolle and Wassermann).

than the middle portion, and the bacillary pigment granules are collected toward the centers. The longer and more slender crescents are usually bent, and the relic of the corpuscle in which they have formed can often be seen forming a line connecting the ends on the concave side. These are the microgametocytes or male elements.

The macrogametocytes are broader, not curved, and sometimes are ovoidal or prolate spheroidal in shape. The pigment granules are more widely scattered throughout the substance. The crescents are most numerous after the fever has lasted for some time or in recurrences of the fever.

The fever in this form of malarial infection may be intermittent with daily—quotidian—paroxysms, or with irregular paroxysms, or the fever may be remittent. The infection is sometimes mild, but may be so severe as to be rapidly fatal. In such cases the number of parasites is enormous, the cerebral capillaries become filled with them, and coma quickly comes on and is soon followed by death. Such cases are described as "congestive chills" or "algid" cases.

Cultivation of the Parasites.—The parasites have been successfully cultivated in blood, prevented from coagulation, by Bass.

In the first paper, Bass announced that the cultivation of these parasites was made possible by the maintenance of the culture at 40°C., the selection of such an elevated temperature being based upon the theory that in the bloods of infected human beings, there were specific amboceptors directed against the invading organisms, but unable to effect their destruction until complement is formed. Complement soon appears in the drawn blood, according to Bass, unless the temperature be sufficiently elevated to prevent it, and he finds 40°C. sufficient for the purpose. A later paper by Bass and Johns† gives the details of cultivation as follows:

> When blood is to be taken from a malarial patient for the purpose of cultivating the parasites, one prepares a sterile 50 per cent. solution of Merck's dextrose, in distilled water, and measured into a sterilized test-tube, 1 inch in diameter 0.1 cc. for each 10 cc. of blood to be collected. The tube, which is called the "defibrinating tube" is provided with a glass rod that passes through the cotton plug to the bottom of the tube. A needle is plunged into the arm vein of the patient, and the infected blood is permitted to flow into the defibrinating tube until the requisite quantity has been collected. The needle is then withdrawn, the arm dressed, and the blood gently stirred or whipped until defibrinated. In the process of collecting and whipping, the admixture of air with the blood is to be avoided.
>
> If only one generation of parasites is to be cultivated, the culture may be grown in the defibrination tube, provided that the contained column of blood be not greater than 1–2 inches. There is no advantage in having a deeper column of blood, but there is danger in having less depth as under such circumstances the parasites die before the stage of segmentation is reached. In case the column is more than the required depth, some of the blood can be pipetted to other tubes and several cultures made. The plasmodia grow in the top layer of the sedimented cells, near the clear supernatant serum above. The thickness of the layer of cells in which they live is said to be not more than $\frac{1}{20}$ of an inch.
>
> If the cultures are to be continued for numerous generations, precautions must be taken to exempt the parasites from the destructive activities of the leukocytes. The method is therefore varied in this manner: The defibrinated blood is centrifugalized until three layers are formed, clear serum above, leukocytes in a thin layer below, and red corpuscles at the bottom. The clear serum is pipetted off and filled into small culture tubes to make a column not deeper than 1½ inches. Red blood corpuscles and plasmodia are then drawn up from

* Jour. Amer. Med. Asso., 1911, LVII, 1534.
† "Jour. Exp. Med.," 1912, XVI, 567.

the deeper part of the corpuscular layer, thus escaping the leukocytes at the top, and planted at the bottom of each tube of serum. It is thought to be advantageous to use culture tubes with flat bottoms. A still better method is the introduction of a paper disk into a half-inch tube, about half an inch below the surface of the serum, and then place one- or two-tenths of a cubic centimeter of corpuscles upon it. Under these circumstances all of the plasmodia are said to grow and segment. Two or three generations of parasites grow in such cultures, then the plasmodia begin to die out, so that if the culture is to be perpetuated, they must be transplanted to freshly prepared blood-corpuscle tubes of the same kind. The method of transplantation recommended is so very simple: a drop of the culture is drawn into a fine (not capillary) glass pipette and then followed by about five times the volume of the fresh corpuscle suspension. These are mixed in the pipette, care being taken not to mix air with the blood, and are then transferred to the new media in the same manner as in making the original inoculation. The transplantation should be done within five hours of the time of maximum segmentation, and therefore every forty-eight hours for the tertian and æstivo-autumnal parasites. All species of the plasmodia have been successfully cultivated by these means. The parasites have also been grown in red blood-cells in Lock's solution, free of calcium chlorid and in the presence of ascitic fluid.

According to Bass and Johns, the parasites grow in the corpuscles, not upon them as believed by Koch. They are destroyed in a few minutes *in vitro* by normal human serum or by all the modifications of it that they have tested. This fact, together with numerous observations of parasites in all stages of development apparently within the corpuscles render untenable the idea of extra-corpuscular development. Leukocytes phagocytize and destroy malarial parasites growing *in vitro* only when they escape from their red-corpuscle capsule or when the latter is perforated or becomes permeable.

The substance of the malarial plasmodium is very different in consistency from that of the blood-cells, and therefore they cannot pass through the smallest capillaries like the more yielding fluid-like red blood-cells. That the consistency of the protoplasm of the parasite is less yielding than that of the red blood-cell is shown by the fact that when a small quantity of a culture containing large parasites is spread over a slide with the end of another slide, the parasites are dragged to the end of the spread though the red blood-cells are left behind. Large æstivo-autumnal plasmodia are round or oval; the tertian variety are more or less flattened. As a result of their unyielding consistency, malarial parasites lodge in the capillaries of the body, especially where the current is weakest, and remain and segment. In the meantime other red corpuscles are forced against them and if in a favorable situation, one or more merozoites pass directly into the other cells. When the segmented parasite has become sufficiently broken up it can pass through the capillary into the circulating blood where the remaining merozoites are almost instantly destroyed.

They further observed that calcium salts added to cultures of æstivo-autumnal parasites caused hemolysis of the infected, possibly also of non-infected red blood-cells. Such salts have no effect on the corpuscles of normal blood, possibly because of the precipitation of other substances from the serum. The amount of calcium necessary to cause hemolysis of malarial blood is only slightly in excess of the quantity present in normal blood and possibly might be reached by the ingestion of considerable quantities of calcium in drinking water or food. They speculate that malarial hemoglobinuria may be the result of the presence of an excess of calcium in drinking water.

Bass and Johns believe that quinine has no direct effect upon the malarial parasites, but effects its curative influence by rendering the substance of the corpuscles more permeable to the all-sufficient destructive influence of the serum. The quinine would then affect only the parasites in the circulation, and not those lodged in the capillaries, which would not be reached until they had segmented. The effect of quinine is said to be defeated by influences such as diet, exertion, etc., which increase the dextrose content of the blood, whereby the permeability of the red blood-cells seems to be decreased. It is hoped that a better understanding of the principles involved in the treatment of malaria may result from the study of the organism in culture by which empiricism may be exchanged for rationalism.

Animal Inoculation.—The human malarial parasites cannot be successfully transmitted by experimental inoculation to any of the lower animals.

Human Inoculation.—The blood of one human being containing schizonts, when experimentally introduced into another human being in doses of 1 to 1.5 cc. transmits the disease. When thus transmitted, an incubation period of from seven to fourteen days intervenes before the disease, which is of the same type as that from which the blood was taken, makes its appearance.

Pathogenesis.—The pathogenic effects wrought by the malarial parasite are imperfectly understood. The synchrony of the segmentation of the parasite with the occurrence of the paroxysms seems to indicate that a toxic substance saturates and disturbs the economy at that time. Whether it be an endotoxin liberated by the dividing parasite is not, however, known.

The anemia that follows infection can be referred to the destruction of the red blood-corpuscles by the parasites which feed upon them and transform the hemoglobin into melanin (?). When great numbers of the parasites are present the destruction is enormous, and the number of corpuscles and the quantity of hemoglobin in the blood sink far below the normal. Leukopenia instead of leukocytosis is the rule, and while the leukocytes have an appetite for the spores of the parasites and often phagocyte and destroy them, their activity is not sufficiently rapid or universal to check their rapid increase.

The melanin granules set free during sporulation are also taken up by the leukocytes and endothelial cells, the latter becoming deeply pigmented at times.

The spleen enlarges as the disease continues until it forms the "ague-cake." The enlargement may cause the organ to weigh 7 to 10 pounds. It appears to result from hypertrophy. The tissue is pigmented. The liver and kidneys are also enlarged and pigmented.

Prophylaxis.—With the knowledge of the rôle of the mosquito in the transmission of malaria, its prophylaxis becomes a matter of simplicity when certain measures can be systematically carried out. There are two equally important factors to be considered—the human being and the mosquito. The measures must be directed toward preventing each from infecting the other.

1. *The Human Beings.*—In districts where malarial fever prevails, the first part of the campaign had perhaps best be directed toward finding and treating all cases of malarial fever, so that the parasites in their blood may be destroyed and the infection of mosquitoes prevented. This is done by the systematic and general use of quinin.

All cases of malarial fever should be required to sleep in mosquito-proof houses under nets, and as the mosquitoes are nocturnal and

begin to fly at dusk, the patients should shut themselves in before that time. By thus killing the parasites in the blood, and keeping the mosquitoes from the patients in the meantime, much can be done. But where malarial fever prevails, the mosquitoes are already largely infected, hence the healthy population should also learn to respect the habits of the insects and not expose themselves to their bites, should screen their houses and their beds, and should take small prophylactic doses of quinin to prevent the development of the parasites when exposure cannot be avoided.

2. *The Mosquitoes.*—It is not known that the parasites can pass from one generation of mosquitoes to another, hence the mosquitoes to be feared are those that are already infected. By

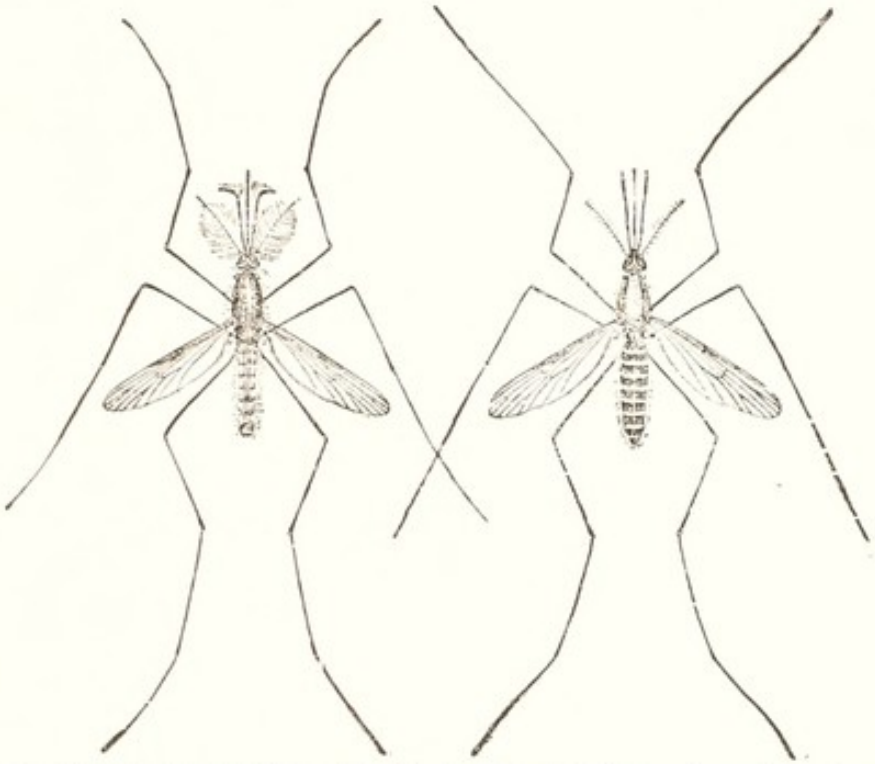


Fig. 190.—Anopheles maculipennis: Adult male at left, female at right (Howard).

making the houses mosquito-proof most of the insects can be kept out, while those that get in can be caught and killed.

By draining the swamps and destroying all the breeding places in and near human habitations, the number of mosquitoes can be greatly diminished. Fortunately this is particularly true with reference to the mosquitoes most concerned—the anopheles—which fly but short distances. By closing all the domestic cisterns and reservoirs, cesspools, etc., so that no mosquitoes can get in to breed or get out to bite, and by draining the pools for half a mile in all directions from human habitations, the number of anopheles mosquitoes can be made almost negligible. If at the same time no mosquitoes are any longer permitted to infect themselves by biting infected human beings, the spread of the disease must be greatly restricted or checked.

Mosquitoes and Malarial Fever

In order that the student may be able to differentiate with reasonable accuracy such mosquitoes as come under his observation,

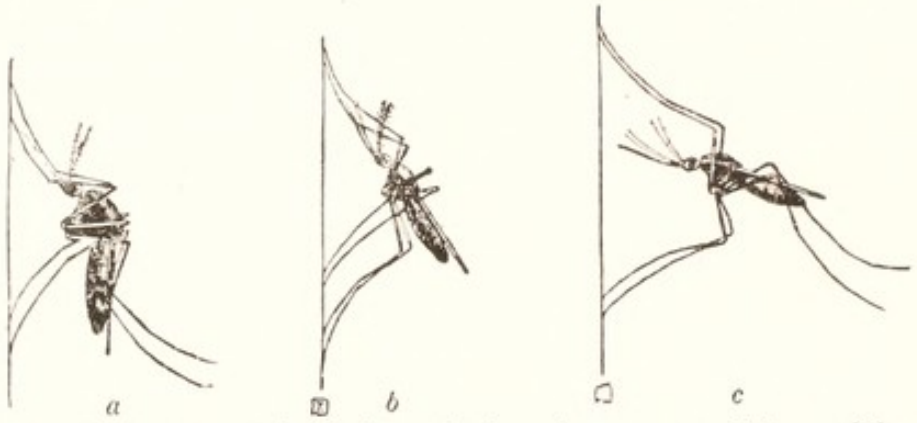


Fig. 191.—Various mosquitoes in attitudes of repose: *a*, Culex pipiens; *b*, Myzorrhynchus pseudo-pictus; *c*, Anopheles maculipennis (Manson).

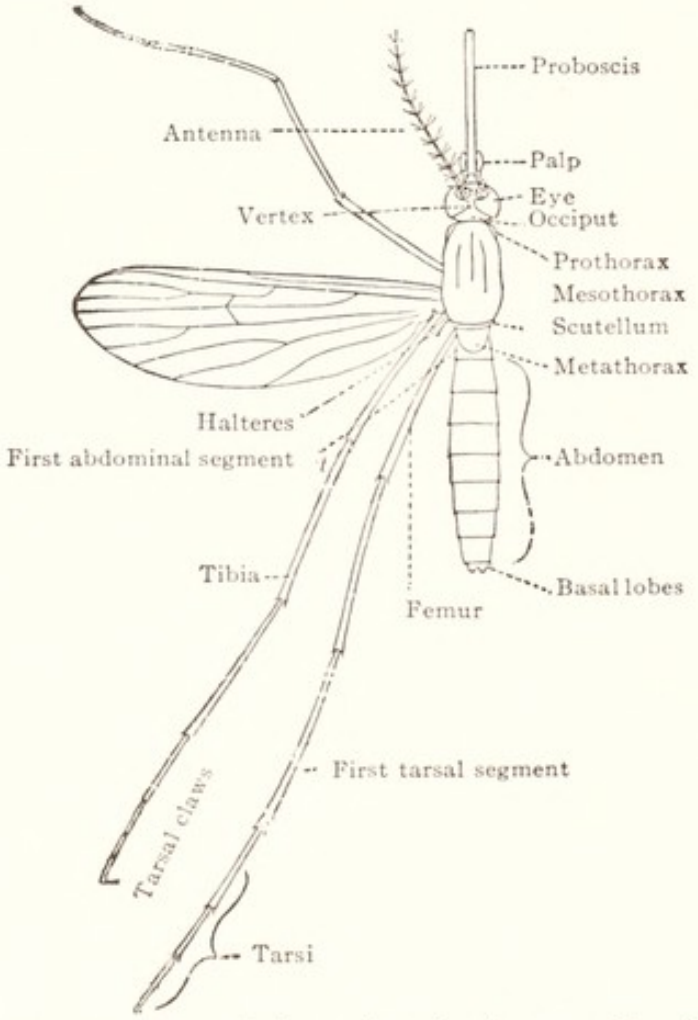


Fig. 192.—External morphology of a female mosquito (Manson).

use must be made of tabulations, to correctly use which, however, the student should have some familiarity with insect structure and

the general principles of entomology. The best works of reference for this purpose, that have come under observation to the present time are "A Text-book of Medical Entomology" by Patton and Cragg, published by the Christian Literature Society for India, London, Madras and Calcutta, 1913, and the "Handbook of Medical Entomology" by Riley and Johannsen, the Comstock Publishing Co., Ithaca, New York, 1915.

The mosquitoes comprise a family of dipterous or two-winged insects, included in the family Culicidæ. They can be recognized, first by their well-known general form, and second by the presence of *scales* upon some part of the head, thorax, abdomen, and wings. For the rough and ready identification of the larger groups and principal genera, the following table compiled from various authors may answer. For more precise information and for the identification of the species, of which hundreds are now described, reference must be made to the large works recommended above.

CLASSIFICATION (Stitt)

There are four subfamilies of CULICIDÆ, differentiated according to the palpi:

I. Palpi as long or longer than the proboscis in the male.
  1. Palpi as long as the proboscis in the female; proboscis straight..... ANOPHELINÆ.
  2. Palpi as long or shorter than the proboscis; proboscis curved..... MEGARRHININÆ.
  3. Palpi shorter than the proboscis..... CULICINÆ.

II. Palpi shorter than the proboscis in the male and female ÆDINÆ.

Of these the Anophelinæ is the one family concerned in the transmission of malarial fever, so that it is important to be able to differentiate the genera included in the family.

ANOPHELINÆ

1. Scales on head only; hairs on thorax and abdomen.
  1. Scales on wings large and lanceolate. Palpi only slightly scaled..... *Anopheles.*
  2. Wing scales small, narrow, and lanceolate. Only a few scales on palpi..... *Myzomyia.*
  3. Large inflated wing scales..... *Cycloleppteron.*
2. Scales on head and thorax. Scales narrow and curved. Abdomen with hairs, not scales.
  1. Wing scales small and lanceolate..... *Pyretophorus.*
3. Scales on head, thorax, and abdomen. Palpi covered with thick scales.
  1. Abdominal scales on ventral surface only. Thoracic scales like hairs. Palpi rather heavily scaled.... *Myzorrhynchus.*
  2. Abdominal scales narrow, curved or spindle shaped, in tufts and dorsal patches..... *Nyssorrhynchus.*
  3. Abdomen almost completely covered with scales and also having lateral tufts..... *Cellia.*
  4. Abdomen completely scaled..... *Aldrichia.*

Species of the genera Anopheles, Myzomyia, and Myzorrhynchus, are known to transmit malarial parasites. The Culicinæ include Stegomyia and Culex, which have some medical interest, as the former transmits yellow fever and the latter, filarial worms.

CULICINÆ

I. Posterior cross-vein nearer the base of the wing than the mid-cross-vein.

1. Proboscis curved in the female..... *Psorophora.*
2. Proboscis straight in the female:
   A. Palpi with three segments in the female.
      a. Third segment somewhat longer than the first two..... *Culex.*
      b. The three segments are equal in length..... *Stegomyia.*
   B. Palpi with four segments in the female.
      a. Palpi shorter than the third of the proboscis. Spotted wings..... *Theobaldia.*
      b. Palpi longer than the third of the proboscis. Irregular scales on the wings..... *Mansonia.*
   C. Palpi with fine segments in the female.... *Tæniorrhynchus.*

II. Posterior cross-vein in line with the mid-cross-vein... *Joblotina.*

III. Posterior cross-vein further from the base of the wing than the mid-cross-vein..... *Mucidus.*

Male mosquitoes can at once be recognized by the pennate antennæ which appear like plumes on each side of the head. They commonly "swarm" in flocks, do not suck blood, and are not commonly found in or about human habitations. Comparatively little is known of their habits. Cohabitation of the sexes occurs but once after which the males commonly die. The females after fecundation require a meal of blood before they become gravid and ready to oviposit. Oviposition takes place in water. During the winter many gravid females hibernate in cellars in a very inactive condition, but are immediately ready to fly to appropriate places and lay their eggs with the return of warm weather. In hot climates some of them estivate—*i.e.*, become similarly inactive during the dry period, but are ready to fly to the water and oviposit as soon as the rains begin again. The breeding places vary with the species. Fresh water is the usual preference, but a few select pools of brackish water, and one or two species prefer salt water. Most of the malaria-bearing species of anopheles prefer pools of fresh clear water, some prefer running water in small streams with a slow current. A few breed in large rivers. Some species are notably domestic and oviposite in wells, cisterns, water-butts, cans and any other available collection of water.

The eggs are laid as the female hovers upon the surface, touching the water from time to time, with the tip of the abdomen, each time depositing an egg. Culex eggs are fastened together side by side to form a kind of minute raft, but anopheles eggs are laid singly and float away independently of one another. If at the time the waters are receding, the eggs catch upon the leaves and stems of plants they may remain alive until the waters rise again before hatching. Dry eggs are sometimes able to remain alive for long periods, and may even be frozen without being killed. Cazeneuve hatched eight larvæ from eggs obtained by thawing a block of ice taken from a swamp in North China, where the temperature had gone as low as $-32°C$. When conditions are favorable the eggs hatch in two or three weeks. The anopheles larvæ feed at

the surface of the water along the banks where they are protected by the vegetation. They are voracious feeders and satisfy their appetites with all kinds of minute vegetable and animal organisms or remnants. In a day or two the larvæ molt for the first time. In

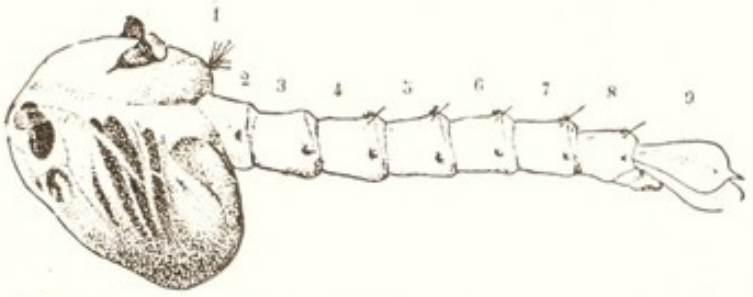


Fig. 193.—Pupa of Anopheles maculipennis (Brumpt).

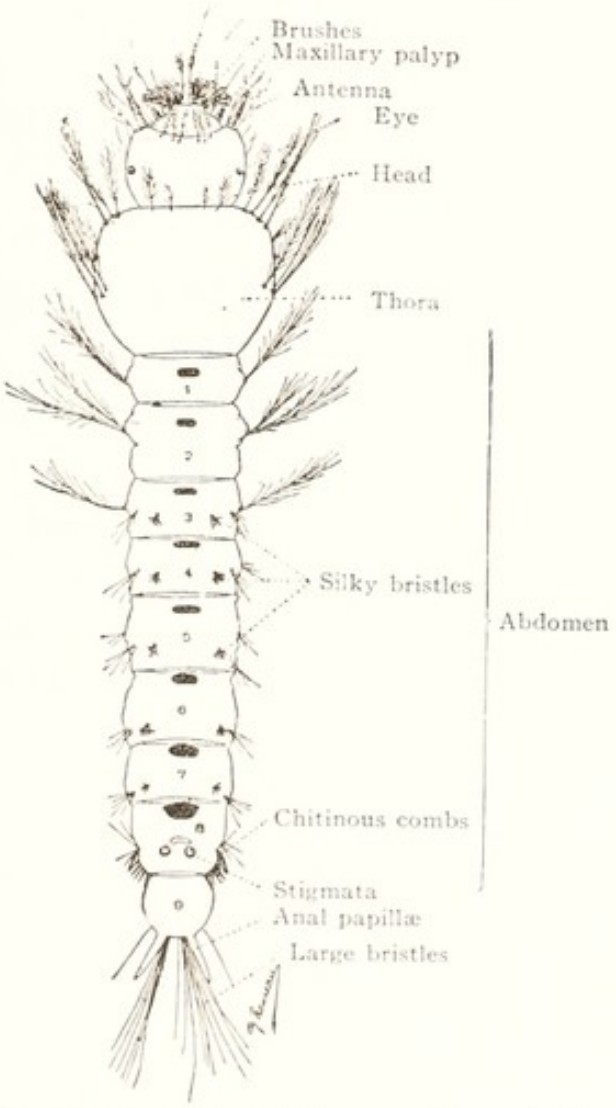


Fig. 194.—Larva of Anopheles maculipennis (Brumpt).

five or six days, having grown larger, they molt a second time and pupate. The appearances of the larvæ and pupæ are shown in the accompanying diagrams. The pupa floats at the surface of the water, is comparatively inactive and does not feed. If disturbed, it

is capable of swimming vigorously to escape. In about three days the imago issues and is ready to fly. Anopheles do not fly great distances; a few hundred yards is the common range of their activities. They do not always return to the same pools from which they issued, any similar pool or stream is good enough for ovi-

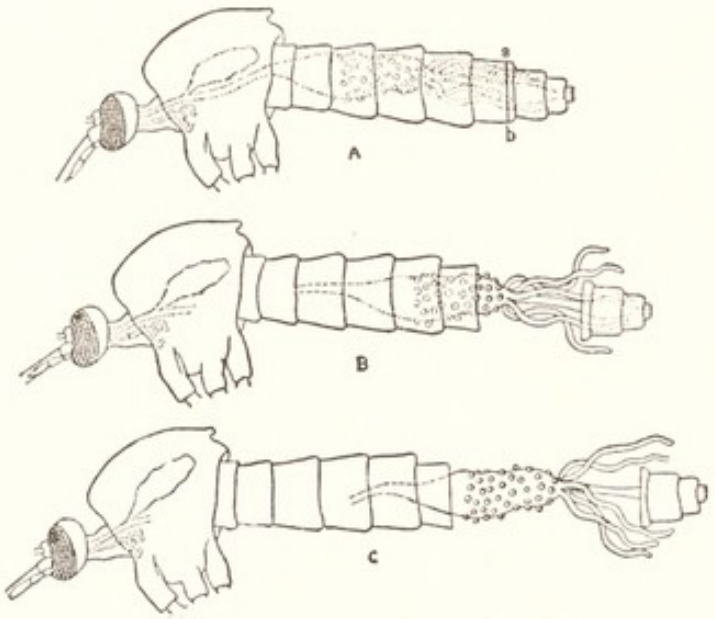


Fig. 195.—Method of withdrawing the digestive tube of the mosquito for study (Blanchard).

position. After having deposited the first lot of eggs, the female is ready to feed again and produce a new lot. This can go on for a number of broods. How long the insects can live, probably depends upon their activities. When actively engaged in reproductive activities they probably live a shorter time than when hibernating

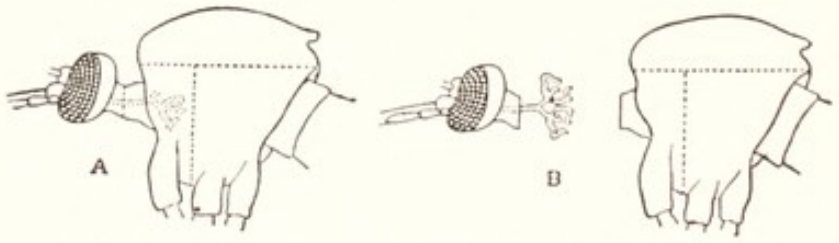


Fig. 196.—Method of withdrawing the salivary glands of the mosquito for study (Blanchard).

or estivating. It is known that some of them can live the greater part of a year.

The mosquitoes used for study and for classification should be mounted dry in the usual way well known to all entomologists.

Fine entomologic pins (00–000) should be employed for the purpose. The insects should be caught in a wide-mouth bottle containing some fragments of cyanid of potassium, covered with a layer of sawdust, over which a thin layer of plaster of Paris is allowed to solidify. The insects die in a moment or two, can be emptied upon a table, and the pin carefully thrust through the central

part of the thorax. As soon as the insect is impaled, the pin should be passed through an opening in a card or between the blades of a forceps until the insect occupies a position at the junction of the middle and upper third. The insect should not be touched with the fingers, as the scales will be brushed off and the limbs broken. Mounted insects must be handled with entomologic forceps, touching the pins only. Every insect thus mounted should have placed upon the pin, at the junction of the middle and lower thirds, a small bit of card or paper, telling where and when and under what circumstances it was taken.

The dissection of fresh mosquitoes for determining whether or not they are infected with malarial organisms must be made with the aid of needles mounted in handles. The position of the stomach, intestines, and the salivary glands, and the mode of pulling the insect apart to show them can be learned from the diagram. The organs thus withdrawn and separated from the unnecessary tissue can be fixed to a slide with Meyer's glycerin-albumin or other albuminous matter, and then stained like a blood-smear, but should be cleared after staining and washing, and mounted in Canada balsam under a cover-glass.

Fig. 197.—Imago of Anopheles maculipennis escaping from the pupa case upon the surface of the water (Brumpt).

A more certain and more elegant manner of showing the parasites in infected mosquitoes is by pulling off the legs and wings, embedding the insect in paraffin and cutting serial longitudinal vertical sections.

To infect mosquitoes and study the development of the malarial parasites in their bodies, the insects should be bred from the aquatic larva in the laboratory, to make sure that they do not already harbor parasites. The mosquitoes are allowed to enter a small cage made with mosquito netting, and are taken to the bedside of the malarial patient, against whose skin the cage is placed until the insects have bitten and distended themselves with blood, when they are taken back to the laboratory, kept as many days as may be desired, then killed and sectioned. In this way, remembering that the entire mosquito cycle of development takes about a fortnight, any stage of the cycle may be observed.

CHAPTER XX

RELAPSING FEVER

Spirillum Obermeieri or Spirochæta Obermeieri or Spirochæta Recurrentis (Obermeier)

General Characteristics.—An elongate, flexible, flagellated, non-sporogenous, actively motile spiral organism, pathogenic for man and monkeys, susceptible of cultivation in special media, stained by ordinary methods, but not by Gram's method.

In 1868 Obermeier* first observed the presence of actively motile spiral organisms in the blood of a patient suffering from relapsing fever. Having made the observation, he continued to study the organism until 1873, when he made his first publication. From 1873 until 1890 it was supposed that spirochæta rarely played any pathogenic rôle. Miller† had, indeed, called attention to the constant presence of Spirochæta dentinum in the human mouth, but it had not been connected with any morbid condition. In 1890 Sacharoff‡ discovered a spirillary infection of geese in the Caucasus, caused by an organism much resembling Spirochæta obermeieri and called Spirochæta anserinum. In 1903 Marchoux and Salimbeni§ found a third disease, fatal to chickens, caused by Spirochæta gallinarum, and found that the spread of the disease was determined by the bites of a tick, Argas miniatus. In 1902 Theiler,‖ in the Transvaal, observed a spiral organism in a cattle plague. This has been named after him by Laveran, Spirochæta theileri. It was found to be disseminated by the bites of certain ticks—Rhipicephalus decoloratus. Later, what was probably the same organism, was found in the blood of sheep and horses. In 1905 Nicolle and Comte** found a spiral organism infecting certain bats. By this time, therefore, it became evident that spirochætal infections were fairly well disseminated among the lower animals and that the spirochæta were of different species with different hosts and intermediate hosts.

In 1904 Ross and Milne†† and Dutton and Todd‡‡ studied a peculiar African fever which they were able to refer to a spirochæta

* "Centralbl. f. d. med. Wissenschaft," 1873.
† Microorganisms of the Human Mouth, Phila., 1890, p. 44 et seq.
‡ "Ann. de l'Inst. Pasteur," 1891, XVI, No. 9, p. 564.
§ Ibid., 1903, XVII, p. 569.
‖ "Jour. Comp. Path. and Therap.," 1903, XLVII, p. 55.
** "Compt.-rendu de la Soc. de Biol. de Paris," July 22, 1905, LIX, p. 200.
†† "British Med. Jour.," Nov. 26, 1904, p. 1453.
‡‡ "Memoir XVII, Liverpool School of Tropical Medicine," "Brit. Med. Jour.," Nov. 11, 1905, p. 1259.

for which Novy* has proposed the name Spirochæta duttoni in memory of Dutton, who lost his life while studying it. In 1905 Koch† while working in Africa discovered a spirochæta that he regarded as identical with that already described by Ross and Milne and Dutton and Todd. Later studies of the organism convinced C. Fränkel‡ that it was a separate species. For it Novy later suggested the name Spirochæta kochi. In 1906 Norris, Pappenheimer and Flournoy§ found a spirochæta in the blood of a patient suffering from relapsing fever in New York. This having been extensively studied by Novy, has since been called Spirochæta novyi.

With the work of Schaudinn and his associate, Hoffmann,‖ the spirochæta came to be regarded as protozoan parasites because of the presence of an undulating membrane; the refusal of most of the organisms to grow upon artificial media, the rôle of an intermediate host (ticks, etc.) in transmitting them, and the longitudinal mode of division.

Fevers characterized by relapses and by the presence of spiro-

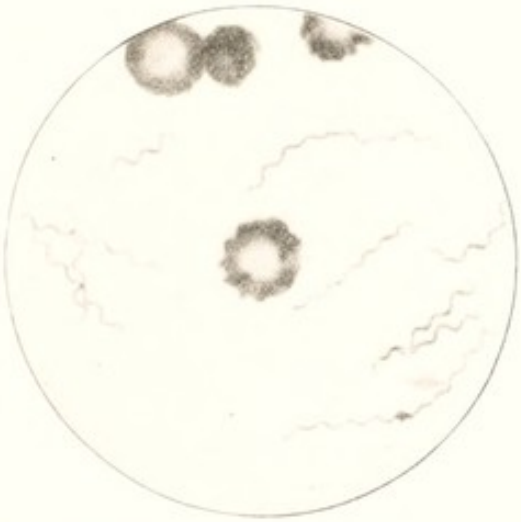


Fig. 198.—Spirochæta obermeieri from human blood (Kolle and Wassermann).

chæta in the blood have been found in northern and northeastern Europe (true relapsing fever with Spirochæta obermeieri), in various parts of equatorial Africa (African relapsing fever with Spirochæta duttoni); in North Africa (Spirochæta berbera); in Bombay and in other parts of India (Spirochæta carteri); in Persia (Spirochæta persica); and in America (Spirochæta novyi). The question, therefore, arises whether these similar diseases are slight modifications

* "Jour. Infectious Diseases," 1906, III, p. 295.

† "Deutsche med. Wochenschrift," 1905, XXXI, p. 1865; "Berliner klinische Wochenschrift," 1906, XLIII, 185.

‡ "Med. klin.," 1907, III, 928; "Münchener med. Wochenschrift," 1907, LIV, 201.

§ "Jour. Infectious Diseases," 1906, III, 266.

‖ "Deutsche med. Wochenschrift," Oct., 1905, XXXI, p. 1665; "Arbeiten aus dem kaiserlichen Gesundheitsamte," 1904, XX, pp. 387–439.

of the same thing caused by the same parasite, or whether they are different diseases caused by slightly different parasites.

If Nuttall be correct, there are no adequate grounds upon which to conclude that the spirochetes are really different species. On this account, and as the differences between the organisms are minute, it scarcely seems well to devote space to the consideration of each, but better to select the oldest and the best known—Spirochæta obermeieri—as the type, describe it, and then point out such variations as are shown by its close relations.

Morphology.—The Spirochæta obermeieri is extremely slender, flexible, spirally coiled, like a corkscrew, and pointed at the ends.

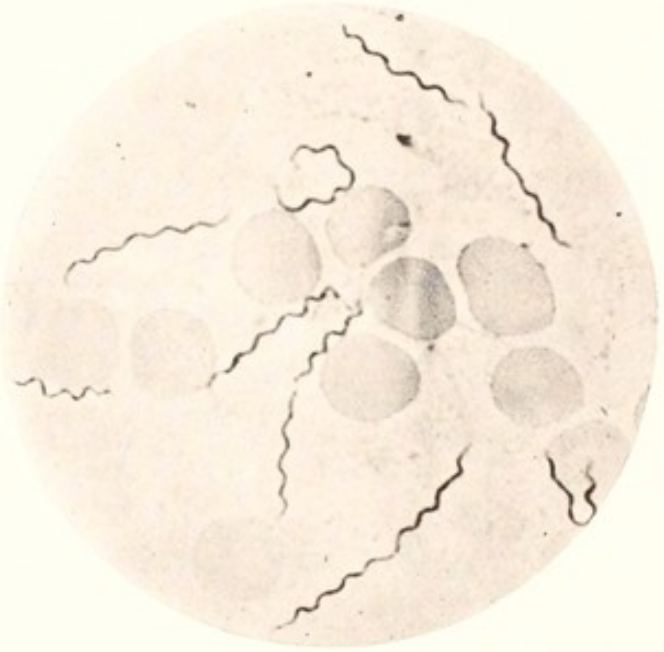


Fig. 199.—Spirochæta obermeieri (Novy). Rat blood No. 321a. × 1500.

It measures approximately 1 μ in breadth and 10, 20, or even 40 μ in length. The number of spiral coils varies from 6 to 20; the diameter of the coils varies so greatly that scarcely any two are uniform. Wladimiroff* doubts the existence of a flagellum, but flagella-like appendages are usually to be seen at one or both ends of the organisms. An undulating membrane attached nearly the entire length of the organism, very narrow, and inconspicuous, forms the chief means of locomotion. The organism is actively motile, and darts about in fresh blood with a double movement, consisting of rotation about the long axis and serpentine flexions. No structure can be made out by our present methods of staining and examining the spirochæta. No spores are found. Multiplication is thought to take place by longitudinal division, though some believe the division to be transverse.

* "Kolle and Wassermann's Handbuch der pathogene Mikroörganismen," 1903, III, p. 82.

The Spirochæta duttoni is said by Koch,* in his interesting studies of "African Relapsing Fever," to resemble the Spirochæta obermeieri in all particulars.

The Spirochæta novyi with which Novy and Knapp† experimented, and which they believed to be identical with Spirochæta obermeieri, measured 0.25 to 0.3 μ in breadth by 7 to 19 μ in length. The number of coils varies from three to six. The shorter forms are pointed, with a long flagellum at one end and a short one at the other.

Staining.—The spirochæta can be stained with ordinary anilin dye solutions, by the Romanowsky and Giemsa methods, and by the silver methods (see Treponema pallidum). It does not stain by Gram's method.

Fig. 200.—Spirochæta duttoni (Novy). Tick fever, No. 520. Rat blood. × 1500.

Cultivation.—Following the suggestion of Levaditi, Novy and Knapp‡ cultivated Spirochæta obermeieri in collodion sacs in the abdominal cavity of rats, and succeeded in maintaining it alive in this way through twenty consecutive passages during sixty-eight days. They were able to do this in rat serum from which all corpuscles had been removed by centrifugation, and so proved that no intercellular developmental stage of the organism takes place. Organisms thus cultivated attenuate in virulence.

Norris, Pappenheimer, and Flournoy§ believe that they succeeded in securing multiplication of the spirochæta by placing several drops

* "Berliner klin. Wochenschrift," Feb. 12, 1906, XXXIV, No. 7, p. 185.
† "Jour. Infectious Diseases," 1906, III, p. 291.
‡ "Jour. Amer. Med. Assoc.," Dec. 29, 1906, XLVII, p. 2152.
§ "Journal of Infectious Diseases," 1906, III, 266.

of blood containing them in 3 to 5 cc. of citrated rat or human blood. A third generation always failed.

Noguchi* was the first to achieve the successful cultivation of the spirochæta in artificial culture media. The best success was obtained as follows: Into each of a number of sterile test-tubes 2 × 20 cm. in size is placed a fragment of fresh sterile rabbit kidney and then a few drops of citrated blood from the heart of an infected mouse or rat. Following this, about 15 cm. of sterile ascitic or hydrocele fluid are quickly poured into the tubes and the contents of some of the tubes are covered with a layer of sterile paraffine oil, while the rest are left without the oil. The tubes are placed in the incubating oven at 37°C. By these means cultures of Spirochæta duttoni, Spirochæta kochi, Spirochæta obermeieri and Spirochæta novyi were secured. The maximum growth was obtained in 7, 8 or 9 days at 37°C. The presence of some oxygen seemed to be essential. By transplantations to fresh media of the same kind they were all kept growing for many generations during which they did not lose their virulence.

Mode of Infection.—The means by which Spirochæta obermeieri is transmitted from individual to individual is not definitely known. Tictin† seems to have been the first to believe that the transmission of the disease was accomplished through the intermediation of some blood-sucking insect. He investigated lice, fleas, and bed-bugs, in the latter of which he was able to find the organisms, and through blood obtained from which he was able to transmit the disease to an ape. He was not able to infect apes by permitting infected bed-bugs to bite them. Breinl and Kinghorn and Todd‡ made a careful study of the subject, but, like Tictin and their other predecessors, were unable to infect monkeys by permitting infected bed-bugs to bite them.

Mackie,§ Graham-Smith,‖ Bousfield,** Ed. Sergent and H. Foley,†† studied the louse and found that it was undoubtedly capable of acting as a transmitting agent, and possibly was the only definitive host of the parasite. Nicolle, Blaizot and Conseil‡‡ studied the North African relapsing fever of Tunis and Algeria, and proved that the body and head lice are undoubtedly the common definition hosts of its spirochæte. When the lice were fed upon blood of infected patients, the spirochætes rapidly disappear in their bodies, but after eight days reappear and remain for almost twelve days during which time the insects can transmit the disease. They also found that the

* "Journal of Experimental Medicine," 1912, XVI, 199.
† "Centralbl. f. Bakt. u. Parasitenk.," 1894, I Abt., XV, p. 840.
‡ Ibid., Oct., 1906, XLII, Heft 6, p. 537.
§ "Brit. Med. Jour.," Dec. 14, 1907.
‖ "Ann. de l'Inst. Pasteur," 1910, p. 63.
** Report of the Wellcome Tropical Research Laboratories, 1911, p. 63.
†† "Ann. de l'Inst. Pasteur," 1910, p. 337.
‡‡ "Ann. de l'Inst. Pasteur," Mar. 25, 1913, vol. XXVII, No. 3, p. 204.

infectious agent passes to a new generation of the lice, which are also infective. They also studied a tick, Ornithodorus savignyi, found in those countries, thinking that it might behave like Ornithodorus moubata toward Spirochæta duttoni, and found that it could transmit the spirochæte of the Tripolitan relapsing fever, though apparently not that of the Tunisian fever.

When we come to consider Spirochæta duttoni, however, we find our knowledge much further advanced. On Nov. 26, 1904, Dutton and Todd announced that they had discovered a spirillum to be the specific agent in the causation of tick fever in the Congo, and on the same date Ross and Milne* published the same fact. Dutton and Todd subsequently withdrew their claim to priority of the discovery. On Feb. 4, 1905, Ross published in the "British Medical Journal" the following cablegram from Dutton and Todd, then working on the Congo: "Spirilla cause human tick fever; naturally infected ornithodorus infect monkey." It was not until Nov. 11, 1905, that the paper upon the subject was read and published in the same journal by Dutton and Todd, and the etiology of the disease made clear. These observers found that the horse-tick, *Ornithodorus moubata* (Murray) is the intermediate host of the spirilla or spirochæta causing the disease, and that when these ticks were permitted to bite infected human beings, and then subsequently transferred to monkeys, the latter sickened with the typical infection.

The matter received confirmation and addition through the studies of Koch,† who studied the ticks, observed the distribution of the micro-organisms in their bodies, and found that they collected in large numbers in the ovaries, so that the eggs were commonly infected and the embryo hexapod ticks hatched from them were infective. Not only is this second generation of ticks infected, but Möller has found the third generation also infected by the spirochæta, and it is not improbable that the infection is kept on passing from female to offspring through many generations. Leishman, who followed the spirochæta throughout the body of the tick, observed that it entered the ovaries and appeared in the ova in the spiral form, but that in the ova it not infrequently became transformed to "coccoid" granules which held together more or less closely like tiny streptococci. He supposed that it was in the granular form that the micro-organism found its way into the embryo and so infected the developing nymph. There is reason to believe that this was an error and that the spirals alone are the sources of transmission and infection. What is true of the tick seems to be equally true of the lice, the infective micro-organisms being passed down from generation to generation. Thus, in regard to Spirochæta duttoni we are able to say quite definitely that the tick is the usual if not the only means of dissemination. How the ticks and lice

* "British Medical Journal," Nov. 26, 1904.
† "Berliner klin. Wochenschrift," Feb. 12, 1906.

effect the transmission of micro-parasites is to a certain extent in dispute. It was at first supposed that the spirochætes entered the human hosts with the saliva of the respective arthropods, but there is some reason to think that this is a mistake, and that the scratching of the itching bite conveys the spirochæta deposited upon the skin in the excrement of the arthropod, into the deeper layers and lymphatics through which it reaches the blood.

Pathogenesis.—The spirochæta of relapsing fever are pathogenic for man and monkeys, some of them for smaller animals. Novy and Knapp* found their organism and Spirochæta duttoni to be infectious for mice and rats, and attribute the failure of others to discover this to their failure to examine the blood during the first and second days. Fulleborn and Meyer, and Martin† were able successfuly to transmit the spirochæta of Russian relapsing fever to mice after first passing it through apes. Rabbits and guinea-pigs seem to be refractory; white mice susceptible. Man, monkeys, and mice suffer from infection characterized by relapses, and in them the disease may be fatal. Rats never die of the disease and rarely have relapses.

The micro-organisms are free parasites of the blood in which they swim with a varying rapidity, according to the stage of the disease. They are present during the febrile paroxysms only, disappearing completely as soon as the crisis is reached.

The course of relapsing fever in man is peculiar and characteristic. After a short incubation period the invasion comes on with chill, fever, headache, pain in the back, nausea and vomiting, and sometimes convulsions. The temperature rises rapidly and there are frequent sweats. The pulse is rapid. By the second day the temperature may be 104° to 105°F. and the pulse 110 to 130. There is enlargement of the spleen. Icteroid discoloration of the conjunctiva may be observed. The fever persists with severity and the patient appears very ill for five or six days, when a crisis occurs, and the temperature returns to normal; there is profuse sweating and sometimes marked diarrhea, and the patient at once begins to improve. So rapid is the convalescence that in a few days he may be up and may desire to go out. The disease is, however, not at an end, for on or about the fourteenth day the *relapse* characteristic of the affection makes its appearance as an exact repetition of what has gone before. This is followed by another apyretic interval, and then by another relapse, and so on. The patient usually recovers, the mortality being about 4 per cent. The fatal cases are usually old or already infirm patients. The Indian, African, and American varieties present variations of no great importance. The European fever usually ends after the second or third relapse, the African not until after a greater number.

* Loc. cit.

† Loc. cit.

The spirochæta are present in the blood in great numbers during the febrile stages, but entirely disappear during the intervals.

Lesions.—There are no lesions characteristic of relapsing fever.

Bacteriologic Diagnosis.—This should be quite easily made by an examination of either the fresh or stained blood, provided the blood be secured during a febrile paroxysm. The readiness with which the organisms take the stain leaves little to be desired.

Novy and Knapp have found that the serum of recovered cases can be used to assist in making diagnosis because of its agglutinating, germicidal, and immunizing powers.

Immunity.—The phenomena of immunity are vivid and important. At the moment of decline of the fever a powerful bacteriolytic substance appears in the blood and dissolves the organisms. At the same time an immunizing substance appears. The two do not appear to be the same.

The immunizing body affords future protection to the individual for an indefinite length of time. It can be increased by rapidly injecting the animal with blood containing spirochæta. Serum containing the immunizing body imparts passive immunity to other animals into which it is injected, and, according to Novy and Knapp, establishes a solid basis for the prevention and cure of relapsing fever in man.

THE VECTORS OF RELAPSING FEVER

I. Ticks

The ticks thus far known to act as vectors of relapsing fever are two species of the genus Ornithodorus. Thirteen species of this genus are described in "A Text-book of Medical Entomology," by Patton and Cragg, who give excellent tables for their identification and additional valuable information is to be found in the excellent "Monograph of the Ixodoidea," by Nuttall. Ornithodorus ticks of various species are to be found pretty widely distributed throughout tropical and semitropical regions of both hemispheres. In general, they are most numerous where the temperature is highest and the soil driest.

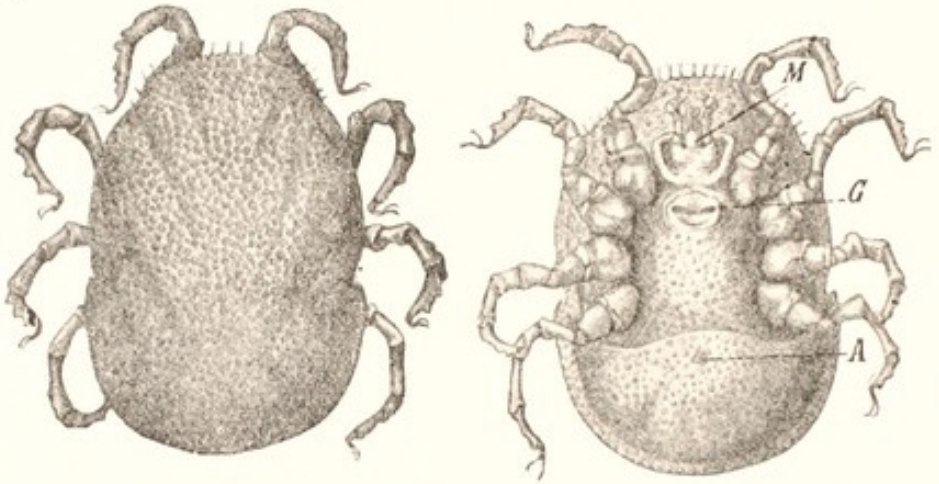
The genus Ornithodorus was described by C. L. Koch and characterized as follows: "The body is flat when starving and convex when replete, and may be nearly as broad anteriorly as posteriorly, or pointed and beak-like anteriorly. The margin of the body is not distinct but is of a similar structure to the rest of the integument which is generally mamillated. On the ventral surface there are two well-marked folds, one internal to the coxæ, the coxal fold, and the other above the coxæ, the supracoxal fold; there is also a transverse pre-anal groove, as well as a transverse postanal groove. Eyes are either absent or present in pairs on the supracoxal fold; one pair between coxæ I and II, and the other between coxæ III and IV.

The Ornithodorus savignyi is the transmitting agent of Spirochæta berbera; Ornithodorus moubata of Spirochæta duttoni.

Ornithodorus savignyi.—The description given by Patton and Cragg ("A Text-book of Medical Entomology," 1913, p. 586) is as follows: Integument leathery and covered by distinct non-contiguous mammillæ and numerous short hairs interspersed. Supracoxal folds well marked, with two eyes on each side. Coxal folds less well marked. Pre-anal groove distinct. The basis capituli broader than long and shorter than the rest of the rostrum. Hypostome with six principal rows of teeth, the external the stoutest. Palps with first and second segments of equal length, third segment the shortest. Coxæ contiguous; protarsus and tarsus of legs I, II and III with three well-marked humps; the two proximal humps on tarsus of leg IV are close to each other, while the third is

separated by an interval of about two and a half times the distance between the first and second.

Length 5–12 mm. Width 4–8.5 mm. The female and male resemble each other except that the latter are smaller. Its genital orifice is markedly smaller. In the female the genital orifice is a broad transverse slit which can be made to gape and is guarded by two flaps like valves; in the male the orifice is oval and the valves are absent. The eggs number 50–100, measure 1.3–1.5 mm. in length and 0.8–1 mm. in breadth. They are oval, smooth and of a dark brown or black color.



a *b*

Fig. 201.—Ornithodorus moubata. Tick that transmits African relapsing fever: *a*, Viewed from above; *b*, viewed from below (Murray from Doflein).

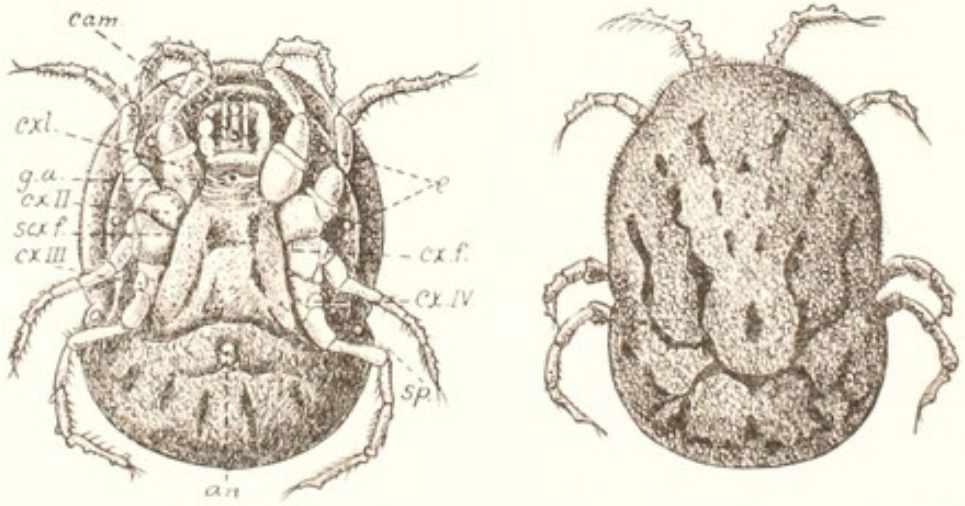


Fig. 202.—Ornithodorus savignyi. *An*, anus; *cam*, camerostome; *cx.I*, coxa I; *cx.II*, coxa II; *cx.III*, coxa III; *cx.IV*, coxa IV; *cx.f.*, coxal fold; *e*, eye; *g.a.*, genital aperture; *g.g.*, genital groove.

Habitat.—Arabia, Nubia, Egypt, Somaliland, Abyssinia, German East Africa, Cape Colony, Rhodesia, Bechuanaland and Portuguese East Africa. In India it is common in the Madras Presidency, in Gujarat, and in many parts of the Bombay Presidency. In Aden it is widely distributed throughout the Hinterland, where its principal host is the camel.

Ornithodorus moubata.—Patton and Cragg describe this tick as follows: Body almost as broad anteriorly as posteriorly; covered with non-contiguous mamillæ, but with fewer hairs than *savignyi*. Basis capituli broader than long and shorter than the palps; hypostome with six principal rows of teeth. Tarsi of legs I, II and III with three humps as in *savignyi;* those on the pro-tarsus are

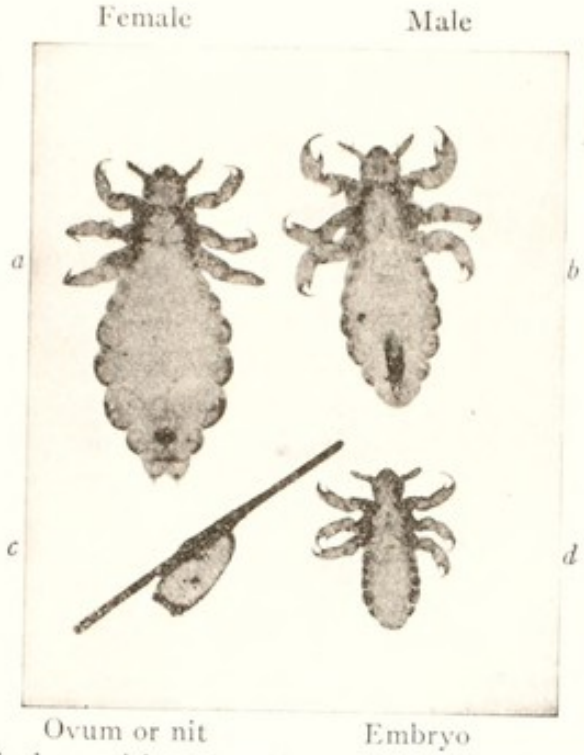


Fig. 203.—Pediculus capitis, or head-louse. × 10. *a*, Female; *b*, male; *c*, egg cemented to a hair; *d*, nymph. (From Beattie and Dickson's "A Text-book of General Pathology," by kind permission of William Heinemann, Publisher.).

Fig. 204.—Pediculus vestimenti, the clothes or body louse. × 10. *a*, Male, *b*, female; *c*, nymph; *d*, egg. (From Beattie and Dickson's "A Text-book of General Pathology," by kind permission of William Heinemann, Publisher.)

subequal, more pointed and about equidistant, while those of *savignyi* are unequal, less pointed and not equidistant. The tarsus of leg IV in *moubata* is shorter and thicker than in *savignyi*, and its humps are nearly equidistant. Eyes absent. Length 8–12 mm.; breadth 6–10 mm. The eggs are ovoid, measure 0.8–0.9 mm. in length, are smooth on the surface and dark yellow in color.

Habitat.—Africa: from British East Africa to the Transvaal, and across to the Congo; southward to German East Africa and Cape Colony. It is common in Egypt, Abyssinia and in parts of Somaliland and in Portuguese East Africa.

Ornithodorus savignyi is chiefly a parasite of the camel and only occasionally bites man; Ornithodorus moubata is essentially a human pest.

The eggs of these ticks hatch in eight to fourteen days. The larval stage which has six legs is spent in the eggs and the creature that emerges is usually a first nymphal instoη, which has eight legs. After hatching it remains inactive for several days, then becomes very active and ready to suck blood. As it grows it becomes voracious, distending itself with blood, then dropping off, hiding itself for a time,

Male Female

Fig. 205.—Pediculus pubis, Phthirius inguinalis or crab-louse. × 17. (From Beattie and Dickson's "A Text-book of General Pathology," by kind permission of William Heinemann, Publisher.)

molting, then being ready to feed again. This continues for a number of months, the ticks molting four times before passing from the nymph to the adult stage.

Ornithodorus moubata is a common inhabitant of the native African huts along the caravan routes. To avoid it and escape relapsing fever R. Koch in his African expedition camped near but not in the villages, and avoided the native houses. It lives in the cracks in the mud walls, in the thatch, in the mats and sometimes simply upon the ground where its small size and dull color make it difficult to see. From these hiding places it crawls at night and like a bed-bug attacks the sleeping host. When handled it feigns death, remaining quiet for so long a time that it is hard to believe it alive.

The Ornithodorus savignyi is less adapted to the requirements of the spirochæta than its relative. Brumpt* found that the spirochæta did not pass through the eggs of O. savigyni to subsequent generations, and that the infectivity of the tick itself soon was lost. The spirochætæ remain indefinitely in O. moubata, and are passed through their eggs to at least three generations. It is, therefore, difficult to be certain that any particular tick is uninfected unless its progenitors be known.

The spirochæta pass from female to the ovum and infect the young nymphs as such. The granules observed in the eggs of infected ticks, also occur in those of non-infected ticks and have nothing to do with the spirochæta.

* "Precis de Parasitologie," 1910, 538.

II. Lice

Lice are apterous insects formerly classed in the order Hemiptera, but now placed in a separate order, the Anoplura. Two genera, and three species are common upon human beings.

I. **Pediculus** (Linn, 1758). In this genus there are two species:

1. *Pediculus capitis* (de Geer, 1778). This is the head-louse. It is of a gray color. The abdomen is composed of eight and not of seven segments as was stated by Piaget, and is blackened along the edges. The males and females look much alike, but the male measures 1.8 mm. in length and 0.7 mm. in breadth, while the female measures 2.7 mm. in length by 1 mm. in breadth.

   These parasites live in the hair, close to the scalp. Rarely they pass from the scalp to the beard. Still more rarely do they occur upon other hair-covered surfaces. The female produces large eggs, one at a time, which are firmly anchored to the hairs by a mucilaginous secretion. In them the embryo develops in about sixteen to eighteen days then escapes as a nymph with proportionally smaller body and larger legs than the adult. There are three molts before the insect reaches maturity. The full and empty eggs occur in great numbers upon the hairs and are known as "nits."

   The insects are sometimes present on the head in great numbers and cause intolerable itching.

2. *Pediculus vestimenti* (Nitzsch, 1818). This is a larger louse of much the same appearance and structure as P. capitis. Indeed there are such minute differences between the two that there is some dispute as to whether they should not form subspecies of the same insect instead of different species of insects.

   The size is, however, larger. The male measures 3 mm. in length and 1 mm. in breadth; the female 3.3 mm. in length and 1.14 in breadth.

   The "body louse" as this is commonly called, lives in the clothing, and passes to the skin to feed, then returns again to the seams of the garments. Its eggs are fastened to the fabric of the clothing, not to the skin or hairs. It is sometimes present in great numbers and its bites cause much annoying itching.

Both of these lice have been found to be capable of effecting the transmission of the spirochæta of relapsing fever. The infection in the lice is transmitted to its offspring as in the case of Ornithodorus moubata.

II. **Phthirius** (Leach, 1815). In this genus there is only one human parasite. Phthirius inguinalis (Ridi, 1668). This pubic louse or "crab louse" is often incorrectly called Pediculus pubis. It is a shorter, stouter-bodied creature with more powerful legs terminating in large tarsal hooks that give it a crab-like appearance. The thorax and abdomen are compressed and shortened to a heart-like body. The abdomen is composed of six segments, each of which has a pair of stigmata, but the stigmata of the first, second, third, fourth, and fifth segments appear to be in one broad segment. The males measure 1 mm. in length, the females 1.5 mm. These lice live chiefly in the pubic hair and that of the perineum. Rarely they are found in the axilla, the beard, the eye-brows and even upon the eye-lashes. The eggs are fixed to the bases of the hairs as in P. capitis. They hatch in about seven days and the nymphs grow to maturity fifteen days later.

The bites of these lice are very irritating and cause severe itching and the eruption of pink papules that sometimes become bluish spots nearly a centimeter in diameter. Such spots known as " "taches ombrées" are frequent in typhoid fever when lice are present.

It is not known that this louse can harbor spirochæta or any pathogenic bacteria or protozoa.

CHAPTER XXI

SLEEPING SICKNESS

Trypanosoma Gambiense (Dutton) Trypanosoma Rhodesiensi (Stephens and Fantham)

Sleeping sickness, African lethargy, Maladie du sommeil, Schlafkrankheit, or human trypanosomiasis is a specific, infectious, endemic disease of equatorial Africa characterized by fever, lassitude, weakness, wasting, somnolence, coma, and death. The first mention of the disease seems to have been made by Winterbottom.*

Sir Patrick Manson† says that "For upward of a century students of tropical pathology have puzzled over a peculiar striking African disease, somewhat inaccurately described by its popular name, the sleeping sickness. Its weirdness and dreadful fatality have gained for it a place not in medical literature only, but also in general literature. The mystery of its origin, its slow but sure advance, the prolonged life in death that so often characterizes its terminal phases, and its inevitable issue, have appealed to the imagination of the novelist, who more than once has brought it on his mimic stage, draping it, perhaps, as the fitting nemesis of evil-doing. The leading features of the strange sickness are such as might be produced by a chronic meningo-encephalitis. Slow irregular febrile disturbance, headache, lassitude, deepening into profound physical and mental lethargy, muscular tremor, spasm, paresis, sopor, ultimately wasting, bed-sores, and death by epileptiform seizure, or by exhaustion, or by some intercurrent infection.

"In every case the lymphatic glands, especially the cervical, are enlarged, though it be but slightly. In many cases pruritus is marked. In all, lethargy is the dominating feature.

"In some respects this disease, which runs its course in from three months to three years from the oncoming of the decided symptoms, resembles the general paralysis of the insane. It differs from this, however, in the absence, as a rule, of the peculiar psychic phenomenon of that disease. There are exceptions, but generally, though the mental faculties in sleeping sickness are dull and slow acting, the patient has no mania, no delusions, no optimism. So far is the last from being the case, that he is painfully aware of his condition and of the miserable fate that is in store for him; and he looks as if he knew it."

* "An Account of Native Africans in the Neighborhood of Sierra Leone," 1803.

† "The Lane Lectures for 1905," Chicago, 1905.

Specific Organism.—The discovery of the specific organisms was foreshadowed by Nepveu,* who recorded the existence of trypanosomes in the blood of several patients coming from Algeria, by Barron,† and by Brault.‡ In 1901 Forde received under his care at the hospital in Bathurst (Gambia), a European, the captain of a steamer on the River Gambia, who had navigated the river for six years, and who had suffered several attacks of fever that were looked upon as malarial. The examination of his blood revealed the presence not of malarial parasites, but of *small worm-like bodies*, concerning the nature of which Forde was undecided.§ Later, Dutton, in conjunction with Forde, examined this patient, whose condition had become more serious, and recognized that the worm-like bodies seen by Forde were trypanosomes. Of these parasites he has written an excellent description, calling them Trypanosoma

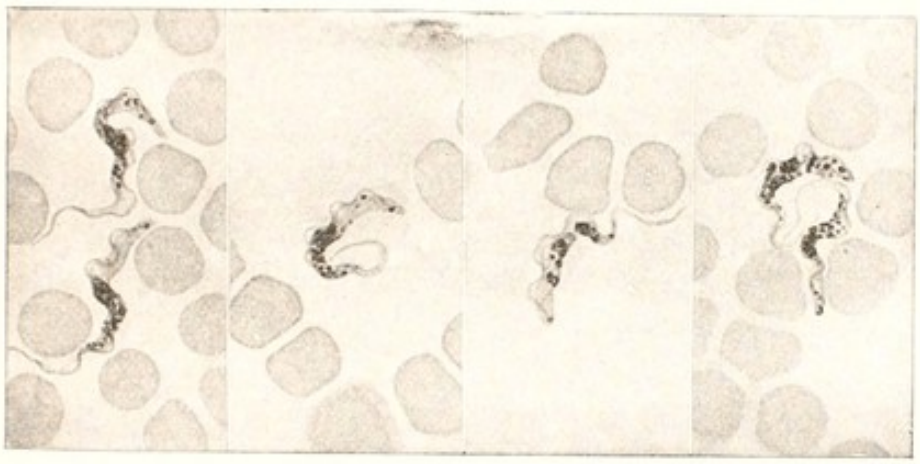


Fig. 206.—Trypanosoma gambiense (Todd).

gambiense.‖ The patient thus studied by Forde and Dutton died in England January 1, 1903. In 1903 Dutton and Todd** examined 1000 persons in Gambia and found similar trypanosomes in the bloods of 6 natives and 1 quadroon. In the same year Manson†† discovered 2 cases of trypanosomiasis in Europeans that had become infected upon the Congo. Brumpt‡‡ also observed T. gambiense at Bounba at the junction of the Ruby and the Congo, and Baker§§ observed 3 cases at Entebbe in Uganda.

During all this time no connection was suspected between these

* "Memoirs, Soc. de Biol. de Paris," 1891, p. 49.
† "Transactions of the Liverpool Medical Institute," Dec. 6, 1894.
‡ "Janus," July to August, 1898, p. 41.
§ "Trypanosomes and Trypanosomiasis," Laveran and Mesnil, 1907.
‖ See Forde, "Jour. Trop. Med.," Sept. 1, 1902; Dutton, Ibid., Dec. 1, 1902; Dutton, "Thompson-Yates Laboratory Reports," 1902, v, 4, part II, p. 455.
** "First Report of the Trypanosomiasis Expedition to Senegambia," 1902, Liverpool, 1903.
†† "Jour. Trop. Med.," Nov. 1, 1902, and March 16, 1903; "Brit. Med. Jour.," May 30, 1903.
‡‡ "Acad. de Med.," March 17, 1903.
§§ "Brit. Med. Jour.," May 30, 1903.

micro-organisms and African lethargy, and much interest was being taken in a coccus—the hypnococcus—that was being studied by Castellani in Uganda. As Castellani was prosecuting the investigation of this organism, he chanced to examine the cerebro-spinal fluid of several negroes in Uganda who were suffering from sleeping sickness, and in it found trypanosomes. Even then, though Castellani* realized that these organisms were connected with sleeping sickness, he did not identify them in his mind with the Trypano-

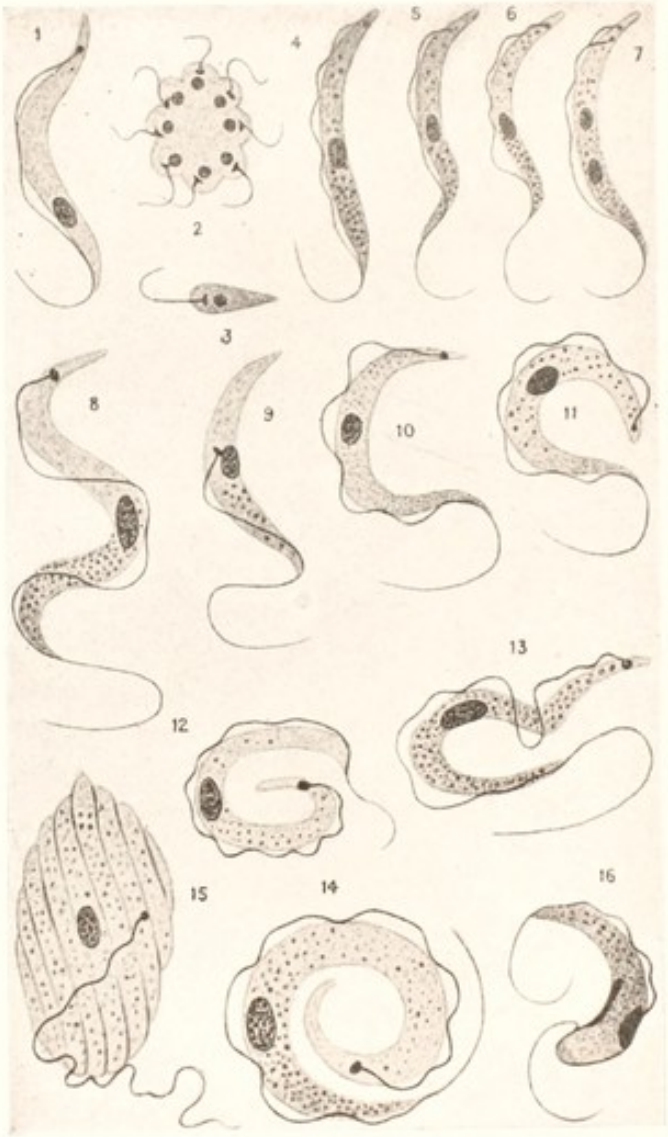


Fig. 207.—Various species of trypanosomes: 1, *Trypanosoma lewisi* of the rat; 2, *Trypanosoma lewisi*, multiplication rosette; 3, *Trypanosoma lewisi*, small form resulting from the disintegratoin of a rosette; 4, *Trypanosoma brucei* of nagana; 5, *Trypanosoma equinum* of caderas; 6, *Trypanosoma gambiense* of sleeping sickness; 7, *Trypanosoma gambiense*, undergoing division; 8, *Trypanosoma theileri*, a harmless trypanosome of cattle; 9, *Trypanosoma transvaliense*, a variation of *T. theileri;* 10, *Trypanosoma avium*, a bird trypanosome; 11, *Trypanosoma damoniæ* of a tortoise; 12, *Trypanosoma soleæ* of the flat fish; 13, *Trypanosoma granulosum* of the eel; 14, *Trypanosoma rajæ* of the skate; 15, *Trypanosoma rotatorium* of frogs; 16, *Cryptobia borreli* of the red-eye (a fish). (From Laveran and Mesnil.)

* Ibid., May 23, 1903; June 20, 1903.

soma gambiense discovered in the blood by Forde and Dutton, and described the newly discovered organism as Trypanosoma ugandense. Kruse,* thinking to honor the discoverer, called it Trypanosoma castellani. Bruce and Nabarro† found the new trypanosome in each of 38 cases of sleeping sickness in the cerebrospinal fluid, and 12 out of 13 times in the blood. These observers also found that 23 out of 28 natives from parts of Uganda where sleeping sickness is endemic had trypanosomes in their blood, while in 117 natives from uninfected areas the blood examination was negative in every case. They also declared that, contrary to what had been stated, there were no appreciable morphologic differences between Trypanosoma gambiense and Trypanosoma ugandense. Dutton, Todd, and Christy‡ arrived at the same conclusion. The matter was finally settled by Thomas and Linton§ and Laveran,‖ who, by means of animal experiments, determined not only the complete identity of the organisms, but their uniform virulence.

Early in 1910 J. W. W. Stephens** studied the blood of a rat inoculated with blood from a patient suffering from sleeping sickness, with which he had become infected in North Eastern Rhodesia, and observed certain definite morphological differences between trypanosomes in it, and Trypanosoma gambiense. Later he and Fantham†† studied this organism with great care and came to the conclusion that it was a new and separate species, and gave it the name Trypanosoma rhodesiense. In this they received the support of Mesnil.‡‡

Morphology.—Trypanosoma gambiense is a long, slender, spindle-shaped, flagellate micro-organism that measures 17 to 28 μ in length and 1.4 to 2 μ in breadth. From the anterior end (that which moves forward as the organism swims) a whip-like flagellum projects about half the length of the organism. The terminal third of the flagellum is free in most cases. The proximal two-thirds are connected with a band of the body substance, which is continued like a ruffle along one side of the organism to within a short distance of its blunt posterior end, where the flagellum abruptly ends at the blepharoplast. This thin ruffle is known as the undulating membrane. By means of the flagellum and the undulating membrane the organism swims rapidly with a wriggling and rotary movement that gives it the name Trypanosome, which means "boring body."

* "Gesell. f. natur. Heilkunde," 1903.
† "Brit. Med. Jour.," Nov. 21, 1903.
‡ Ibid., Jan. 23, 1904, also "Thompson-Yates and Johnson Lab. Reports," 1905, v, 6, part 1, pp. 1–45.
§ "Lancet," May 14, 1904, pp. 1337–1340.
‖ "Compt.-rendu de l'Acad. des Sciences," 1906, v, 142, p. 1056.
** "British Medical Journal," 1912, II, 1182.
†† "Proceedings of the Royal Society," 1910, LXXXIII, 28, 31; 1912, LXXXV, 223; "Bulletin of the Sleeping-sickness Bureau," 1911–1912, Nos. 33, 38.
‡‡ "Brit. Med. Jour.," 1912, II, 1185.

The protoplasm is granular and often contains chromatin dots that are remarkable for their size and number. There is a distinct nucleus of ovoid form that is always well in advance of the centrosome or blepharoplast, and not infrequently is near the center of the organism. There is also a centrosome or blepharoplast, which appears as a distinct, deeply staining dot near the posterior blunt end and from which the flagellum appears to arise. Near this a vacuole is sometimes situated.

Trypanosoma rhodesiense differs from Trypanosoma gambiense in that the nucleus is never near the center, rarely far in advance of the blepharoplast, and not infrequently is posterior to the blepharoplast.

Staining.—The organisms are best observed when stained with one of the polychrome methylene-blue combinations—Leishman's, Wright's, Jenner's, Romanowsky's, Marino's. To stain them a spread of the blood or cerebro-spinal fluid is made and treated precisely as though staining the blood for the differential leukocyte count or for the malarial parasite.

Cultivation.—Trypanosoma lewisi of the rat and Trypanosoma brucei of "nagana" or "tsetse-fly" disease of Africa have been cultivated by Novy and McNeal* in mixtures composed of ordinary culture agar-agar and defibrinated rabbit-blood, combined as necessary, 1:1, 2:1, 1:2, or 2:3, etc. The actual culture was made chiefly in the water of condensation collected at the bottom of obliquely congealed media.

Laveran and Mesnil found that when blood containing Trypanosoma gambiense was mixed with salt solution or horse-serum, the trypanosomes remain alive for five or six days at the temperature of the laboratory. They live much longer in tubes of rabbit's blood and agar, sometimes as long as nineteen days, and during this time many dividing forms but no rosettes were observed. But subcultures failed, and eventually the original culture died out.

Bayon† has found it easy to cultivate Trypanosoma rhodesiense in Clegg's ameba-agar (*q.v.*) and in blood agar-agar containing dextrose. The organisms thus cultivated retain their virulence for rats for a long time.

Reproduction.—Multiplication takes place by binary division, the line of cleavage being longitudinal and beginning at the posterior end. The centrosome and nucleus divide, then the flagellum and undulating membrane divide longitudinally, and finally the protoplasm divides, the two organisms hanging together for some time by the undivided tip of the flagellum.

In addition to this simple longitudinal fission, the trypanosomes seem to possess a sexual mode of reproduction. When the well-stained organisms are carefully studied, it is possible to divide them

* "Contributions to Medical Research dedicated to Victor Clarence Vaughan," Ann Arbor, Michigan, 1903, p. 549; "Journal of Infectious Diseases," 1904, I, p. 1.

† "Proc. Royal Society, Series B," 1912, LXXXV, 482.

into three groups—those that are peculiarly slender, those that are peculiarly broad, and those of ordinary breadth. The fact that conjugation takes place between the first two has led to the opinion that they represent the male and female gametocytes respectively, while the others are asexual. All forms multiply by fission, and conjugation between the gametes is observed to take place only in the body of the invertebrate host. It has not yet been accurately followed in the case of Trypanosoma gambiense, but there is no reason to think that the organism differs in its method of reproduction from Trypanosoma lewisi. Prowazek found that when rat blood containing the latter organism was taken into the stomach of the rat louse, Hematopinus spinulosus, the male trypanosome enters the female near the micronucleus and the various parts of the two individuals become fused. A non-flagellate oökinete results, and, after passing through a spindle-shaped gregarine-like stage, can develop into an immature trypanosome-like form in the cells of the intestinal epithelium, after which the parasite is thought to enter the general body cavity, and, migrating to the pharynx, enter the proboscis, through which it is transmitted to a fresh host.

Another form of multiplication consists in the "shedding" of infective granules. This has been studied by Ranken.* The organisms from which this is about to take place are observed to contain three or four, sometimes five or six granules of small size, highly refractile and spherical in shape. They are distinctly within the protoplasm of the trypanosome and swing backward and forward as it makes its lashing movements. When these are closely watched a time comes when one of the granules shoots out. At first the granule is carried about by whatever currents of fluid it happens to meet, having no motility of its own, but soon a dot appears, then a flagellum, and provided with means of locomotion, and now having a pyriform shape, the new embryo parasite swims away. Ranken thinks these granular forms develop in the internal organs and has found them of pyriform shape in the liver, spleen, and lungs.

Transmission.—It is well known that the disease does not spread from person to person. In the days when African negroes were imported into America as slaves, the disease often reached our shores, and though freshly arrived negroes and those in the country less than a year frequently died of it, there was no spread of the affection to those that were acclimated. The Europeans that carried the disease from Africa to England and were the first in whose bloods the trypanosomes were found, did not spread it among their fellow countrymen. A case from the Congo that died in a hospital in Philadelphia and came to autopsy at the hands of the author, did not spread the disease in this city.

* "Brit. Med. Jour.," 1912, II, 408.

Yet the disease is infectious, and the transfer of a small quantity of the parasite-containing blood to appropriate experiment animals perfectly reproduces it.

The present knowledge of the mode of transmission came about through the knowledge of other trypanosome infections that had already been carefully studied and understood. In speaking of nagana, or tsetse-fly disease, Livingstone, as early as 1857, recognized that the flies had to do with it. For years, however, the supposition was that the fly was poisonous and that its venom was responsible for the disease. In 1875 Megnin stated that the tsetse-fly carries a virus, and does not inoculate a poison of its own. In 1879 Drysdale suggested that the fly might be an intermediate host of some blood parasite, or the means of conveying some infectious poison. In 1884 Railliet and Nocard, who suspected the same thing, proved

Fig. 208.—Glossina palpalis. A perfect insect just escaped from the pupa (Brumpt). Showing how the wings close over one another like the blades of a pair of scissors.

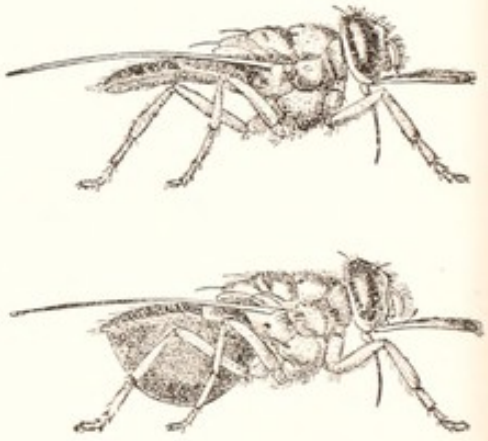


Fig. 209.—Glossina palpalis before and after feeding (Brumpt).

that inoculations with the proboscis of the tsetse-flies were harmless. The exact connection between the flies and the disease was worked out by Bruce,* who found, first, that flies fed on infected animals, *kept in captivity for several days*, and afterward placed upon two dogs, did not infect; second, that flies fed on a sick dog, and immediately afterward on a healthy dog, conveyed the disease to the latter. The flies were infectious for twelve, twenty-four, and even for forty-eight hours after having fed on the infected animal. It was, therefore, shown that the flies could and did infect, not through something of which they were constantly possessed, but through something taken from the one animal and put into the other; this, of course, proved to be the trypanosome. Further, it was shown that where there were no tsetse-flies, there never was nagana.

* "Preliminary Report on the Tsetse-fly Disease or Nagana in Zululand, Ubombo, Zululand," Dec., 1895; "Further Report," etc., Ubombo, May 29, 1896; London, 1897.

So soon as African lethargy was shown to be a form of trypanosomiasis, the question arose, Was it spread by tsetse-flies? Sambon* and Brumpt† both suggested it, but it was soon discovered that the geographic distribution of the tsetse-fly, Glossina morsitans, that distributes nagana, does not coincide with the geographic distribution of sleeping sickness. There are, however, different kinds of tsetse-flies, and Bruce and Nabarro‡ first showed that it was not Glossina morsitans, but a different tsetse-fly, Glossina palpalis, that is the most important source of the spread of human trypanosomiasis. They submitted a black-faced monkey (Cercopithicus) to the bites of numerous tsetse-flies caught in Entebbe, Uganda, and found trypanosomes in its blood. Bruce, Nabarro, and Greig§ allowed Glossina palpalis to suck the blood of negroes affected with sleeping sickness and afterward to bite five monkeys (Cercopithicus). At the end of about two months trypanosomes appeared in the blood of these monkeys. They also made maps showing the geographic distribution of African lethargy and of Glossina palpalis, which were found perfectly to correspond.

But the natural history of sleeping sickness is less simple than these facts make it appear. Kinghorn and Yorke‖ observed that in the Luangwa Valley where tsetse-flies (Glossina morsitans) abound, there is much game but few domestic animals. This led them to study the bloods of all the game animals in an attempt to discover how many harbored trypanosomes and what kind they were. The results are interesting, but two are of great importance in the present connection. They discovered that antelopes harbored Trypanosoma rhodesiense, and that it could be transmitted by Glossina morsitans. As Trypanosoma rhodesiense is the more virulent parasite, and as the antelope regularly harbors it and the widely distributed Glossina morsitans distributes it, the likelihood of an early and successful outcome of the campaign against sleeping sickness becomes improbable.

The flies are found to become infective in from eleven to twenty-five days after consuming infected blood, and to remain so as long as they continue to live.

Bruce, Hamerton, Bateman and Mackie, the members of the "Royal Society Sleeping-sickness Commission" for 1908–9** have found that under experimental conditions the development of the parasites takes place only in about 5 per cent. of infected flies. The shortest time in which their flies became infective was 18 days, the longest 53 days, the average 34 days. An infected fly was kept

* "Jour. Trop. Med.," July 1, 1903.
† "C. R. Soc. de Biol.," Jan. 27, 1903.
‡ "Reports of the Sleeping Sickness Commission of the Royal Society," 1903, I, II, 11.
§ Ibid., 1903, No. 4, VIII, 3.
‖ "Brit. Med. Jour.," 1912, II, 1180.
** "British Medical Journal," 1910, I, 1312.

alive in the laboratory for 75 days and remained infective all that time. Experiments directed toward finding out how long the flies might remain infective in nature indicate that the flies may be able to transmit the parasites for at least two years.

It is, of course, not impossible that other flies, especially other species of tsetse-flies, may act as distributing hosts of the trypanosomes, but there is no doubt about the chief agents being Glos-ina palpalis and Glossina morsitans. With increased entomologic and geographic information it has been found that there are certain districts where these flies abound though the disease is unknown, but that only shows that in those districts the flies are not infected. Tsetse-flies are not, as was formerly supposed, peculiar to Africa, but have been found in Arabia, where African lethargy could no doubt spread should the flies become infected through imported cases of the disease. The inability of the disease to spread in England and America depends upon the absence of tsetse-flies from those countries.

It is possible for the disease to be transmitted from human being to human being through such personal contacts as may afford opportunity for interchange of blood. Thus, Koch observed that in certain parts of Africa where there were no tsetse-flies the wives of men that had become infected in tsetse-fly countries sometimes developed the disease, probably through sexual intercourse, a probable explanation when one remembers that it is solely or chiefly by such means that a trypanosome disease of horses—Dourine or Maladie du coit, caused by Trypanosoma equiperdum—is transmitted.

Transmission to Lower Animals.—Trypanosoma gambiense is infectious for monkeys as well as for human beings. In the monkeys a disease indistinguishable from the sleeping sickness is brought about. It is also infective for dogs, cats, guinea-pigs, rabbits, rats, mice, marmots, hedgehogs, goats, sheep, cattle, horses, and asses. The lower animals are not, however, so far as is known, subject to natural infection.

Trypanosoma rhodesiense, being a more virulent parasite than its close relative, probably infects a greater variety of animals. Among these, in nature, antelopes seem to be commonly infected.

Pathogenesis.—The first effect of human trypanosomiasis seems to be fever of an irregular and atypical type, occurring in irregular paroxysms. It was in this early febrile stage of the disease that Forde and Dutton first found the trypanosomes in the circulating blood. The number of organisms in the peripheral circulation is, however, usually so small that it is tedious to look for them. The search may be made in thick smears stained by any blood stain, but it is better to proceed by washing the corpuscles in citrated blood as in preparing to calculate the opsonic index, and to collect the "leukocyte cream" for staining and examination. The trypano-

somes, which seem to have much the same specific gravity of the leukocytes, appear in greatest numbers where the leukocytes collect. In African natives the trypanosomes may be present in the blood for a long time before any symptoms are discovered, but in Europeans their presence is soon followed by fever. As the infection progresses, the micro-organisms increase in great numbers in the organs, and almost entirely disappear from the blood. The lymph nodes swell and Winterbottom, who first described the disease, called particular attention to the enlargement of those of the posterior cervical triangle, which he regarded as of diagnostic significance.

When the blood examination fails to reveal trypanosomes, they may frequently be found by puncturing an enlarged lymph node with a dry needle and examining the drop of fluid obtained.

Wolbach and Binger* found that the trypanosomes invade the connective-tissue structure of all organs, the reticular tissue of lymph nodes and spleen, and the substance of the brain. The lesions are due to the presence of the flagellated form of the parasite in the tissues. They found the initial cell reaction to be the proliferation of endothelial cells. They believe the discovery of numerous intravascular mitoses of endothelial cells in the lung, liver, spleen and kidney to indicate the source of the increase of the large mononuclear leukocytes of the blood in human trypanosomiasis.

Lymphocytosis is the rule in trypanosomiasis but is of no diagnostic importance.

As the invasion of the body continues, the trypanosomes disappear in large measure from the blood to multiply in the organs. In the spleen, in particular, the parasites assume a different form: a deep band makes its appearance between the nucleus and the blepharoplast. The former becomes surrounded by a large vacuole, and the trypanosome becomes disintegrated and reduced to a nucleus, which represents the latent form of the organism. The nucleus later divides giving rise to a new blepharoplast from which a new flagellum arises, an undulating membrane later forms, and the usual appearance of a trypanosome again develops. When perfected, this new trypanosome enters the circulating blood. At the time that the first indications of somnolence appear, the parasites are present in the cerebro-spinal fluid. The fluid is collected by the technic given in the chapter upon cerebro-spinal meningitis. To find the trypanosomes in the fluid, it should be rapidly centrifugalized for a few minutes and the whitish sediment collected, and examined immediately, when the micro-organisms may be studied alive, or the fluid may be spread upon slides and stained according to the technic for blood spreads, when, the trypanosomes being killed, fixed and stained, their structure can be studied to advantage. In studying the morbid anatomy of sleeping sickness, Mott† came to the con-

* "Jour. Med. Research," 1912–1913, XXVII, 83.
† "British Medical Journal," Dec. 16, 1899, II.

clusion that the essential lesion is an extensive meningo-encephalitis. To the naked eye, there are scarcely any lesions in sleeping sickness, except the enlargement of the lymph nodes, and even in the nervous system when one looks with care, there is but little to be seen. The

Fig. 210.—Photomicrograph of an eosin-methylene-blue-stained section; 1000 diameters. Shows trypanosomes about a small vessel of the cortex of the brain (Wolbach and Binger, in "Jour. of Med. Research").

histological examination of the nervous tissues, on the contrary, shows that in both the brain and spinal cord there is proliferation and overgrowth of neuroglia cells, especially those connected with the sub-

Fig. 211.—Photomicrograph of a Giemsa-stained section; 1000 diameters, showing a trypanosome deep in the cortex of the brain (Wolbach and Binger, in "Jour. of Med. Research").

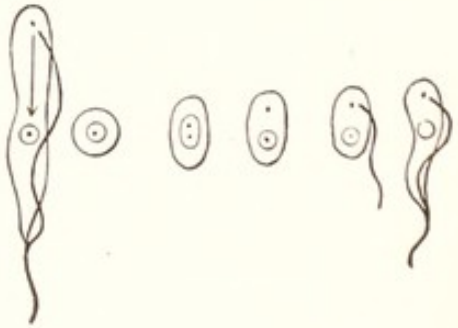


Fig. 212.—Trypanosoma gambiense. Formation of the latent stage and transformation of the latent stage into a trypanosome (after Guiart).

arachnoid space and the perivascular space, with accumulation and probably proliferation of lymphocytes in the meshwork. Wohlbach and Binger found that the trypanosomes actually escape from the blood-vessels and make their way into the nervous tissue. The

period of lethargy seems to coincide with that at which the parasites are invading and injuring the nervous tissue.

Prophylaxis.—Reasoning from knowledge of the successful campaigns that have waged against yellow fever and paludism, it at first appeared as though the prophylaxis of sleeping sickness ought to be based partly upon measures taken to prevent the infection of men by tsetse-flies, and partly upon those taken to prevent the infection of the flies by men.

To prevent the infection of men by the flies is extremely difficult where naked or half-naked savages are to be dealt with. For Europeans, the customary dress, the avoidance of exposure in bathing, the use of mosquito guards, etc., are to be recommended, as well as the erection of habitations and the building of roads, etc., as far as possible from the fly districts. The destruction of the grass and reeds along the river banks, the use of drainage, and the introduction of chickens, to pick up the larvæ and pupæ, have been recommended.

To prevent infection of the flies with Trypanosoma gambiense is impossible where, as in some sections of Africa, 50 per cent. of the population of some of the villages already harbor the parasites, and still more impossible when, as is the case with Trypanosoma rhodesiense, the wild animals, especially antelopes which are extremely numerous, continually harbor the parasites and act as reservoirs from which the flies receive a continuous supply.

The importance of undertaking radical measures for the prevention of the disease may be imagined when it is understood that in the last few years no less than a half-million of the natives of the infected districts have died of sleeping sickness.

Tsetse Flies

The Tsetse flies are dipterous insects belonging to the family Glossininæ, and included in a single genus Glossina. With one exception, G. tachinoides, the entire family lives in tropical and subtropical Africa. About sixteen species of Glossina are now described, for the rough and ready identification of which the following table from Brumpt ("Précis de Parasitologie" 1910, p. 630) will be found useful. For those who desire more accurate information, Austin's "Handbook of the Tsetse Flies," the "Sleeping Sickness Bulletin," and Patton and Cragg's "Text-book of Medical Entomology" will prove useful books of reference.

Tsetse flies are easily recognized by their fly-like appearance, by their horizontal proboscis, slender but swollen at the base, and by their habit of resting with the wings crossed like the blades of a closed pair of scissors.

The greater number of the flies occupy sections of country, spoken of as "fly belts" or "fly districts," some of which are permanently infected, others temporarily infected. Such "belts" are usually deep forests along the banks of streams or on the shores of lakes. The adult flies seem to love the shade, though they fly from it into the hot sun to seek their prey. The large game animals seem to be the natural prey of the flies, though a number of them bite human beings, and one, Glossina palpalis, seems to prefer human blood to all others. The flies seem to attack moving animals by preference. So long as the creature moves they pursue. When it stands, many of them fly away to the shade again.

Both males and females bite. The latter distend themselves with blood until

they are so heavy that they can scarcely fly and drop off to the ground. Biting is almost entirely confined to bright sunny weather. On dull or cloudy days the flies remain in the brush. Exceptions are found among the few species that live in arid sections. Such may bite at night. Few of the flies fly far from their native haunts where they seem to prefer to await the coming of their prey, rather than to make excursions after it. Clouds of the flies often arise at the same time and attack the animals in swarms.

The flies are larviparous and do not lay eggs. Copulation of the sexes takes place but once, the sperm being retained in a spermatotheca. The eggs are fertilized as they descend from the oviduct to the uterus where they hatch into a larva on the fifth day. The larva grows rapidly, molts three times and attains its full size by the tenth day, when it is born. The larva at the time of birth is cylindrical in shape, consists of thirteen segments and measures 6–7 mm. in length. It is nearly white but has a black head which is small and inconspicuous. The larvæ are usually deposited on the sand of the banks of streams or lakes, and at once burrow into the ground to a depth of an inch or so. In a half hour or an hour the larva changes to a pupa in which state it continues for about a month. The imago or fly then emerges. The average duration of life of the imago fly is about three months, during which time each female bears an average of ten new larvæ.

Glossina palpalis is commonly infested by a flagellate called Crithidia grayi, that seems in some way to pass from fly to fly, and to have nothing to do with the bloods upon which it feeds. It is to be regarded as a parasite of the fly, and should be known lest it be confused with the Trypanosoma of which the fly is the vector.

Table for the Identification of the Common Tsetse Flies

Large Species; body measuring more than 12 mm. in length.
- Pattern on thorax faint; four very distinct black spots... G. longipennis.
- Pattern on thorax sharp and distinct, no black spots..... G. fusca.

Small species; body in general measuring less than 12 mm. in length.
- All five tarsal joints of the third pair of legs black.
  - Colors dark; antennæ black; last two tarsal joints of the first pair of legs black..... G. palpalis.
  - All of the tarsal joints of the first pair of legs yellow... G. bocagei.
  - Very small species; markings like those of G. morsitans on abdomen..... G. tachinoides.
  - Colors dark; antennæ yellow..... G. pallicera.
- Only the last two tarsal joints of the third pair of legs black; all the others yellow.
  - The fifth tarsal joint of the first and second pairs of legs is yellow..... G. pallidipes.
  - The last two joints of the tarsi of the first and second pairs of legs are black.
    - The yellow band on the abdominal segments takes up one-third of the segment..... G. morsitans.
    - The yellow band on the abdominal segments, takes up one-sixth of the segment..... G. longipalpis

AMERICAN TRYPANOSOMIASIS

Schizotrypanum Cruzi (Chagas)

No sleeping sickness has thus far been found to occur upon either of the American continents, though human trypanosomiasis in another form has been observed in Brazil where it has been studied by Chagas.*

* "Archives für schiffs u. tropen Hygiene," 1909, Heft 4; abstract "Centralbl. f. Bacteriologie etc. Ref.," 1909, XLIV, 639; "Bull. de l'Inst. Pasteur," 1910, VIII, 373.

The disease, which in Minas Gaeras often attacks the entire population, chiefly affects the children and goes by the local name of

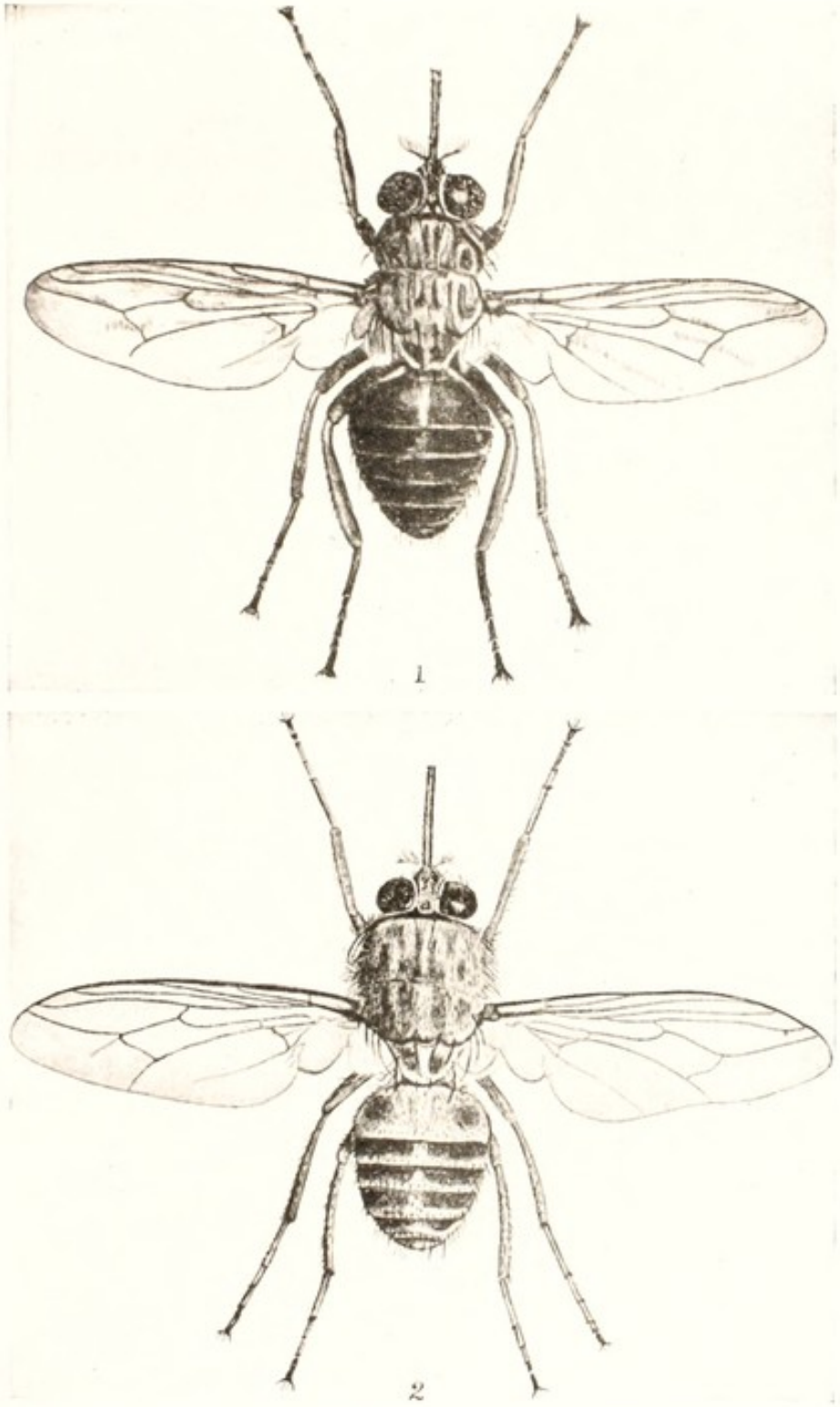


Fig. 213.—1. Glossina palpalis ♂ × 6. 2. Glossina morsitans, ♀ × 6. (Patton and Cragg).

Opilaçao. In childhood it usually assumes the form of an acute malady characterized by an incubation period of ten days, and by high continued fever, puffiness of the face, enlargement of the

thyroid gland, of the lymph nodes and spleen. In some cases meningitis occurs. It is extremely fatal.

In adults it is apt to take a more chronic course in which the chief symptoms are enlargement of the thyroid gland, and a myxedematous condition of the skin. The lymph nodes usually enlarge. If the adrenal glands become affected, symptoms resembling Addison's disease make their appearance. If the heart muscle be invaded by the parasites, its power is diminished and the pulse becomes feeble and irregular. If the nerve-cells or neuroglia cells of the central nervous system be affected through parasitic invasion, symptoms occur according to the extent and localization of the disturbance. There is always irregular fever and marked anemia.

Chagas found a trypanosome in the peripheral blood of patients suffering from Opilaçao, and gave it the name Trypanosoma cruzi. Later studies of the micro-parasite have, however, shown that its method of reproduction differs so strikingly from that of the trypanosomes, that it was necessary to make a generic distinction between the two, and it is now called Schizotrypanum cruzi.

Morphology.—The Schizotrypanum is present in the peripheral circulation only during the febrile stages of the disease, when it may be found by the usual methods of staining for trypanosomes. It is a long slender trypanosome-like organism, with the characteristic fusiform shape, with a nucleus, a large blepharoplast, a flagellum and an undulating membrane. No measurements are given, but the parasite is rather small. No dividing forms are observed in the circulating blood. The trypanosomes may be free, may be attached to the erythrocytes or may be partly or entirely in the red corpuscles. They show sexual dimorphism, the males being long and slender, the females shorter and stouter.

Reproduction.—Gametogony takes place in the lungs. Such of the trypanosome forms as are caught and retained there, lose the undulating membranes, the two ends curve toward one another forming first a crescent, them unite and form a ring. The female parasites shed the blepharoplasts, and in both male and female parasites the nucleus breaks up into eight secondary nuclei, giving rise to eight merozoits. The merozoits derived from the female parasites have a single nucleus, those derived from the male parasites, a nucleus and a blepharoplast connected by a fine thread of chromatin. The merozoits thus formed enter into erythrocytes where they eventually develop into the trypanosome forms. Hence is explained the peculiar relation of the trypanosomes to the erythrocytes mentioned above.

The chief multiplication of the parasites, however, takes place in the cells of the voluntary muscles, the heart muscle, the central nervous system, the thyroid, the adrenal glands and the bone marrow. In these situations, according to Chagas, the parasites take on a

rounded form, and by schizogony give rise to a great number of daughter parasites, each having a nucleus and a blepharoplast. For a time the schizonts are quiescent, then develop flagellæ and undulating membranes. The infected cells are destroyed. Chagas thinks that gametes are formed only in the lungs.

In the definitive host, the Lamus (or Conorhinus) megistis, the sexual conjugation occurs in the mid-gut. The blepharoplast approaches and seems to blend with the nucleus, the undulating membrane disappears and the parasites assume a spherical form. Actual conjugation does not seem to have been observed. Multiplication takes place by division of these rounded organisms, the daughter parasites becoming flagellated, the flagellum originating from the blepharoplast. Numerous flagellated trypanosome and crithidia forms of the parasite are observed in the hind-gut of the insect. Chagas observed trypanosome forms in the body cavity and in the salivary glands by the insect, and it is probable that it is through these that the infection is transmitted when the insect bites a susceptible animal, though Brumpt thinks the infection may take place through the feces of the bug, especially when these are in some way brought to the conjunctiva.

Transmission.—Chagas was able to show that a large bug, Lamus (Conorhinus) megistus, common in the neighborhood in which Opliaçao occurs, is the principal definitive host of the parasite. Both males and females of this flying bug are vicious biters and both live upon human blood as well as upon the bloods of other warm-blooded vertebrates. The bugs are common in the thatch and in the cracks between the timbers of the native houses. Whether other species of Lamus may also harbor the parasites is not known. Brumpt* found that Cimex lectularius, Cimex boneti and Ornithodorus moubata could also act as definitive hosts. A study of Cimex lectularius, the common bed-bug, as a definitive host of the parasite, was made by Blacklock† who found that only a very occasional bug becomes so infected as to be able to effect the transmission.

Cultivation.—The parasites are easily cultivated *in vitro* in the medium recommended for trypanosomes by Novy and McNeal. In culture the organisms resemble those found in the bugs, *i.e.*, round and crithidial forms, or pear-shaped rapidly dividing forms. More than two subcultures can rarely be made before the organisms die out.

Pathogenesis.—The Schizotrypanum is pathogenic for certain monkeys (Callithrix), dogs, rabbits and guinea-pigs. Guinea-pigs usually die in five to ten days, though the trypanosome forms are not usually found in the peripheral blood. They are, however, present in larger numbers in the lungs. Monkeys live longer. Trypano-

* "Centralbl. f. Bakt. etc. Ref.," LV, No. 3, p. 75.
† "British Medical Journal," 1914, I, 912.

some forms of the parasite appear in the blood in about a week, then may disappear. The animals live a month or two.

Diagnosis.—As the trypanosomes are present in the circulating

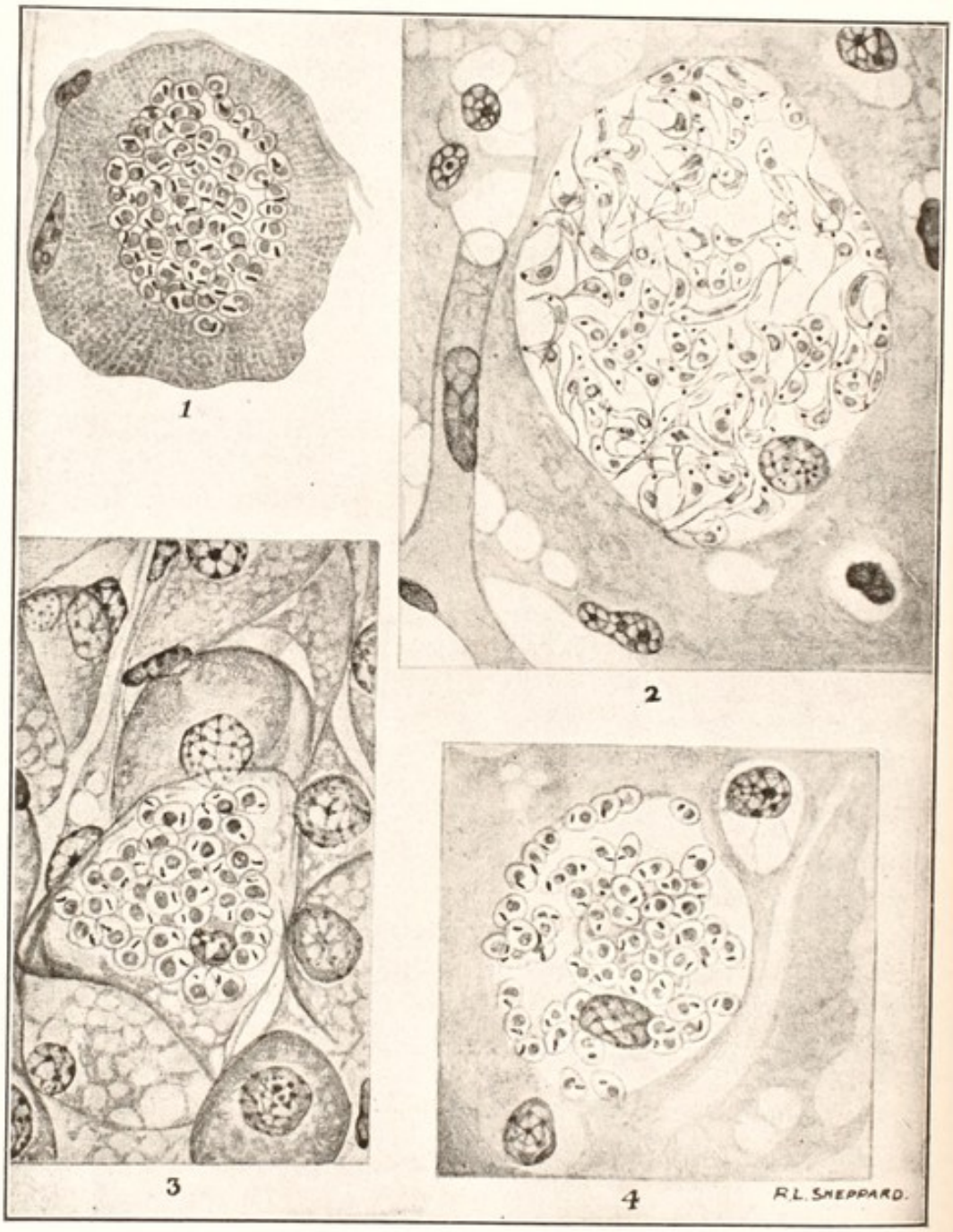


Fig. 214.—*Schizotrypanum cruzi* developing in the tissues of the guinea-pig. 1. Cross-section of a striated muscle fiber containing *Schizotrypanum cruzi:* Note dividing forms. 2. Section of brain showing a *Schizotrypanum* cyst within a neuroglia cell, containing chiefly flagellated forms. 3. Section 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.)

blood of human beings in somewhat small numbers, and only at certain times, it is unwise to rely upon them as a means of making

the diagnosis, though if they be found the diagnosis is certain. It is usually much better to inoculate 1 or 2 cc. of the blood of the suspected case into a guinea-pig and then make frequent examinations of its blood. Here, again, the common absence of trypanosome forms from the blood complicates matters. If none can at any time be found, the muscles of the guinea-pig must be examined for the dividing forms of the parasites, which are usually quite numerous.

Prophylaxis.—As the bugs fly it is somewhat difficult to defend the sleeping patient against them, so long as he lives in a carelessly built and thatched country house. Sulphur fumigation and whitewashing may help. Well-built habitations with screened windows and the use of mosquito bars should constitute the best defense.

Lamus (Conorhinus) Megistis (Burn)

Patton and Cragg* describe this bug as follows: "Dark brown to black. Pronotum broadly expanded, with two broad raised red lines extending from the

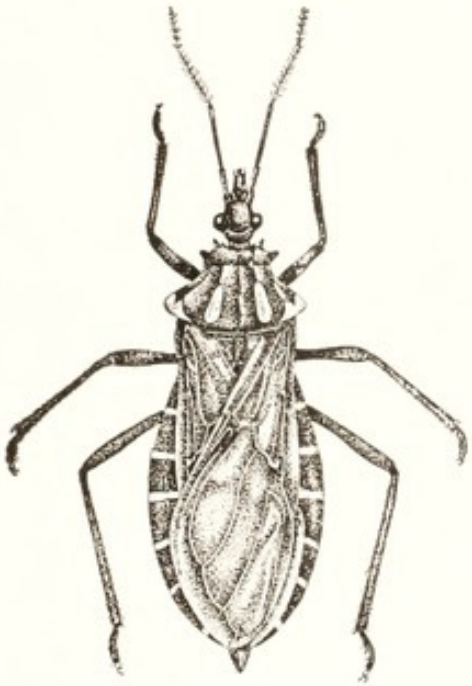


Fig. 215.—Lamus (Conorhinus) megistus (female), the insect host and distributing agent of Schizotrypanum cruzi (Chagas). × 2.

middle of the posterior border, and a red spot on the postero-lateral angles of pronotum. At the anterior border of the pronotum there are six short spines, three on each side; the most anterior are the longest and project on each side of the eyes; two are situated further back, one on each side of the middle line at the origin of the two admedial ridges; the third spine is situated on a ridge at the junction of the middle and anterior third of the pronotum just above the first pair of legs. Scutellum dark brown with two short red lines converging toward the apex, where they meet; apex red, turning upward and bluntly rounded off. Corium and membrane fuscous, the former with one or more red streaks. Connexivum with six well-marked bright red lines, broader in the male; in both sexes the lines extend round to the ventral border. In the male the last segment, except for a central black mark, is entirely red. Length 30 to 32 mm."

The L. megistus "is almost entirely a domestic insect." "The adults enter

* "A Text-book of Medical Entomology," 1913, p. 492.

inhabited houses but never those that have been abandoned. In houses which are old or badly kept they are to be found in cracks and holes in the walls, where they lay their eggs; the early stages, which are wingless, crawl out of their resting places in the walls so soon as the lights are put out and make their way to the beds of the occupants of the house. The adults behave in the same manner, but as they are powerful fliers, they can reach the people who sleep in hammocks. The bite is said to be painless and to leave no mark."

"The eggs of L. megistus are of a creamy white color and are laid in batches of from eight to twelve, and as many as forty-five such batches may be laid. According to Neiva they hatch in twenty-five to forty days. The larva is of a uniform light color when it emerges, becoming darker later; it takes its first feed from five to eight days after emerging from the egg, and the second from the fifteenth to the twentieth day; it changes its skin (first nymphal stage) after about forty-five days. The second molt takes place during the second or third month, and the third during the fourth or sixth month. The fourth molt occurs about the 190th day after the larva has hatched out from the egg; this stage lasts at least forty-two days. Neiva states that this time is the most critical period in its life, and that large numbers of them die. After the next molt the adult stage is reached, and eight days later they are ready to suck blood; egg-laying commences about the fifty-fifth day after the first feed. One female kept under observation by Neiva for about three and a half months laid 218 eggs in thirty-eight batches. Under favorable conditions of food supply the cycle from egg to egg is completed in about 324 days."

This bug, when experimentally infected with Schizotrypanum cruzi, transmitted the infection to monkeys, guinea-pigs, rabbits and dogs. Both males and females bite and may transmit the parasites.

CHAPTER XXII

KALA-AZAR (BLACK SICKNESS)

LEISHMANIA DONOVANI (LAVERAN AND MESNIL)

"KALA-AZAR," "Dumdum fever," "Febrile tropical splenomegaly," "Non-malarial remittent fever," is a peculiar, fatal, infectious disease of India, Assam, certain parts of China, the Malay Archipelago, North Africa, the Soudan and Arabia, caused by a protozoan micro-organism known as Leishmania donovani, and characterized by irregular fever, great enlargement of the spleen, anemia, emaciation, prostration, not infrequent dysentery, occasional ulcerations of the skin and mucous membranes, and sometimes cancrum oris.

Because of its protean manifestations the disease has been given many names, and has been confused with the various diseases which its symptoms may resemble. It was not until 1900 that it was finally differentiated from malarial fever and came to be regarded as a distinct entity.

In 1900 Leishman* noticed in the spleen of a soldier returned from India and suffering from "dumdum fever"—a fever acquired at Dumdum, an unhealthy military cantonment not far from Calcutta—certain peculiar bodies. He reserved publishing the observation until 1903, so that it appeared almost simultaneously with a paper upon the same subject by Donovan.† As the publications came from men in different parts of the world, appeared so nearly at the same time, and showed that they had independently arrived at the same discovery, the parasite they described became known as the *Leishman-Donovan body*. For a long time its nature was not known and its proper classification impossible, but after it had been carefully studied by Rogers,‡ Ross,§ and others, and its developmental forms observed, it was agreed that it belonged in a new genus of micro-organisms, not far removed from the trypanosomes, and eventually Ross, and then Laveran and Mesnil, honored both of its discoverers by calling it Leishmania donovani, which name has been generally accepted.

Morphology.—As seen in a drop of splenic pulp the organism is a minute round or oval intracellular body measuring 2.5 by 3.5 μ. When properly stained with polychrome methylene blue (Wright's,

* "Brit. Med. Jour.," 1903, I, 1252.

† Ibid., 1903, II, 79.

‡ "Quarterly Jour. Microscopical Society," XLVIII, 367; "Brit. Med. Jour.," 1904, I, 1249; II, 645; "Proceedings of the Royal Society," LXXVII, 284.

§ "Brit. Med. Jour.," 1903, II, 1401.

Leishman's, or Jenner's stains) and examined under a high magnification, it is found that the protoplasm takes a pinkish color and contains two well-defined bright red bodies. The larger of these is ovoid and lies excentrically, its long diameter corresponding to

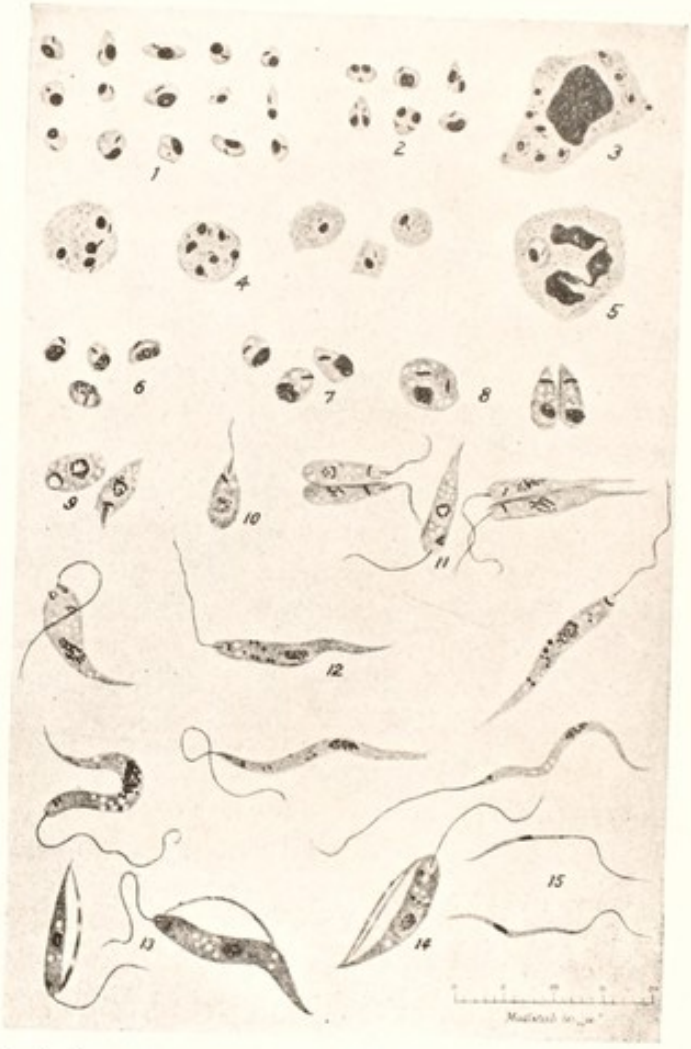


Fig. 216.—Evolution of the parasite of kala-azar: 1 to 5. Parasites of kala-azar. 1 Isolated parasites of different forms in the spleen and liver; 2, division forms from liver and bone-marrow; 3, mononuclear spleen cells containing the parasites; 4, group of parasites; 5, phagocytosis of a parasite by a polynuclear leukocyte. 6 to 15. Parasites from cultures. 6, First changes in the parasites. The protoplasm has increased in bulk and the nucleus has become larger; 7, further increase in size; vacuolization of the protoplasm; 8, division of the enlarged parasite; 9, evolution of the flagella; 10, small pyriform parasite showing flagellum; 11, further development and division of the parasite; 12, flagellated trypanosoma-like form; 13, 14, flagellated forms dividing by a splitting off of a portion of the protoplasm; 15, narrow flagellated parasites which have arisen by the type of division shown in 13 and 14. (From Mense's "Handbuch," after Leishman.)

the long diameter of the organism. This is regarded as the nucleus. The second body is smaller and of bacillary shape, and usually lies with its long diameter transverse to the nucleus. This is looked upon as a blepharoplast. It stains more intensely than the nucleus.

In addition to these bodies the protoplasm may contain one or two vacuoles.

All of the bodies are intracellular, as can easily be determined by examining sections of tissue, but in smears of splenic pulp the cells are broken and many free bodies may appear. The cells in which they occur are lymphocytes, endothelial cells, and peculiar large cells whose histogenesis is obscure. They are rarely to be found in polymorphonuclear leukocytes, and though there has been much discussion upon this point, probably never appear in the red blood-corpuscles.

The bodies divide by binary and multiple fission, without recognizable mitotic changes. When multiple fission occurs, the nucleus divides several times before the protoplasm breaks up. The organism is not motile and at this stage has no flagella.

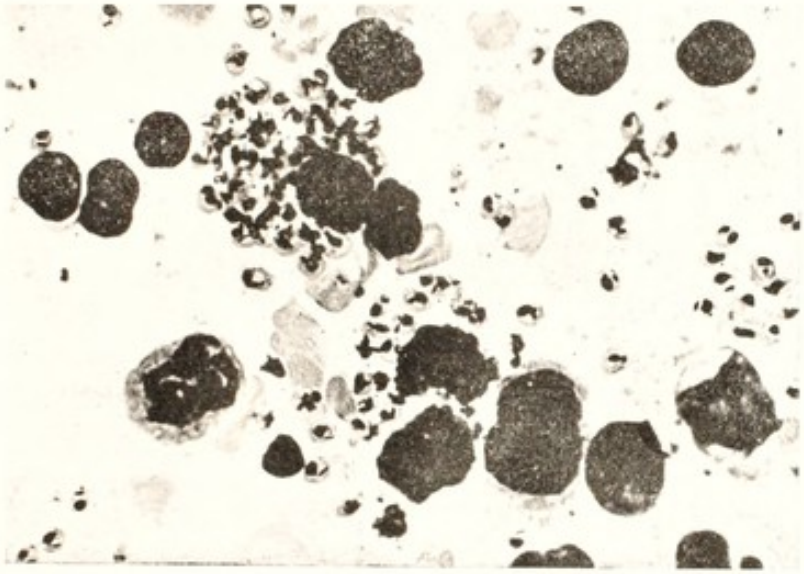


Fig. 217.—Leishman-Donovan bodies from the spleen of a case of kala-azar. × about 1000. (From Beattie and Dickson's "A Text-book of General Pathology," by kind permission of Rebman, Limited, publishers.)

Cultivation.—The organism was first cultivated artificially by Rogers in citrated splenic juice at 17° to 24° C. It can also be cultivated in the blood-serum agar medium used by Novy, McNeal, and Hall for trypanosomes, and in the N. N. N. medium of Nicolle, which has the following composition:

Water	900 c.c.
Salt (NaCl)	6 gm.
Agar-agar	16 gm.

Dissolve, distribute in tubes, sterilize, and add to the medium in each tube after liquefying and cooling to 40°–50° C., one-third of its volume of rabbit's blood obtained by cardiac puncture. Slope the tubes for twelve hours, incubate at 37° C. for five days to test the sterility of the medium, then keep at the ordinary temperature of the laboratory, sealed to prevent evaporation.

It is imperative that the material planted be sterile so far as bacteria

are concerned. Any associated growing bacteria quickly destroy Leishmania donovani.

Under conditions of cultivation the appearance of the organism undergoes a complete change. It enlarges, the nucleus increases greatly in size, and a pink vacuole appears near the blepharoplast. In the course of twenty-four to forty-eight hours the organism elongates, the blepharoplast moves to one end, and from the vacuole near it a flagellum is developed, and the organism becomes in about ninety-six hours a flagellate protozoan resembling herpetomonas. It now measures about 20 μ in length and 3 to 4 μ in breadth, its whip or flagellum measuring about 3 μ additional. It is also motile, and, like the trypanosomes, swims with the flagellum anteriorly. There is no undulating membrane.

This may be regarded as the perfect or adult form of the organism. It multiplies by a peculiar mode of division first observed by

Fig. 218.—Leishmania donovani. Flagellated forms obtained in pure cultures (Leishman).

Leishman. Chromatin granules, a larger and a smaller, appear in the protoplasm in pairs, after which, through unequal longitudinal cleavage, long, slender, almost hair-like individuals, containing one of the pairs of chromatin granules, are separated. These were serpentine at first, but later, as they grew larger, a flagellum was thrust out at one end.

Distribution.—The Leishman-Donovan body is widely distributed throughout the body of the patients suffering from kala-azar. It occurs in great numbers in the cells of the spleen, of the liver, of the bone-marrow, and in the ulcerations of the mucous membranes and skin. In the peripheral blood they are few and only in the leukocytes. They are always intracellular, or when in the circulating blood may be found in indefinite albuminous masses, probably destroyed cells. The number in a cell varies up to several hundred, such great aggregations only being found in the peculiar large cells of the spleen.

Lesions.—The splenomegaly is the most striking lesion. The change by which the enlargement is effected is not specific. The

organ is not essentially changed histologically, but seems to be merely hyperplastic. The liver is enlarged, but here, again, specific changes may be absent. In some cases a pallor of the centers of the lobules may depend upon numbers of parasite-containing cells, partly degenerated.

The yellow bone-marrow becomes absorbed and red tissue takes its place, as in most profound anemias.

Transmission.—Rogers' observation, that the round bodies grew into flagellate bodies at temperatures much below that of the human body, led Manson to conjecture that the extrahuman phase of the life of the organism took place at similar low temperatures in the soil or in water. Patton* found that a number of cases sometimes occurred in the same house, while neighboring houses were free, and thought this suggested that a domestic insect might be the distributing host. Later, Patton† reported a very thorough study of insects in relation to kala-azar, in which after a long series of experimental investigation, he came to the conclusion that the Indian bed-bug, Cimex rotundatus, is the specific invertebrate host of Indian kala-azar. It seems that in order that the parasites shall mature in the bed-bug, and undergo those changes that shall result in the insect's infectivity, the bug must receive one full meal of the infected blood. If a second meal is taken, the digestive condition in the bug's alimentary canal is changed, and instead of continuing to develop, the parasites die out. When the conditions are all favorable, Patton found that the flagellates continued to multiply actively from the fifth to the eighth day. By the twelfth day practically all had reached the postflagellate stage and were only found in the stomach of the bed-bug. These results convince Patton that Cimex rotundatus is the definitive host, but the proof is lacking. No animal is known to be sufficiently susceptible to Leishmania donovani, to acquire anything resembling Kala-azar, therefore there is none that the bug can successfully infect. Human experiment with so fatal a disease being out of the question, the case rests at this point. Row‡ has, however, shown that when a monkey, Macacus sinicus, is inoculated cutaneously or subcutaneously with a three-weeks-old culture of Leishmania donovani, a cutaneous or subcutaneous lesion may result. This may facilitate future studies with biting insects.

It may be, however, that Patton and others are wrong in thinking that the flagellate stage at which the parasites arrive in the bed-bug is the infective stage, and have, therefore, gone astray. Bayon§ points out that Leishmania infantum is infective for dogs and monkeys in the rounded or oval stages, not in the elongate or cultural stages, and that the same may be true of Leishmania dono-

* "Scientific Memoirs of the Government in India," 1907, No. 27.
† "Brit. Med. Jour.," 1912 II, 1194.
‡ "Brit. Med. Jour.," 1912, II, 1196.
§ "Brit. Med. Jour.," 1912, II, 1197.

vani. The fleas, which are the vectors of infantile kala-azar among dogs, show only the rounded and oval forms of the parasites, never the flagellated forms.

Quite recently Patton and Donovan have been successful in infecting puppies with Leishmania donovani, though the mature dogs seem never to be infected, the examination of 2000 street dogs in Madras and other cities failing to reveal any of the parasites in either the liver or spleen. Patton inoculated a white rat with 3 cc. of an emulsion of human spleen containing the oval forms of Leishmania donovani from a case of Indian kala-azar, and fifteen days later found the spleen several times the normal size and containing large numbers of the parasites.

Diagnosis.—The anemia of kala-azar is usually not profound. The erythrocytes number about 3,000,000 in ordinary cases and the hemoglobin is correspondingly diminished. As in malaria, there is leukopenia, but it is usually more severe, the white corpuscles sometimes being as few as 600 to 650 per cubic millimeter of blood. The enlargement of the spleen and liver suggest malaria.

The only certain way to make a diagnosis, except in those rare cases where one has the good fortune to find occasional parasites in the leukocytes of the circulating blood, is by hepatic or splenic puncture. A large hypodermic needle should be used, and it should be carefully sterilized. It should by preference be thrust into the liver and a drop of fluid secured for examination. If nothing be found it may later be necessary to puncture the spleen, though it is dangerous because of the probability of subsequent hemorrhage. If decided upon as a justifiable method of examination, the needle is thrust into the spleen, and a bit of splenic pulp secured by firmly withdrawing the piston of the attached syringe.

Before making such a puncture, leukemia should be excluded, lest hemorrhage occur.

Treatment.—No treatment thus far tried has proved successful. The disease is usually fatal, and in certain parts of India whole towns have been depopulated by it and the fear of it.

INFANTILE KALA-AZAR

Leishmania Infantum (Nicolle)

Pianese* found infantile kala-azar in Italy, and in the children suffering from it he was able to find the Leishmania infantum.

Nicolle,† while in Tunis, observed a form of kala-azar that was peculiar to childhood and most frequent in babies of about two years of age. Mesnil has identified the affection with a disease known as "ponos" in Greece. In the spleens of such patients

* "Gaz. Intern. di Medicin," 1905, VIII, 8.

† "Ann. de l'Inst. Pasteur," 1909, XXIII, 361, 441.

Nicolle found an organism that was not distinguishable either by microscopic examination or by cultivation from Leishmania donovani, but, finding that it was infectious for dogs, he came to the conclusion that it was a separate species, and called it Leishmania infantum. He also found that the dogs in Tunis frequently suffered from spontaneous infection from this parasite, and it is possible that it is from the dogs that the children become infected.

Further experiments with this parasite by Nicolle and Comte have shown that in the form in which it occurs in the human spleen it is capable of infecting monkeys, and Novy has succeeded in cultivating the organism and infecting dogs with artificial cultures containing its flagellate forms.

It is now thought by many that infantile kala-azar and Indian kala-azar are identical diseases, caused by identical parasites. In considering the probable source of the disease Stitt* says: "It has been suggested that the Mediterranean basin may have been the original focus of visceral kala-azar and that it spread thence to India by way of Greece and the Russian Caucasus, cases having been reported from districts which would join the two foci. Just as children bear the brunt of malaria in old malarial districts and adults suffer in places in which the disease has been more recently imported, so by analogy we may consider the disease as of more recent introduction in India . . . . In the Mediterranean basin there is a natural canine Leishmaniasis and some think the human form may be contracted from the dog through the medium of the flea."

TROPICAL ULCER

Leishmania Furunculosa (Firth)

In India, northern Africa, southern Russia, parts of China, the West Indies, South America, and, indeed, most tropical countries, a peculiar intractable chronic ulceration is occasionally observed, and is variously known as Tropical ulcer, Oriental sore, Biscra boil, Biscra button, Aleppo boil, Delhi boil, Bagdad boil, Jericho boil, and Buton d'Orient. It has long been known as a specific ulcerating granuloma. The lesions, which begin as red spots, develop into papules which become covered with a scaly crust which separates, leaving an ulcer upon which a new crust develops. The lesion spreads and is much larger when the crust again separates. A purulent discharge is given off in moderate quantities and the ulcer becomes deep and perpendicularly excavated. It lasts for months—sometimes a year or more—and gradually cicatrizes, forming a contracting scar that is quite disfiguring when upon the face. The lesions may be single, though they are commonly mul-

*Diagnosis and Treatment of Tropical Diseases, 1914, p. 75.

Fig. 219.—"Jericho" boil (Masterman in "Journal of Hygiene").

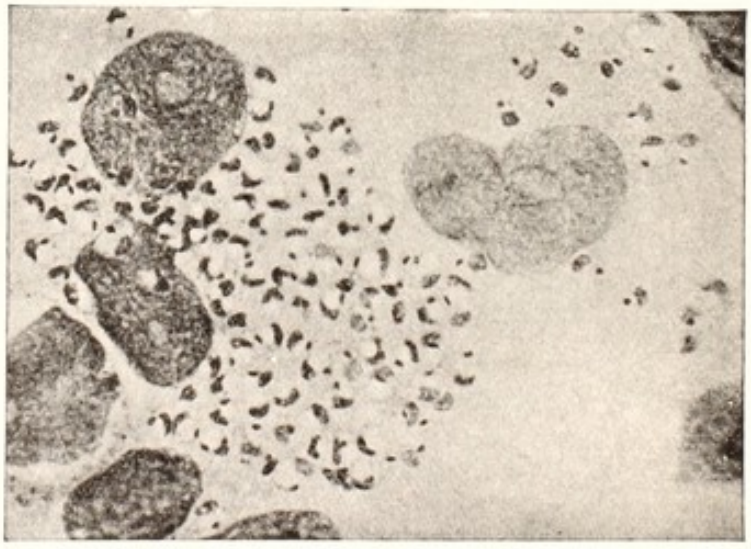


Fig. 220.—Helcosoma tropicum, from a case of tropical ulcer ("Delhi sore") smear preparation from the lesion stained with Wright's Romanowsky blood-staining fluid. The ring-like bodies, with white central portions and containing a larger and a smaller dark mass, are the micro-organisms. The dark masses in the bodies are stained a lilac color, while the peripheral portions of the bodies, in typical instances, are stained a pale robin's egg blue. The very dark masses are nuclei of cells of the lesion. × 1500 approx. (Wright). (From photograph by Mr. L. S. Brown.)

tiple, as many as twenty sometimes occurring simultaneously. It is thought that recovery is followed by immunity.

Organism.—In 1885 Cunningham* described a protozoan organism found in the tropical ulcer, the observation being confirmed by Firth,† who called the bodies Sporozoa furunculosa. Later, J. H. Wright‡ studied a case of tropical ulcer and found bodies precisely like the Leishmania donovani. He gave it the name Helcosoma tropicum. The great similarity to the other organisms has led more recent writers to identify it with Leishmania, but as it induces a local and not a general infection like kala-azar, it is now known as Leishmania furunculosa.

Cultivation.—The organism has been cultivated by Nicolle and

Fig. 221.—Oriental sore (Wellcome Research Laboratory).

Manceaux§ upon the same media and in the same manner as Leishmania donovani and Leishmania infantum with which these investigators believe it to be identical. Cultivation was also successfully achieved by Row.

Pathogenesis.—The virus is pathogenic for man, monkeys such as Macacus simius, M. cynomolgus, M. rhesus and M. inuus, and for dogs. The same effects are produced whether fresh virus from a human ulcer, or from an artificial culture be employed. In dogs the inoculations produce only nodular formations; in monkeys, nodules like those in human beings that go on to ulceration. Intraperitoneal inoculations usually fail. The most successful inocula-

* "Scientific Memoirs by Medical Officers of the Army in India," 1884, I.
† "British Med. Journal," Jan. 10, 1891, p. 60.
‡ "Jour. of Med. Research," 1904, X, 472.
§ "Ann. de l'Inst. Pasteur," 1910, XXIV, 683.

tions are made beneath the skin in the neighborhood of the nose. One successful infection with the parasite usually confers immunity; unsuccessful intraperitoneal introduction of large quantities of culture produce no immunity.

Transmission.—The disease can be transmitted by inoculation from human being to human being.

The usual mode of transmission is not known, but as the lesions usually occur where the body surface is uncovered, it may be that flies or other insects act as vectors of the parasites.

Preventive Inoculation.—Jackson* is authority for the statement that "the Jews of Bagdad recognized that tropical ulcer is inoculable and autoprotective years ago, and practised vaccination of their children upon some portion of the body covered by clothing, in order that their faces and other exposed parts of the body be not disfigured by the ulcers and the resultant scars." Nicolle† sought to vaccinate according to modern methods with killed and living cultures of the organism, and was successful when he first used killed culture, then after a year a live culture, and then three months later another live culture.

Treatment.—Row‡ has endeavored to cure already existing lesions by vaccination, and has met with what seems to be encouraging success. Cultures of the organism were permitted to grow for seven days, then sterilized with glycerin. Patients can bear 0.25 cc. at a dose, there is little febrile reaction, and the lesions proceed to heal nicely.

HISTOPLASMOSIS

Histoplasma Capsulatum (Darling)

In 1906 Darling,§ working at the Isthmus of Panama, observed certain cases presenting pyrexia, anemia, leukopenia, splenomegaly, and emaciation, and bearing a close resemblance to kala-azar. The disease was quite chronic, and it terminated fatally. When examined at autopsy, these cases showed necrosis with cirrhosis of the liver, splenomegaly, pseudo-granulomata of the lungs, small and large intestines, ulceration of the intestines, and necrosis of the lymph nodes draining the injected viscera. The lesions seemed to depend upon the invasion of the endothelial cells of the smaller lymph- and blood-vessels by enormous numbers of a small encapsulated micro-organism.

The organism is small, round or oval in shape, and measures 1 to 4 μ in diameter. It possesses a polymorphous, chromatin nucleus, basophilic cytoplasm, and achromatic spaces all enclosed within an achromatic refractile capsule.

* "Tropical Medicine," Phila., P. Blakiston's Son & Co., 1907, p. 478.
† "Annales de l'Inst. Pasteur," Tunis, 1908.
‡ "British Medical Journal," 1912, 1, 540.
§ "Jour. Amer. Med. Assoc.," 1906, XLVI, 1283; "Archiv of Int. Med.," 1908, II, 107; "Jour. Exp. Med.," 1909, XI, 515.

The micro-organism differs from the Leishman-Donovan body of kala-azar in the form and arrangement of its chromatin nucleus and in not possessing a chromatin rod. The distribution of the parasite in the body is accomplished by the invasion of the contiguous endothelial cells of the smaller blood- and lymph-vessels and capillaries, and by the infection of distant regions by the dislodgment of infected endothelial cells and their transportation

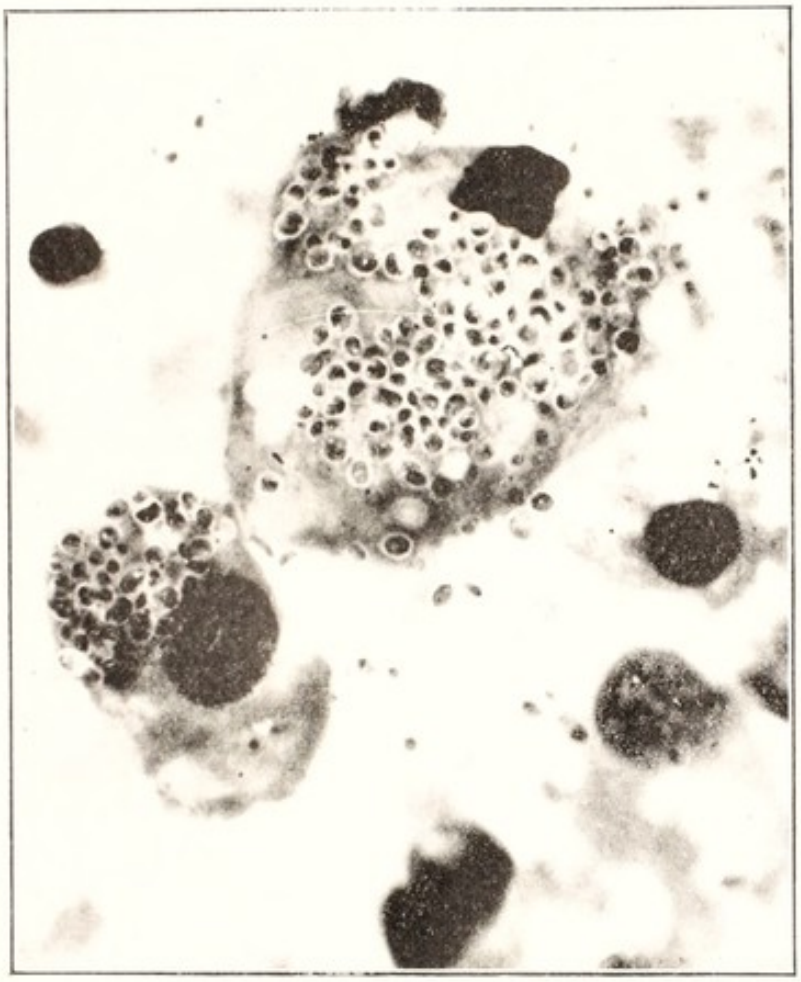


Fig. 222.—Histoplasma capsulatum. Mononuclear cells from the lung containing many parasites (Darling). (Samuel T. Darling in "Journal of Experimental Medicine.")

thither by the blood- and lymph-stream. Thus the skin, intestinal, and pulmonary nodules may be due to secondary distribution of the parasite. The micro-organism apparently lives for a considerable period of time in the tissues, because in the older areas of necrosis there are myriads of parasites all staining well.

The mode of infection and portal of entry are unknown. The parasite has neither been cultivated nor transmitted by inoculation.

Believing it to be a new parasite, Darling has suggested that it be called Histoplasma capsulatum.

CHAPTER XXIII

YELLOW FEVER

THE bacteriology of yellow fever has been studied by Domingos Freire,* Carmona y Valle,† Sternberg,‡ Havelburg,§ and Sanarelli,‖ but all of their work has been shown to be incorrect by the interesting researches and very conclusive results of Finlay,** Carter,†† Reed, Carroll, Lazear, and Agramonte,‡‡ and Reed and Carroll,§§ which have proved the mosquito to be the definitive host of an invisible micro-organism.

Reed, Carroll, Lazear, and Agramonte,‖‖ constituting a Board of Medical Officers "for the purpose of pursuing scientific investigations with reference to the acute infectious diseases prevalent on the island of Cuba," began their work in 1900, at Havana, by a careful investigation of the relationship of Bacillus icteroides to yellow fever. By a most careful technic they withdrew and examined the blood from the veins of the elbow of 18 cases of yellow fever, making 48 separate examinations on different days of the disease, and preparing 115 bouillon cultures and 18 agar plates, every examination being negative so far as Bacillus icteroides was concerned. They were entirely unable to confirm the findings of Wasdin and Geddings,*** that Bacillus icteroides was present in blood obtained from the ear in 13 out of 14 cases, and concluded that both Sanarelli, and Wasdin and Geddings were mistaken in their deductions.

In lieu of the remarkably interesting discoveries of Ronald Ross concerning the relation of the mosquito to malarial infection, the commissioners, remembering the theory of Finlay,††† who in 1881

* "Doctrine microbienne de la fievre jaune et ses inoculation preventives," Rio Janeiro, 1885.

† "Leçons sur l'étiologie et la prophylaxie de la fievre jaune," Mexico, 1885.

‡ "Report on the Etiology and Prevention of Yellow Fever," Washington, 1891; "Report on the Prevention of Yellow Fever by Inoculation," Washington, 1888.

§ "Ann. de l'Inst. Pasteur," 1897.

‖ "Brit. Med. Jour.," July 3, 1897; "Ann. de l'Inst. Pasteur," June, Sept., and Oct., 1897.

** "Amer. Jour. Med. Sci.," 1891, vol. CII, p. 264; "Ann. de la Real Academia," 1881, vol. XVIII, pp. 147–169; "Jour. Amer. Med. Assoc.," vol. XXXVIII, April 19, 1902, p. 993.

†† "New Orleans Med. Jour.," May, 1890.

‡‡ "Phila. Med. Jour.," Oct. 27, 1900; "Public Health," vol. XXVI, 1900, p. 23.

§§ "Public Health," 1901, vol. XXVII, p. 113.

‖‖ "Phila. Med. Jour.," Oct. 27, 1900.

*** "Report of the Commission of Medical Officers Detailed by the Authority of the President to Investigate the Cause of Yellow Fever," Washington, D. C., 1899.

††† "Annales de la Real Academia," 1881, vol. XVIII, pp. 147–169.

published an experimental research showing that mosquitoes spread the infection of yellow fever, and the interesting and valuable observations of Carter* upon the interval between infecting and secondary cases of yellow fever, turned their attention to the mosquito. Securing mosquitoes from Finlay and continuing the work

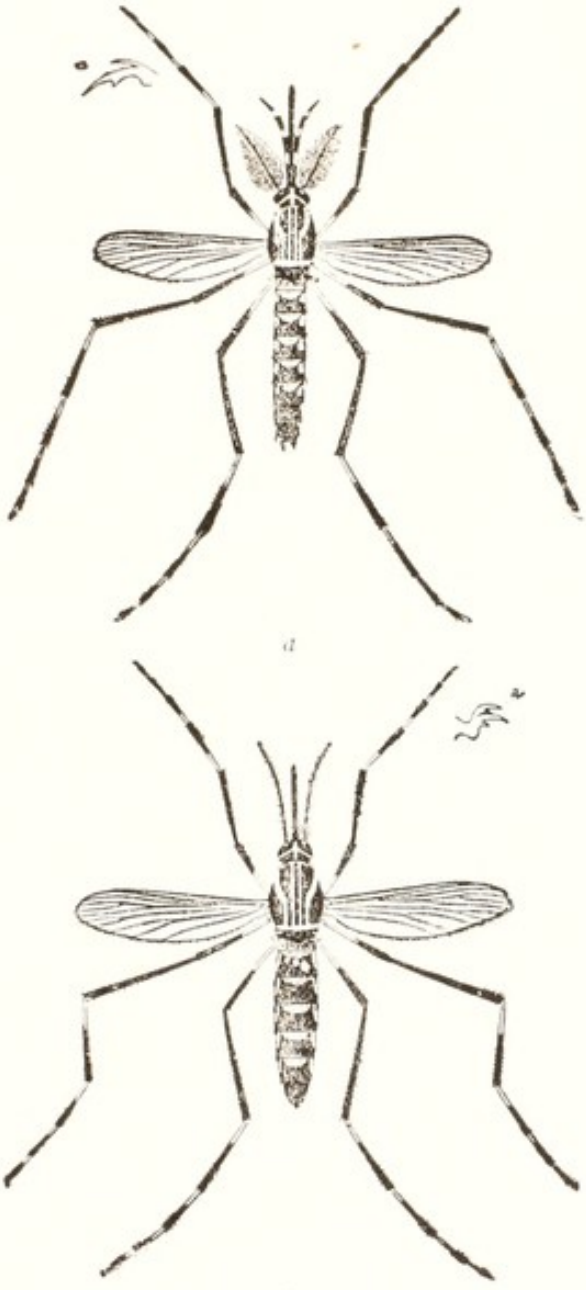


Fig. 223.—*Stegomyia fasciata* (*Stegomyia calopus*): *a*, female; *b*, male (after Carroll).

where he had left it, they found that when mosquitoes (*Stegomyia fasciata seu calopus*) were permitted to bite patients suffering from yellow fever, after an interval of about twelve days they became able to impart yellow fever through their bites. This infectious character, having once developed, seemed to remain throughout the

* "New Orleans Med. Jour.," May, 1900.

subsequent life of the insect. So far as it was possible to determine, only one species of mosquito, Stegomyia calopus, served as a host for the parasite whose cycles of development in the mosquito and in man must explain the symptomatology of yellow fever.

In order to establish these observations, experimental inoculations were made upon human beings in sufficient number to prove their accuracy. Unfortunately, Dr. Lazear lost his life from an attack of yellow fever.

Reed, Carroll, and Agramonte* came to the following conclusions:

1. The mosquito *C. fasciatus* [Stegomyia calopus] serves as the intermediate host of the yellow fever parasite.
2. Yellow fever is transmitted to the non-immune individual by means of the bite of the mosquito that has previously fed on the blood of those sick with the disease.
3. An interval of about twelve days or more after contamination appears to be necessary before the mosquito is capable of conveying the infection.
4. The bite of the mosquito at an earlier period after contamination does not appear to confer any immunity against a subsequent attack.
5. Yellow fever can be experimentally produced by the subcutaneous injection of blood taken from the general circulation during the first and second days of the disease.
6. An attack of yellow fever produced by the bite of a mosquito confers immunity against the subsequent injection of the blood of an individual suffering from the non-experimental form of the disease.
7. The period of incubation in 13 cases of experimental yellow fever has varied from forty-one hours to five days and seventeen hours.
8. Yellow fever is not conveyed by fomites, and hence disinfection of articles of clothing, bedding, or merchandise, supposedly contaminated by contact with those sick with the disease, is unnecessary.
9. A house may be said to be infected with yellow fever only when there are present within its walls contaminated mosquitoes capable of conveying the parasite of this disease.
10. The spread of yellow fever can be most effectually controlled by measures directed to the destruction of mosquitoes and the protection of the sick against the bites of these insects.
11. While the mode of propagation of yellow fever has now been definitely determined, the specific cause of the disease remains to be discovered.

The probability that Bacillus icteroides is the specific cause and is transmitted by the mosquito is so slight that it need scarcely be considered. All analogy points to the organism being an animal parasite similar to that of malarial fever.

With this positive information before us, the prophylaxis of yellow fever and the prevention of epidemics of the disease where sporadic cases occur becomes very simple and may be expressed in the following rules:

1. Whenever yellow fever is likely to occur, the breeding places of mosquitoes should be destroyed by drainage. Cisterns and other necessary collections of standing water should be covered or secured.
2. Houses should have the windows and doors screened and the inhabitants should use bed nets.
3. So soon as a case of fever appears it should be removed in a mosquito-proof ambulance to a mosquito-proof apartment in a well-screened hospital ward and kept there until convalescent.

* Pan-American Medical Congress, Havana, Cuba, Feb. 4–7, 1901; Sanitary Department, Cuba, series 3, 1902.

4. The premises where such a case has occurred should be fumigated by burning pyrethrum powder (1 pound per 1000 cubic feet) to stun the mosquitoes, which fall to the floor and must afterward be swept up and destroyed.

By these means Major W. C. Gorgas,* without expensive disinfection and without regard for fomites, has virtually exterminated yellow fever from Havana and from the Canal Zone, Panama, where it was for many years endemic.

A practical point connected with the screens is given in the work of Rosenau, Parker, Francis, and Beyer,† who found that to be effective the screens must have 20 strands or 19 meshes to the inch. If coarser than this the stegomyia mosquitoes can pass through.

Reed and Carroll‡ were the first to filter the blood of yellow fever patients and prove that after it had passed through a Berkefeld filter that kept back Staphylococcus aureus, it still remained infective and capable of producing yellow fever in non-immune human beings.

This subject was further investigated by Rosenau, Parker, Francis, and Beyer,§ who found that the virus was even smaller than the first experiment would suggest, as it not only passed through the Berkefeld filter, but also through the Pasteur-Chamberland filter. The filtrates always remained sterile when added to culture-media.

The virus has not been artificially cultivated.

Prophylaxis.—Guiteras‖ has studied the effect of intentionally permitting non-immunes who are to be exposed to the disease to be experimentally infected by being bitten by infected mosquitoes, after which they are at once carefully treated. His first conclusion was that "the intentional inoculation gives the patient a better chance of recovery," but the danger of death from the experimental infection was later shown to be so great that it had to be abandoned.

* International Sanitary Congress held at Havana, Cuba, Feb. 16, 1902: Sanitary Department, Havana, series 4.

† Report of Working Party No. 2, Yellow Fever Institute, Bull. 14, May, 1904.

‡ "Am. Med.," Feb. 22, 1902.

§ "Bull. No. 14, U. S. Public Health and Marine Hospital Service," Washington, D. C., May, 1904.

‖ "Revista de Medicina Tropical," Havana, Cuba, 1902.

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CHAPTER XXIV

TYPHUS FEVER

Typhus fever, also known as jail-fever, ship-fever, army-fever, and by a large number of other names, of which about a hundred have been collected by Murchison,* has long been known, but was probably not recognized as a definite disease before 1760, when Gaultier de Sauvage endeavored to give it individuality, or 1769 when Cullum of Edinburgh defined it. Its eventual separation from typhoid fever, with which it continued to be confused, was the result of the studies of Gerhard "On the Typhus Fever which occurred in Philadelphia in the Spring and Summer of 1836, Etc."† The Germans still speak of *typhus abdominalis*, meaning *typhoid* or enteric fever, and *typhus exanthematicus*, meaning the *typhus* fever of the present day. The Spanish and Mexicans call it *tabardillo*.

The disease is largely a disease of poverty, filth and crowding, and is of frequent occurrence both in sporadic and epidemic form where such conditions occur permanently or temporarily. Its most common epidemic occurrence is therefore among the slums, in jails, in ships, in asylums, in hospitals and in armies. With the improved hygienic conditions of the present time its occurrence in considerable epidemics is much diminished, and it is not to be expected in sanitary dwellings, among cleanly people or in well-regulated institutions.

It is undoubtedly transmissible and therefore infectious, but it early became clear that the infection was not air-borne and did not readily pass from individual to individual. Further, it seems clear that the survival of an attack confers immunity against future infection.

Though its infective and micro-organismal nature is clear, the specific micro-organism has not yet been discovered. This is not because it has not been made the subject of much investigation in many countries by capable men, but rather because of peculiar circumstances that make the discovery difficult, if not impossible.

The early investigations of the subject were confined to demonstrating the truly infectious nature of a disease whose transmissibility was so uncertain as to permit the escape of large numbers of those exposed to it.

In 1876 Moczutkowski‡ inoculated himself with the blood of a

* "A Treatise on the Continued Fevers of Great Britain," 3d edition, 1884, p. 161.

† Amer. Jour. of the Med. Sciences, 1836, xix, p. 283; 1837, xx, p. 289.

‡ "Allgemeine Med. Central Zeitung," 1900, LXVIII, 1055.

patient suffering from typhus fever, and developed the disease eighteen days later. In 1907 Otero* endeavored to induce the disease in human beings by inoculation. In one out of four attempts he was successful.

Experiments with a not infrequently fatal malady made upon human beings being immoral and inexpedient, it became necessary to find some animal susceptible to the disease, with which further experiments could be prosecuted.

In 1909 Nicolle† succeeded in producing the disease in a chimpanzee by inoculating it with human blood. Later‡ he was able to transmit the disease from the chimpanzee, and still later from human beings, to Macacus sinicus by inoculating with human blood. In 1909 Anderson and Goldberger§ were successful in transmitting the disease to monkeys, by inoculating them with human blood. Other workers corroborated these results, and thus it became clear that the suspicion that the disease was infectious was correct, and that the infectious agent was in the blood with which it could be carried over to new men and animals and reproduce the disease. Later Nicolle, Couer and Conseil‖ were able to transmit the disease to guinea-pigs.

In Mexico, Gaveño and Girard** were able to carry the infection through 11 transplantations from guinea-pig to guinea-pig, and still find it infective for monkeys.

Still, however, the micro-organism could not be found. Two additional problems therefore became important for solution. First, what was the nature of this virus that could not be found, second, how did it naturally pass from patient to patient?

In October, 1910, Nicolle, Couer and Conseil†† instead of working with artificially defibrinated blood, permitted the blood to coagulate spontaneously, then passed it through the most porous kind of a Berkefeld filter, and successfully infected one out of two monkeys injected with the filtrate. After other series of experiments, these investigators came to the conclusion that the serum of artificially defibrinated blood, when filtered, was always without infective power, and that of spontaneously coagulated blood, commonly so, and that hence, though the virus of the disease is a filterable virus, it consists of organisms so large as to be commonly held back by the coarsest Berkefeld filters. It may be too small to be visible nevertheless, at least to such methods of observation as are now in vogue.

In regard to the transmission of the disease the investigators had before them the usual exemption of physicians, nurses, attendants

*"Mem. pres. a l'Acad. de Med. de Mex.," 1907.
†"Ann. de l' Inst. Pasteur," 1910, XXIV.
‡"Compt.-rendu Acad. d. Sciences de Paris," 1910.
§"Public Health Reports," 1909, XXIV, p. 1941.
‖"Ann. de l'Inst. Pasteur," 1910, XXV, 97.
**"Publ. de l'Inst. Bact. Noc. Mex.," 1910, Nov. 9.
††"Ann. de l'Inst. Pasteur," 1911, XXV, 97.

and others who cared for patients suffering from the disease, as contrasted with its persistent spread to new patients at the foci of infection. They also had the recently gained knowledge of the part played by insects and arthropods in the transmission of malaria, relapsing fever, African lethargy, etc., the whole matter being of such nature as to make the conclusion that the infection was transmitted by an insect host, a justifiable one.

The first to work upon this problem were Nicolle, Couer and Conseil,* the selected insects being pediculi. They permitted lice to feed upon the blood of an infected monkey, and then upon a healthy monkey. The healthy monkey contracted typhus fever. In the same year, and working independently, Goldberger and Anderson† made two attempts to infect healthy monkeys by permitting lice fed upon cases of typhus fever in men, to bite them. They had partial success—the monkeys became diseased but no immunity tests were made for confirmation of the nature of the disease.

Ricketts and Wilder‡ working in Mexico succeeded in transmitting typhus fever from man to monkeys by means of lice—Pediculus vestimenti. They also succeeded in transmitting the disease to a monkey by scarifying its skin and applying the abdominal contents of some infected lice, so that it was proved by them that the cause of infection was in the lice. Later Nicolle and Conseil§ also succeeded in infecting a monkey by the bites of infected lice.

Wilder‖ further found that the infectious agent passes from the infected lice to a second generation of insects, as does the spirochæta of relapsing fever to subsequent generations of ornithodorus ticks. Wilder failed in experiments directed toward infecting monkeys by fleas or bed-bugs.

In the experiments recorded by Wilder, the transmission of typhus fever to monkeys, by lice, was successful in 7 out of 10 attempts. It required 17 lice to infect a monkey. In one case a monkey seemed to be immunized by being bitten by very young lice.

Goldberger and Anderson** also experimented with the head louse Pediculus capitus and succeeded in showing that it too takes up the typhus fever virus and may pass it on from human being to monkey, and hence probably from man to man.

A description of the lice will be found in the chapter upon "Relapsing Fever."

*"Compt.-rendu de l'Acad. des Sciences de Paris," 1909, CXLIX, 486.
†"Public Health Reports," 1910, XXV.
‡"Jour. Amer. Med. Asso.," 1910, LIV, 1304.
§"Compt.-rendu. de l'Acad. des Sciences de Paris," 1911, CLIII, 1522.
‖"Journal of Infectious Diseases," 1911, IXI.
**"Public Health Reports," 1912, XXVII.

CHAPTER XXV

PLAGUE

Bacillus Pestis (Yersin, Kitasato)

General Characteristics.—A minute, pleomorphous, diplococcoid and elongate, sometimes branched, non-motile, non-flagellated, non-sporogenous, non-liquefying, non-chromogenic, aërobic, pathogenic organism, easily cultivated artificially, and susceptible of staining by ordinary methods, but not by Gram's method.

Plague, bubonic plague, pest, black plague, "black death," or malignant polyadenitis is an acute epidemic infectious febrile disease of an intensely fatal nature, characterized by inflammatory enlargement and softening of the lymphatic glands, marked pulmonary, cerebral and vascular disturbance, and the presence of the specific bacillus in the lymphatic nodes and blood.

The history of plague is so full of interest that many references to it appear in popular literature. The student can scarcely find more profitable reading than the "History of the Plague Year in London," by DeFoe, and readers of Boccacio will remember that it was the plague epidemic then raging in Florence that led to the isolation of the group of young people by whom the stories of the Decameron were told.

During the reign of the Emperor Justinian the plague is said to have carried off nearly half of the population of the Roman Empire. In the fourteenth century it is said to have destroyed nearly twenty-five millions of the population of Europe. Epidemics of less severity but attended with great mortality appeared in the sixteenth, seventeenth, and eighteenth centuries. In 1894 an epidemic broke out in the western Chinese province of Yunnan and reached Canton in January, 1894, thus escaping from its endemic center and began to spread. It can be traced from Canton to Hongkong. In 1895 it appeared also in Amoy, Macao, and Foochoo. In 1896 it had reached Bombay and reappeared in Hongkong. In 1897 Bombay, the Madras Presidency, the Punjab, and Madras were visited. In 1898 the disease spread greatly throughout India and into Turkestan, and by sea went to Madagascar and Mauritius. In 1899 it extended still more widely in India and China, Japan and Formosa, and succeeded in disseminating as widely as the Hawaiian Islands and New Caledonia on the east, Portugal, Russia, and Austria on the west, and Brazil and Paraguay on the south. In 1900 it had spread to nearly every part of the world. In those places in which sanitary measures could not be carried into effect the people died in great numbers—thus in India

in 1901 there were 362,000 cases and 278,000 deaths. In the first six months of the epidemic of 1907, the deaths in India were much more numerous, reaching a total of 1,062,908. Where sanitary precautions are possible and co-operation between the people and the authorities can be brought about, as in New York, San Francisco, and other North American and European ports, the disease remains confined pretty well within limits and does not spread. An interesting account of "The Present Pandemic of Plague" by J. M. Eager, was published in 1908 in Washington, D. C., by the U. S. Public Health and Marine Hospital Service.

Plague is an extremely fatal affection, whose ravages in the hospital at Hongkong, in which Yersin made his original observations, carried off 95 per cent. of the cases. The death-rate varies in different epidemics from 50 to 90 per cent. In the epidemic at

Fig. 224.—Axillary bubo. (Reproduced from Simpson's "A Treatise on Plague," 1905, by kind permission of the Cambridge University Press.)

Hongkong in 1894 the death-rate was 93.4 per cent. for Chinese, 77 per cent. for Indians, 60 per cent. for Japanese, 100 per cent. for Eurasians, and 18.2 per cent. for Europeans. It affects both men and animals, and is characterized by sudden onset, high fever, prostration, delirium, and the occurrence of exceedingly painful lymphatic swellings—buboes—affecting chiefly the inguinal nodes, though not infrequently the axillary, and sometimes the cervical, nodes. Death comes on in severe cases in forty-eight hours. The pneumonic form is most rapidly fatal. The longer the duration of the disease, the better the prognosis. Autopsy in fatal cases reveals the characteristic enlargement of the lymphatic nodes, whose contents are soft and sometimes purulent.

Wyman,* in his very instructive pamphlet, "The Bubonic

* Government Printing Office, Washington, D. C., 1900.

Plague," finds it convenient to divide plague into (*a*) bubonic or ganglionic, (*b*) septicemic, and (*c*) pneumonic forms. Of these, the bubonic form is most frequent and the pneumonic form most fatal.

Specific Organism.—The bacillus of bubonic plague was independently discovered by Yersin* and Kitasato† in the summer of 1894, during an epidemic of the plague then raging at Hongkong. There seems to be little doubt but that the micro-organisms described by the two observers are identical.

Ogata‡ states that while Kitasato found the bacillus in the blood of cadavers, Yersin seldom found it in the blood, but always in the enlarged lymphatic glands; that Kitasato's bacillus retains the color when stained by Gram's method; Yersin's does not; that Kitasato's bacillus is motile; Yersin's non-motile; that the colonies of Kitasato's bacillus, when grown upon agar, are round, irregular,

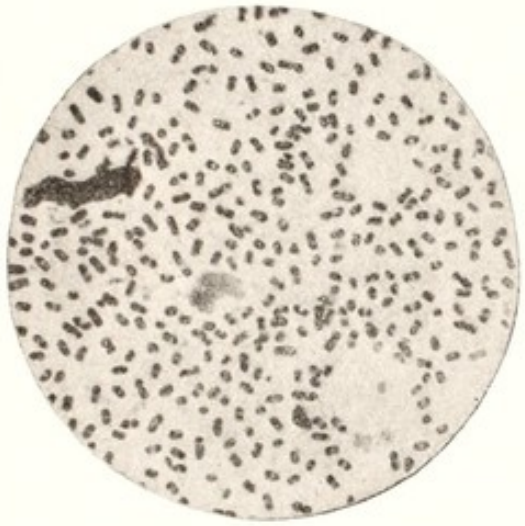


Fig. 225.—Bacillus of bubonic plague (Yersin).

grayish white, with a bluish tint, and resemble glass-wool when slightly magnified; those of Yersin's bacillus, white and transparent, with iridescent edges. Ogata, in his investigations, found that the bacillus corresponded with the description of Yersin rather than that of Kitasato, and it is certain that of the two the description given by Yersin is the more correct.

In the "Japan Times," Tokio, November 28, 1899, Kitasato explains that, his investigations being made upon cadavers that were partly putrefied, he was led to believe that the bacillus first invaded the blood. Later studies upon living subjects showed him the error of this view and the correctness of Yersin's observation that the bacilli first multiply in the lymphatics.

Both Kitasato and Yersin showed that in blood drawn from the

* "Ann. de l'Inst. Pasteur," 1894, 9.

† Preliminary notice to the bacillus of bubonic plague, Hongkong, July 7, 1894.

‡ "Centralbl. f. Bakt. u. Parasitenk.," Sept. 6, 1897, Bd. XXII, Nos. 6 and 7, p. 170.

finger-tips and in the softened contents of the buboes the bacillus may be demonstrable.

Morphology.—The bacillus is quite variable. Uusally it is short and thick—a "coco-bacillus," as some call it—with rounded ends. Its size is small (1.5 to 2 μ in length) and 0.5 to 0.75 μ in breadth. It not infrequently occurs in chains of four or six or even more, and is occasionally encapsulated. It shows active Brownian movements, which probably led Kitasato to consider it motile. Yersin did not regard it as motile, and was correct. Gordon* claims that some of the bacilli have flagella. No spores are formed.

Staining.—It stains by the usual methods; not by Gram's method. When stained, the organism rarely appears uniformly colored, being darker at the ends than at the center, so as to resemble a dumbbell or diplococcus. The bacilli sometimes appear vacuolated,

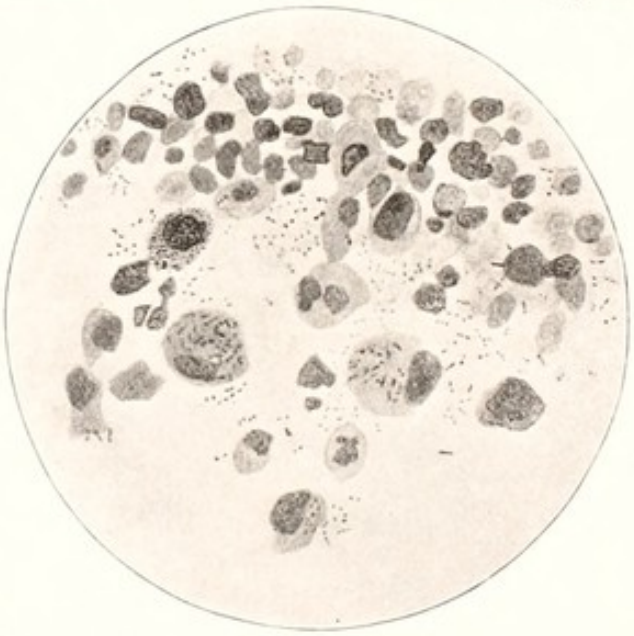


Fig. 226.—Bacilli of plague and phagocytes, from human lymphatic gland × 800 (Aoyama).

and nearly all cultures show a variety of involution forms. Kitasato has compared the general appearance of the bacillus to that of chicken-cholera.

Involution forms on partly desiccated agar-agar not containing glycerin are said by Haffkine to be characteristic. The microbes swell and form large, round, oval, pea-shaped, spindle-shaped or biscuit-like bodies which may attain twenty times the normal size, and gradually lose the ability to take the stain. Such involution forms are not seen in liquid culture.

Cultivation.—Pure cultures may be from the blood or from the softened contents of the buboes, and develop well upon artificial media. The optimum temperature is about 30°C. The extremes at which growth occurs are 20° and 38°C.

* "Centralbl. f. Bakt. u. Parasitenk.," June 24, 1897, Bd. XXI, Nos. 20 and 21.

Bouillon.—In bouillon a diffuse cloudiness was observed by Kitasato, though Yersin observed that the cultures resembled erysipelas cocci, and contained zoöglea attached to the sides and at the bottom of the tube of nearly clear fluid.

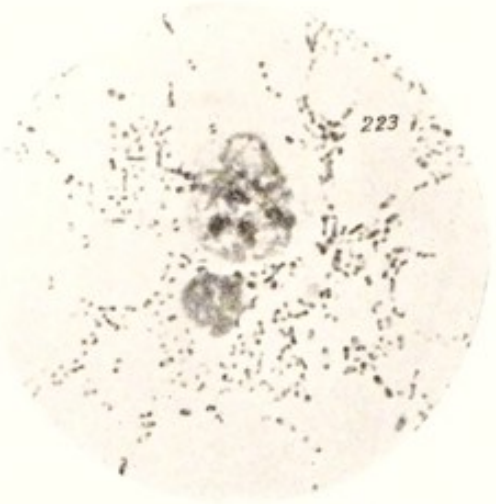


Fig. 227.—Bacillus pestis. Highly virulent culture forty-eight hours old, from the spleen of a rat. Unstained preparation (Kolle and Wassermann).

Haffkine* found that when an inoculated bouillon culture is allowed to stand perfectly at rest, on a firm shelf or table, a characteristic appearance develops. In from twenty-four to forty-eight hours, the liquid remaining limpid, flakes appear underneath

Fig. 228.—Bacillus pestis. Involution forms from a pure culture on 3 per cent. sodium chlorid agar-agar. Methylene-blue (Kolle and Wassermann).

the surface, forming little islands of growth, which in the next twenty-four to forty-eight hours grow into a jungle of long stalactite-like masses, the liquid remaining clear. In from four to six days these islands become still more compact. If the vessels be dis-

* "Brit. Med. Jour.," June 12, 1897, p. 1461.

turbed, they fall like snow and are deposited at the bottom, leaving the liquid clear.

Colonies.—Upon gelatin plates at 22°C. the colonies may be observed in twenty-four hours by the naked eye. They are pure white or yellowish white, spheric when deep in the gelatin, flat when upon the surface, and are about the size of a pin's head. The gelatin is not liquefied. Upon microscopic examination the borders of the colonies are found to be sharply defined. The contents become more granular as the age increases. The superficial colonies are occasionally surrounded by a fine, semi-transparent zone.

Klein* says that the colonies develop quite readily upon gelatin made from beef bouillon (not infusion), appearing in twenty-four hours, at 20°C., as small, gray, irregularly rounded dots. Magnification shows the colonies to be serrated at the edges and made up of

Fig. 229.—Stalactite growth of bacillus pestis in bouillon. (Reproduced from Simpson's "A Treatise on Plague," 1905, by kind permission of the Cambridge University Press.)

short, oval, sometimes double bacilli. Some colonies contrast markedly with their neighbors in that they are large, round, or oval, and consist of longer or shorter, straight or looped threads of bacilli. The appearance was much like that of the young colonies of Proteus vulgaris. At first these were regarded as contaminations, but later their occurrence was regarded as characteristic of the plague bacillus. The peculiarities of these colonies cannot be recognized after forty-eight hours.

Gelatin Punctures.—In gelatin puncture cultures the development is scant. The medium is not liquefied; the growth takes place in the form of a fine duct, little points being seen on the surface and in the line of puncture. Sometimes fine filaments project into the gelatin from the central puncture.

Abel found the best culture-medium to be 2 per cent. alkaline

* "Centralbl. f. Bakt. u. Parasitenk.," July 10, 1897, XXI, Nos. 24 and 25.

peptone solution containing 1 or 2 per cent. of gelatin, as recommended by Yersin and Wilson.

Agar-agar.—Upon agar-agar the bacilli grow freely, but slowly, the colonies being whitish in color, with a bluish tint by reflected light, and first appearing to the naked eye when cultivated from the blood of an infected animal after about thirty-six hours' incubation at 37°C. Under the microscope they appear moist, with rounded uneven edges. The small colonies are said to resemble tufts of glass-wool. Microscopic examination of the agar-agar culture shows the presence of chains resembling streptococci.

Upon glycerin-agar the development of the colonies is slower, though in the end the colonies attain a larger size than those grown upon plain agar.

Hankin and Leumann* recommended, for the differential diagnosis of the plague bacillus, a culture-medium prepared by the addition of 2.5 to 3.5 per cent. of salt to ordinary culture agar-agar. When transplanted from ordinary agar-agar to the salt agar-agar, the involution forms so characteristic of the bacillus occur with exceptional rapidity. In bouillon containing this high percentage of salt the stalactite formation is beautiful and characteristic.

Blood-serum.—Upon blood-serum, growth, at the temperature of the incubator, is luxuriant and forms a moist layer, of yellowish-gray color, unaccompanied by liquefaction of the serum.

Potato.—Upon potato no growth occurs at ordinary temperatures. When the potato is stood in the incubator for a few days a scanty, dry, whitish layer develops.

Vital Resistance.—Kitasato found that the plague bacillus did not seem able to withstand desiccation longer than four days; but Rappaport† found that they remained alive when kept dry upon woolen threads at 20°C. for twenty-three days, and Yersin found that although it could be secured from the soil beneath an infected house at a depth of 4 to 5 cm., the virulence of such bacilli was lost.

Kitasato found that the bacillus was killed by two hours' exposure to 0.5 per cent. carbolic acid, and also by exposure to a temperature of 80°C. for five minutes. Ogata found the bacillus instantly killed by 5 per cent. carbolic acid, and in fifteen minutes by 0.5 per cent. carbolic acid. In 0.1 per cent. sublimate solution it is killed in five minutes.

According to Wyman, the bacillus is killed by exposure to 55°C. for ten minutes. The German Plague Commission found that the bacilli were killed by exposure to direct sunlight for three or four hours; and Bowhill‡ found that they are killed by drying at ordinary room temperatures in about four days.

* "Centralbl. f. Bakt. u. Parasitenk.," Oct., 1897, Bd. XXII, Nos. 16 and 17, p. 438.

† Quoted by Wyman.

‡ "Manual of Bacteriological Technique and Special Bacteriology," 1899, p. 197.

Wilson* found the thermal death-point of the organism one or two degrees higher than that of the majority of non-sporulating pathogenic bacteria, and that the influence of sunlight and desiccation cannot be relied upon to destroy it.

Rosenau† found temperature the most important factor, as it dies quickly when kept dry at 37°C., but remains alive for months when kept dry at 19°C. Sunlight kills it in a few hours. A temperature of 70°C. is invariably fatal in a short time.

Metabolism.—The bacillus develops best under aërobic conditions though it develops to a slight extent also under anaërobic conditions. In glucose-containing media it does not form gas. No indol is formed. Ordinarily the culture-medium is acidified, the acid reaction persisting for three weeks or more.

Ghon,‡ Wernicke,§ and others who have studied the toxic products of the bacillus all incline to the belief that it forms only endotoxin.

Kossee and Overbeck,‖ however, believe that there is, in addition, a soluble exotoxin that is of importance.

Bielonovsky** finds that broth, agar, and serum cultures of the plague bacillus possess the property of hemolyzing the blood of normal animals. The hemolytic power of filtrates of plague cultures increases up to the thirteenth or fourteenth day, then gradually diminishes, but without completely disappearing. The hemolysins are notably resistant to heat, not being destroyed below 100°C.

Experimental Infection.—Mice, rats, guinea-pigs, rabbits, and monkeys are all susceptible to experimental inoculation. When blood, lymphatic pulp, or pure cultures are inoculated into them, the animals become ill in from one to two days, according to their size and the virulence of the bacillus. Their eyes become watery, they show disinclination to take food or to make any bodily effort, the temperature rises to 41.5°C., they remain quiet in a corner of the cage, and die with convulsive symptoms in from two to seven days. If the inoculation be made intravenously, no lymphatic enlargement occurs; but if it be made subcutaneously, the nearest lymph nodes always enlarge and suppurate if the animal live long enough. The bacilli are found everywhere in the blood, but not in very large numbers.

Rats suffer from both an acute septicemic and a chronic form of the disease. In the former an infiltration or watery edema can be observed in a few hours about the point of inoculation. The autopsy shows the infiltration to be made up of a yellowish gelatinous exuda-

* "Journal of Medical Research," July, 1901, vol. VI, No. 1, p. 53.
† Bulletin No. 4 of the Hygienic Laboratory of the U. S. Marine Hospital Service, 1901.
‡ Wien, 1898.
§ "Centralbl. f. Bakt.," etc., 1898, XXIV.
‖ "Arbeiten aus d. Kaiserl. Gesundheitsamte," 1901, XVIII.
** "Arch. des Sci. Biol.," Petersb., 1904. St. Tome X, No. 4.

tion. The spleen and liver are enlarged, the former often presenting an appearance similar to that observed in miliary tuberculosis. Sometimes there is universal enlargement of the lymphatic glands. Bacilli are found in the blood and in all the internal organs. Skin eruptions may occur during life, and upon the inner abdominal walls petechiæ and occasional hemorrhages may be found. The intestine is hyperemic, the adrenals congested. Serosanguinolent effusions may occur into the serous cavities.

In the latter, they sometimes have encapsulated caseous nodules in the submaxillary glands, caseous bronchial glands, and fibroid pneumonia, months after infection. In all such cases virulent plague bacilli are present.

In and about San Francisco the extermination of rats for the eradication of the plague was unexpectedly complicated by the discovery that other rodents with which the rats came into contact also harbored the plague bacilli. McCoy and Smith* found this to be true of the prairie dog, the desert wood rat, the rock squirrel, and the brush rat. To insure security against the recurrence of the disease among men necessitates continued observation of these animals and the extermination of diseased colonies, as well as their complete extermination in the neighborhood of human habitations.

Devell† has found frogs susceptible to the disease.

Mode of Infection.—The plague bacillus may enter the body by *inhalation*, from an atmosphere through which it is disseminated, under which circumstances it usually causes the pneumonic type of the disease which is not unlike other forms of pneumonia. The lung is consolidated, enormous numbers of plague bacilli occur in the sputum, the fever is high, and death occurs in a few days.

Plague pneumonia does not necessarily imply infection through inhalation of the bacilli, however, for it occasionally occurs as a complication in the bubonic form of the disease.

Klein found that animals fed upon cultures of the bacillus or upon the flesh of animals dead of the disease became ill and died with typical symptoms. Simond has confirmed his results and it is not improbable that the disease is sometimes acquired by rats through feeding upon their companions that have died of it. The micro-organisms seem able to penetrate any of the mucous membranes, so that infection usually follows their application to the uninjured conjunctiva, nasal, buccal, vaginal or gastro-intestinal surfaces.

Cutaneous and Subcutaneous Inoculation.—All susceptible animals quickly become infected if a needle infected with a culture of the bacilli or with material from a bubo or other infective lesion be used to puncture or scratch the skin. Wyssokowitsch and Zabolotny† found monkeys highly susceptible to plague, especially

* "Journal of Infectious Diseases," 1910, VII, p. 374.
† "Centralbl. f. Bakt. u. Parasitenk.," Oct. 12, 1897.

when subcutaneously inoculated. When an inoculation was made with a pin dipped in a culture of the bacillus, the puncture being made in the palm of the hand or sole of the foot, the monkeys always died in from three to seven days. In these cases the local edema observed by Yersin did not occur. They point out the interest attaching to infection through so insignificant a wound and without local lesions. Weichselbaum, Albrecht and Gohn have found that rats may be infected by rubbing the infective material upon the surface of the shaved skin, the method being employed for making a diagnosis of the disease in suspected cases. Rats and mice in-

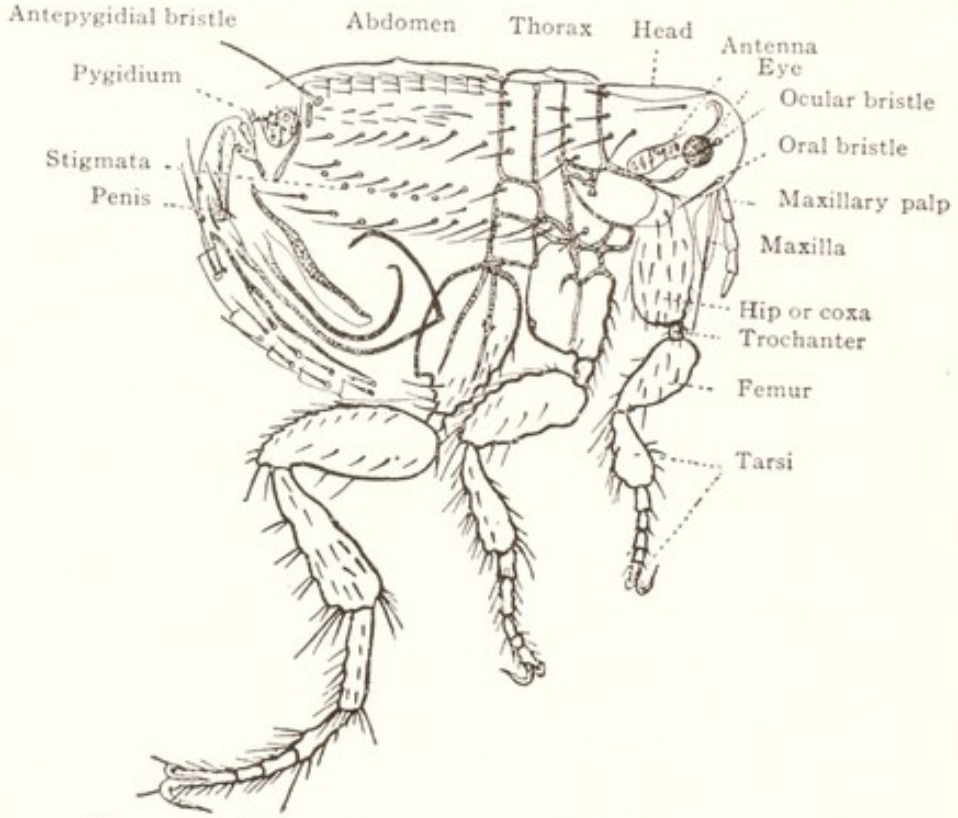


Fig. 230.—Xenopsylla cheopis (male) (from Rothschild).

fected through the skin usually die in two or three days, guinea-pigs in two to five days, rabbits in three to eight days.

The facility with which dermal infection could be brought about, quickly suggested that the skin might be the common route, and that biting insects might act as vectors.

Yersin showed that flies taking up the bacilli may die of the infection. Macerating and crushing a fly in bouillon, he not only succeeded in obtaining the bacillus, but infected an animal with it.

Nuttall,* in repeating Yersin's fly experiment, found his observation correct, and showed that flies fed with the cadavers of plague-infected mice die in a variable length of time. Large numbers of plague bacilli were found in their intestines. He also found that bed-bugs allowed to prey upon infected animals took up large

* "Centralbl. f. Bakt. u. Parasitenk.," XXI, No. 24, Aug. 13, 1897.

numbers of the plague bacilli and retained them for a number of days. These bugs did not, however, infect healthy animals when allowed to bite them; but Nuttall was not satisfied that the number of his experiments upon this point was great enough to prove that plague cannot be thus spread. Vergbitski,* however, was more successful and a bed-bug that he caused to bite a patient suffering from plague, subsequently transmitted the disease to a rat. It is quite possible that mosquitoes and biting flies may transmit it. As epidemics of human plague are commonly preceded by epidemics among the rats which die in great numbers, it early became a question whether the plague among them was not caused by the bites of fleas, and whether it might not also be fleas that infected man.

M. Herzog† has shown that pediculi may harbor plague bacilli and act as carriers of the disease.

Ogata found plague bacilli in fleas taken from diseased rats. He crushed some fleas between sterile object-glasses and introduced the juice into the subcutaneous tissues of a mouse, which died in three days with typical plague, a control-animal remaining well. Some guinea-pigs taken for experimental purposes into a plague district died spontaneously of the disease, presumably because of flea infection.

Galli-Valerio‡ and others think that the fleas of the mouse and rat are incapable of living upon man and do not bite him, and that it is only the *Pulex irritans*, or human flea, that can transmit the disease from man to man. Tidswell,§ however, found that of 100 fleas collected from rats—there were four species, of which three—the most common kinds—bit men as well as rats. Lisbon‖ found that of 246 fleas caught on men in the absence of plague, only one was a rat flea, but out of 30 fleas caught upon men in a lodging-house, during plague, 14 were rat fleas. This seems to show that as the rats die off their fleas seek new hosts, and may thus contribute to the spread of the disease.

That fleas can cause the transmission of plague from animal to animal has been proved by experiments made in India. These experiments, which are published as "Reports on Plague Investigations in India," issued by the Advisory Committee appointed by the Secretary of State for India, the Royal Society, and the Lister Institute, appear in the "Journal of Hygiene" from 1906 onward.** It seems from these experiments that human fleas (Pulex irritans) do not bite rats, but that the rat fleas of all kinds do, though not

* "Jour. of Hygiene," 1904, VIII, 185.
† "Amer. Jour. Med. Sci.," March, 1895.
‡ Ibid., XXVII, No. 1, p. 1, Jan. 6, 1900.
§ "British Medical Journal," June 27, 1903.
‖ "Times of India," Nov. 26, 1904.
** "Journal of Hygiene," Sept., 1906, vol. VI, p. 421; July, 1907, vol. VII, p. 324; Dec., 1907, vol. VII, p. 693; May, 1908, vol. VIII, p. 162; 1909, vol. IX; 1910, vol. X; 1911, vol. XI.

willingly, bite men. By placing guinea-pigs in cages upon the floor of the infected houses, the fleas of all kinds quickly attack them with resulting infection, but if the guinea-pigs are kept in flea-proof cages, or if the cages are surrounded by "tangle-foot," or "sticky fly-paper," the fleas, not being able to spring over the barrier, are caught on the sticky surfaces and do not reach the guinea-pigs, which then remain uninfected. What is true of the guinea-pigs is undoubtedly true of the rats; the disease is transmitted from rat to rat by the fleas. When the rats die, the fleas being hungry, jump upon any convenient warm-blooded animal to satisfy their appetites, and when human beings become their victims, infection may follow the bites. It is now clearly demonstrated that though Pulex irritans, the human flea, prefers to bite human beings, and Xenopsylla cheopis, the rat flea, prefers to bite rats, under stress of necessity preferences are set aside and miscellaneous feeding practised by these and probably all other fleas.

A peculiar circumstance attending flea infection has been discovered by Bacot and Martin* who find that when Xenopsylla cheopis and Ceratophyllus fasciatus are fed upon septicemic plague blood, the respective fleas suffer from a temporary obstruction at the entrance of the stomach, caused by a massive growth of the plague bacilli. This culture appears to start in the intercellular recesses of the proventriculus and grows so abundantly as to choke this organ and extend into the esophagus. Fleas in this condition are not prevented from sucking blood, as the pump is in the pharynx, but they only succeed in distending an already contaminated esophagus, and on the cessation of the pumping act, some of the blood is forced back into the wound. Such fleas are persistent in their endeavors to feed and this renders them particularly dangerous.

Bacot† found that infected fleas remained infectious when starved for forty-seven days, and that when they were subsequently permitted to feed upon mice, another period of twenty days might supervene before the mice became infected.

The cutaneous and subcutaneous inoculation in man is followed by lymphatic invasion with bubo formation. Beyond this lymphatic barrier but few bacilli get so that in the greater number of cases with buboes there is little blood infection. However, should the bacilli be highly virulent or the patient exceptionally susceptible, the septicemic form of the disease may supervene, and the case progress to a rapidly fatal termination.

Intravenous and Intraperitoneal Inoculations produce rapidly fatal septicemic forms of plague.

Klein‡ found that intraperitoneal injection of the bacillus into guinea-pigs was of diagnostic value, producing a thick, cloudy,

* "The Journal of Hygiene," Plague Supplement, III, 1914, p. 423.
† "Journal of Hygiene," Plague Supplement, No. IV, Jan., 1915, p. 770.
‡ "Centralbl. f. Bakt. u. Parasitenk.," XXI, No. 24, July 10, 1897, p. 849.

peritoneal exudate rich in leukocytes and containing characteristic chains of the plague bacillus, occurring in from twenty-four to forty-eight hours.

Diagnosis.—It seems possible to make a diagnosis of the disease in doubtful cases by examining the blood, but it is admitted that a good deal of bacteriologic practice is necessary for the purpose.

Abel found that blood-examinations may yield doubtful results because of the variable appearance of the contained bacilli, which may easily be mistaken for other bacteria. He deems the best tests to be the inoculation of broth cultures and the subsequent inoculation into animals, which, he advises, should have been previously vaccinated against the streptococcus.

Kolle* has suggested a method valuable both for the diagnosis of the disease and for estimating the virulence of the bacillus. It is as follows: "The skin over a portion of the abdominal wall of the guinea-pig is shaved, care being taken to avoid the slightest injury of the skin. The infective material is carefully rubbed into the shaved skin. Important, in order rightly to understand the occurrence of plague infection, is the fact disclosed here in the case of guinea-pigs, that by this method of inoculation the animals present the picture of true bubonic plague—that is to say, the production of nodules in the various organs, principally in the spleen. In this manner guinea-pigs, which would not be affected by large subcutaneous injections, even amounting to 2 mg. of agar culture (equal to a loop) of low-virulence plague bacillus, may be infected and eventually succumb."

The *postmortem* appearance of the body of a plague-infected rat is as follows:† Subcutaneous hemorrhages occur in about 40 per cent. of the animals and are most frequently to be seen in the submaxillary region. Buboes are present in the majority of cases, usually in some one locality, and commonly about the neck. The liver may show necrotic changes which have the appearance of an excessive deposit of fat, and a condition of the greatest importance in diagnosis is the occurrence of small necrotic foci scattered over its surface and throughout its substance. The spleen is firm and does not collapse like a soft normal spleen; granules or nodules may be well marked in it and may be confluent. The kidneys and suprarenal capsules are often congested. Hemorrhages are fairly common in the lungs and visceral pleura. The presence of pleural effusion is very characteristic and of great value in diagnosis. In naturally infected plague rats, the most important features for purposes of diagnosis are:

1. A typical bubo—most commonly in the neck.
2. Granular liver—not seen except in plague rats.

* "See Havelburg, "Public Health Reports," Aug. 15, 1902, vol. XVII, No. 33, p. 1863.

† See "Journal of Hygiene," 1907, VII, 324.

3. Hemorrhages beneath the skin and in the internal organs are very suggestive.

4. Pleural effusion.

In putrid rats, bubo, granular liver and pleural effusion may persist and are of great significance. A microscopical examination of scrapings from buboes and spleen and inoculation tests will clinch the diagnosis (Besson).

Virulence.—By frequent passage through animals of the same species the bacillus can be much increased in virulence. Kolle recommended rats for this purpose, and, indeed, declared that without the use of rats it is impossible to keep cultures at a high grade of virulence. According to the researches of the Advisory Committee for the study of plague in India, this is an error. The virulence of plague bacilli for rats is subject to very little change. Their members in investigating the question made twenty-six passages from rat to rat, by subcutaneous inoculation, during eighty-nine days, and found the original virulence of the organism unchanged.

Yersin found that when cultivated for any length of time upon culture-media, especially agar-agar, the virulence was rapidly lost and the bacillus eventually died. On the other hand, when constantly inoculated from animal to animal, the virulence of the bacillus is much increased.

Knorr, Yersin, Calmette, and Borrel* have shown that the bacillus made virulent by frequent passage through mice is not increased in virulence for rabbits.

This no doubt depends upon the sensitivity of the bacillus to the protective substances of the body juices, immunization against those of one animal not necessarily protecting the organism against those of other animals.

Sanitation.—A disease that may be transmitted from man to man by atmospheric infection and inhalation, that can be transported from place to place by fomites, that occurs in epidemic form among the lower animals as well as among men, and that can be transmitted from man to man and from lower animals to man by biting insects, must inevitably become a source of anxiety to the sanitarian.

The preventive measures must take account of men, rats, and goods. If vessels are permitted to visit and leave plague-stricken ports, means must be taken to see that all passengers are healthy at the time of leaving and have remained so during the voyage, and provision should be made at the port of entry for the disinfection of the cargo before the goods are landed. But the rats must be given special consideration, for, so soon as the vessel reaches port some of them jump overboard and swim to the shore, carrying the disease with them. When a vessel visits a plague port, every precaution should be taken to prevent the entrance of rats, first by

* "Ann. de l'Inst. Pasteur.," July, 1895.

anchoring in the stream instead of tying to the dock; by carefully scrutinizing the packages taken from the lighters to see that there are no rats hidden among them; by placing large metal shields or reversed funnels about all anchor chains, hawsers, and cables so that no rats can climb up from the water in which they are swimming at night. Arrangements should also be made for rat destruction on board the ship by means of sulphurous oxid or other poisonous vapors to rid the ship of rats before the next port is reached. Passengers and crew should also be kept in quarantine before mingling with society. It is much more easy to keep plague out of a port than to combat it when it has entered, for under the latter condition are involved the isolation of the patients in rat-free and vermin-free quarters, the disinfection of the premises and goods where the case arose, and an immediate warfare upon the rats and other small animals of the neighborhood. To emphasize how difficult the latter may be it is only necessary to point out that plague reached San Francisco in May, 1907, during which year there were 156 cases and 76 deaths. Every precaution was taken to prevent its spread, and though the extermination of rats was practised at great expense and with the utmost thoroughness, the disease spread to the ground squirrels and other small rodents, and in 1914 plague-infected rodents were still to be found in the outskirts of the city.

Immunity.—An attack of plague usually exempts from future attacks. Artificial immunity may therefore be induced in both man and the lower animals by a variety of methods.

I. *Active Immunity.*—Haffkine* followed his plan of preventive inoculation as employed against cholera, and has invented a method of prophylaxis based upon the use of devitalized cultures. Bouillon cultures are grown in flasks for six weeks; small floating drops of butter being employed to make the "islands" of plague bacilli float. Successive crops of the island-stalacite growth are precipitated by agitating the flasks. In this manner an "intense extracellular toxin," containing large numbers of the bacilli is prepared. After testing the purity of the culture by transplantation to agar-agar, it was killed by exposure to 65°C. for one hour and received an addition of 0.5 per cent. of phenol. The preparation was used in doses of 2 to 3 cc. as a preventive inoculation. A more thorough and prolonged immunity resulted from the administration of a second dose ten days after the first.

An interesting collection of statistics, showing in a convincing manner the value of the Haffkine prophylactic, is published by Leumann, of Hubli. The figures, together with a great deal of interesting information upon the subject, can be found in the paper upon "A Visit to the Plague Districts in India," by Barker and Flint.†

* "Brit. Med. Jour.," June 12, 1897; "India Medical Gazette," 1897.
† "New York Med. Jour.," Feb. 3, 1900.

The German Plague Commission* believed that an important improvement in the vaccine could be brought about by the use of the method now generally employed in making bacterio-vaccines (*q.v.*). They therefore caused the bacilli to grow in Roux bottles upon the surface of agar-agar for forty-eight hours, washed off the bacteria with bouillon or physiological salt solution so that 1 cc. of the suspension contained about 2.5 mg. of bacilli, and then heated the suspension for an hour or so at 65°C. After heating, 0.5 per cent. of phenol was added. This mode of preparation has the advantage of excluding the possibility of the accidental growth of tetanus bacilli and other micro-organisms in the culture. The vaccine appeared to give excellent results in Brazil where it was extensively used. Haffkine, however, considers his method preferable because of the greater quantity of immunizing metabolic products of the bacilli contained in the fluid cultures on account of their prolonged growth.

The immunity conferred by the Haffkine prophylactic is supposed to last about a year. The preparation must never be used if the person has already been exposed to infection, and is in the incubation stage of the disease, as it contains the toxins of the disease, and therefore greatly intensifies the existing condition. When injected into healthy persons it always produces some fever, slight local swellings, and malaise.

Kolle and Otto† from experimental studies of plague immunity in rats, came to the conclusion that a prophylactic injection consisting of a culture of attenuated plague bacilli would have a much more powerful and lasting effect than one consisting of a killed bacilli. The same conclusion was reached by Kolle and Strong‡ and the first use of living cultures for preventive inoculation in human beings was by Strong§ who found them to be devoid of danger, and is hopeful regarding their efficacy.

Besredka‖ advises the use of a killed culture sensitized by the application of immune serum. Such vaccine seems to be productive of long enduring immunity when tried upon experimental animals.

Rowland** is under the impression that the essential immunizing antigen is in the bacterial nucleoproteins. These he extracts from the bacterial cells by treating them while moist with anhydrous sodium sulphate, freezing, permitting the water to be absorbed by the chemical, thawing, and then filtering off the fluid at 37°C. The filtrate thus obtained is highly toxic, fatal to rats in minute doses and capable of effecting immunization.

II. *Passive Immunity* against plague, through the employment

* "Arbeiten aus dem Kaiserl. Gesundheitsamte," 1899, XVI.
† "Deutsche med. Wochenschrift," 1903, p. 493; "Zeitschrift für Hygiene," 1903, XLV, 507.
‡ "Deutsche med. Wochenschrift," 1906, XXXII, 413.
§ "Jour. Medical Research," N. S., 1908, XVIII, 325.
‖ "Bull. de l'Inst. Pasteur," 1910, VIII, 241.
** "Jour. of Hygiene," 1912, XII, 344.

of the serums of experimentally immunized animals for hypodermatic injection into man was tried soon after the discovery of the plague bacillus. Kitasato's experiments first showed that it was possible to bring about immunity against the disease, and Yersin, working in India, and Fitzpatrick, in New York, have successfully immunized large animals (horses, sheep, and goats). The serum of the immunized animals contains specific agglutinins and bacteriolysins as well as an antitoxin, capable not only of preventing the disease, but also of curing it in mice and guinea-pigs and probably in man.

Study of plague serums has been conducted by Yersin, Calmette and Borrel,* but their value as a prophylactic lacks demonstration.

Wyssokowitsch and Zabolotny,† used 96 monkeys in the study of the value of the "plague serums," and found that when treatment was begun within two days from the time of inoculation the animals could be saved, even though symptoms of the disease were marked. After the second day the treatment could be relied upon. The dose necessary was 20 cc. of a serum having a potency of 1 : 10. If too little serum was given, the course of the disease was retarded and the animal improved for a time, then suffered a relapse, and died in from thirteen to seventeen days. The serum also produced immunity, but of only ten to fourteen days' duration. Immunity lasting three weeks was conferred by inoculating a monkey with an agar-agar culture heated to 60°C. If too large a dose of such a culture was given, however, the animal was enfeebled and remained susceptible.

THE PLAGUE FLEAS

Fleas were formerly classed as a suborder of the Diptera, or two-winged insects, and because they had no wings, were known as Aphaniptera. At the present time they constitute an order by themselves, the Siphonaptera.

Every flea undergoes a complete metamorphosis. It begins its life history as a minute, oval, pearly-white egg measuring about 0.6 mm. in length, that falls from the body of the female to the floor or ground. The eggs of fleas are not cemented to the hairs like those of lice, but drop to the ground where the larva lives. More or less eggs are therefore always scattered about where dogs, cats, rats, mice or other animals that harbor fleas are to be found, and more or less larvæ and pupæ are likewise to be found in such places. In the course of from five to ten days, a minute, active caterpillar-like larva emerges from the egg to feed upon such organic matter as it may find for the six to eight weeks of this stage. During the larval period the skin is shed three or four times. When full grown, the larva empties its alimentary canal, spins itself a tiny silken cocoon, sometimes including minute bits of rubbish or grains of sand in its structure, sheds its skin for the last time, and becomes a pupa. As such it is inactive for from two to eight weeks, according to external conditions of temperature and moisture, then opens the cocoon and emerges from the pupa shell, a perfect insect—the flea proper.

The adult fleas, both males and females, have soft exoskeletons at first, but soon they harden, through the formation of chitin, to the well-known tough and brittle armor.

The male differs from the female in being smaller and in its shorter abdomen.

* "Ann. de l'Inst. Pasteur," 1895, IX, 589.
† Loc. cit.

Both insects hop about in search of the appropriate warm-blooded hosts upon whose blood they are to live. Each kind of flea has a preferred host, but the tastes of all are more or less cosmopolitan, so that in the absence of the preferred host, another kind of warm-blooded creature will do. Adult fleas live solely by sucking blood.

The longevity of a flea varies according to conditions of temperature and moisture Life is longest when the temperature is high and the ground not too dry. They may live for months without feeding; when regularly fed they can live at least a year and a half. The longevity of the fleas in the adult stage, the long periods of abstention from food that they may suffer without dying, and the accessions to their numbers that may occur through the perfection of their embryonal fellows in the same place, explain why families returning to their closed city houses, or going to their closed country houses, sometimes find them after months of desertion, occupied by a welcoming host of fleas. They are the progeny of

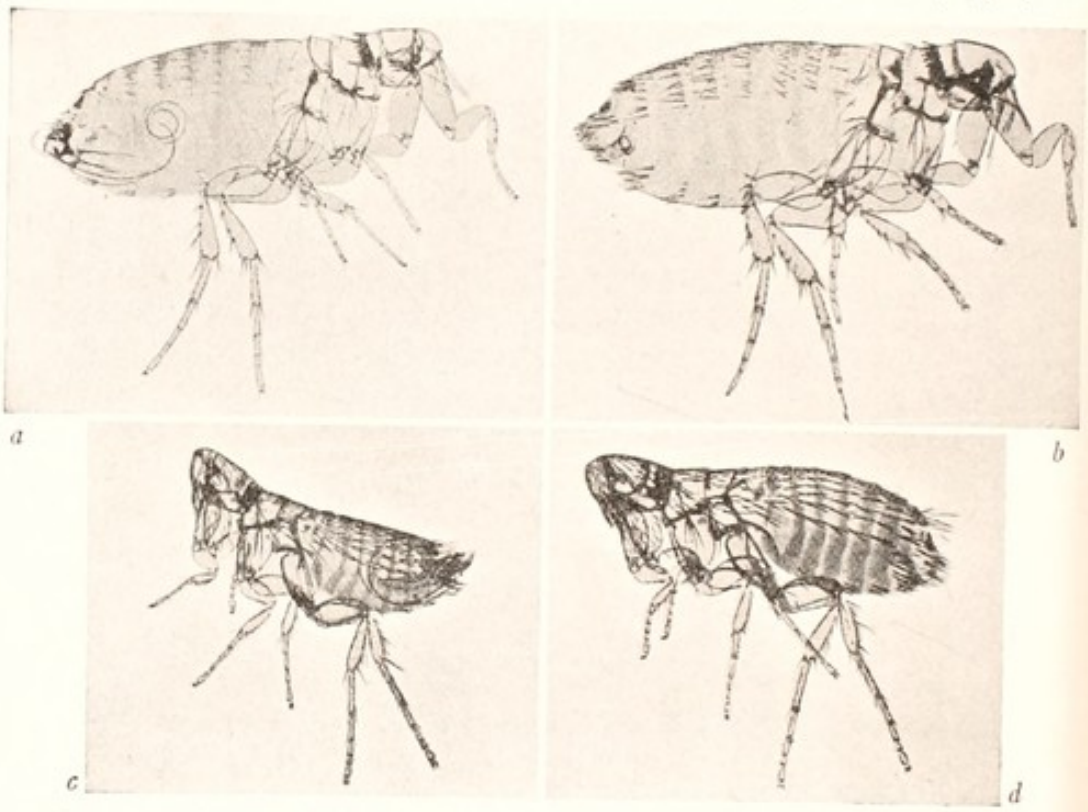


Fig. 231.—Various fleas, magnified about 30 diameters. The specimens are treated with hot 20 per cent. caustic potash for a few minutes, dehydrated in alcohol, cleared in xylol and mounted in balsam. *a*, Ceratophyllus fasciatus ♂; *b*, Ceratophyllus fasciatus, ♀; *c*, Leptopsylla musculi, ♂; *d*, Leptopsylla musculi, ♀ (Bacot, in Journal of Hygiene, "Plague Supplement III, 1914").

the fleas of the former dog, cat, rat or mouse tenants, that have matured or survived the interval and are now hungry because the removal of the family months before, was probably followed by the withdrawal of the rats and mice no longer able to find food in the deserted habitation.

To get rid of such fleas is often a perplexing question. A way to accomplish it is to place a cage containing a cat or a guinea-pig, or a trap containing living rats or mice on the floor of a room and surround it by sticky fly-paper. Fleas when empty and hungry, were found by Strickland* to be able to jump 4 inches; those recently fed only 3 inches. In their endeavors to reach the caged animals the fleas jump upon the fly-paper and are caught. This can be done in several rooms of the house and soon cleans up the fleas.

During such periods of fasting the sexes do not copulate and no ova are produced. As soon as blood is taken, copulation takes place, and if the blood be

* "Journal of Hygiene," 1914, XIV, p. 129.

that of the preferred host, ovulation follows in about twenty-four hours. The eggs are relatively large, and small numbers are produced.

In the case of Sarcopsylla penetrans, a flea that has no known interest in connection with plague transmission, the female after copulation imbeds itself in the skin of the host and suffers an enormous saccular distension of the abdomen where many ova are produced. Ordinary fleas never imbed themselves but simply bite and suck blood, leaping off of the host when satisfied.

Epidemics of plague among men are commonly preceded by epizootics of plague among rats. The mortality of the rats being high and their number diminishing, many fleas are unprovided for and seek human hosts upon whom to satisfy their appetites. In this way, the plague which was at first transmitted by the fleas to the rats, is now transmitted to men. Human fleas may also trans-

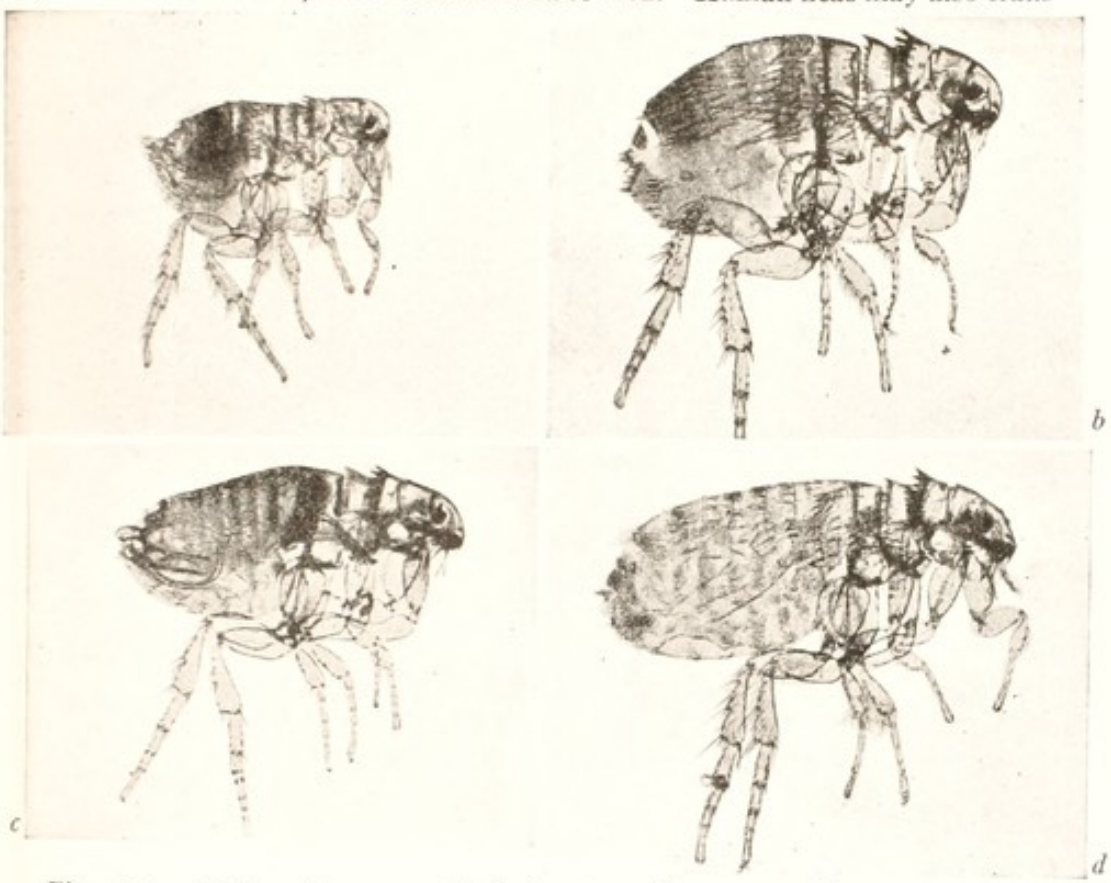


Fig. 232.—Various fleas, magnified about 30 diameters. The specimens are treated with hot 20 per cent. caustic potash for a few minutes, dehydrated in alcohol, cleared in xylol and mounted in balsam. *a*, Ctenocephalus canis, ♂; *b* Ctenocephalus canis, ♀; *c*, Ctenocephalus felis, ♂; *d*, Ctenocephalus felis, ♀ (Bacot, in Journal of Hygiene, "Plague Supplement III, 1914").

mit the infection from man to man, but the bulk of the transmission probably takes place through rat fleas.

When the plague spreads from the rat to ground squirrels or to marmots, rare fleas may engage in the transmission of the disease from animal to animal and from man to man, but ordinarily it is the common rat fleas that are responsible for it.

Both rats and fleas vary in prevalence and in relative frequency in different parts of the world. Thus there are three common rats: Mus decumanus, the brown or sewer rat, Mus rattus, the black or house rat and Mus norvegius, the Norway rat. In Northern Europe, the Mediterranean coast, Egypt and North America, the Norway rat has colonized more or less successfully. Where it preponderates Ceratophyllus fasciatus is a common flea. Where Mus decumanus and Mus rattus alone are found, or are preponderant, Xenopsylla cheopis is the common flea. In the Orient, Xenopsylla cheopis is the chief flea that is to be taken into account in plague transmission. The dog flea Ctenocephalus canis

is common everywhere as is Pulex irritans, the human flea. It is likely that any or all of these engage in plague transmission when once an epidemic has started, but the most active vector of the disease, the world over, and the most important agent in starting human epidemics of plague is Xenopsylla cheopis.

Much interesting and valuable information concerning the biology, bionomics

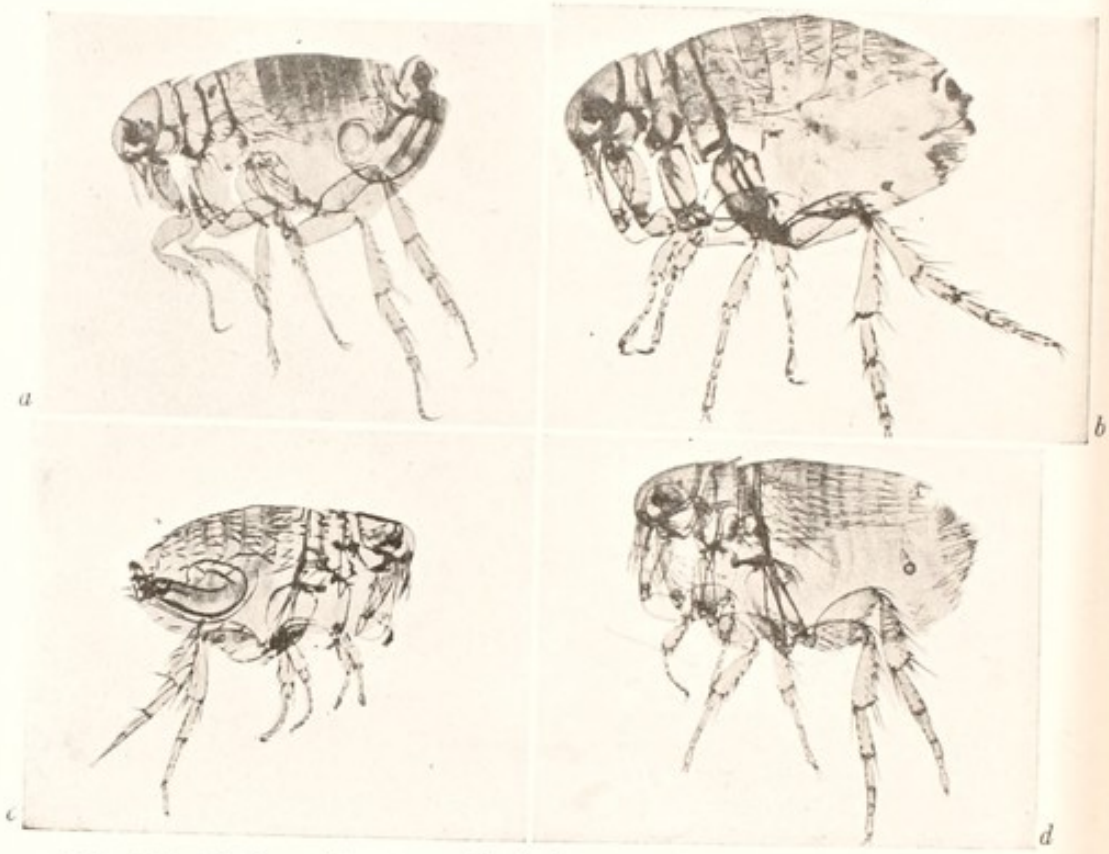


Fig. 233.—Various fleas, magnified about 30 diameters. The specimens are treated with hot 20 per cent. caustic potash for a few minutes, dehydrated in alcohol, cleared in xylol and mounted in balsam. *a*, Pulex irritans, ♂; *b*, Pulex irritans, ♀; *c*, Xenopsylla cheopis, ♂; *d*, Xenopsylla cheopis, ♀ (Bacot, in Journal of Hygiene, "Plague Supplement III, 1914").

and relation of rats and fleas to plague, will be found in the "Reports of the India Plague Commission" many of which are to be found in the "Journal of Hygiene," vols. I–XIV.

The following illustrations and tabulations will enable the student to identify the common genera of fleas. For more intimate systematic study he must be referred to "A Text-book of Medical Entomology," by Patton and Cragg.*

* "Christian Literature Society of India, London, Madras and Calcutta," 1913.

Table for the Identification of the Fleas Concerned in Plague Transmission

Family—PULICIDÆ.
Subfamily—*PULICINÆ*.
All have eyes.

A. Have no combs or spines on head, thorax or abdomen.

a. The meso-sternite is narrow and has no rod-like incrassation from the insertion of the coxa upward..... *Pulex*.

b. The meso-sternite has a rod-like incrassation from the insertion of the coxa upward..... *Xenopsylla*.

B. With combs.

c. Combs on the prothorax only..... *Ceratophyllus*.

d. Combs on the prothorax and on the gena or lower margin of the face..... *Ctenocephalus*.

OTHER MICRO-ORGANISMS OF THE PLAGUE GROUP

The Bacillus pestis is a member of a group of organisms collectively known as the bacilli of hemorrhagic septicemia. Two of these organisms are of sufficient interest to deserve special mention.

Bacillus Choleræ Gallinarum (Perroncito); Bacillus Choleræ; Bacillus Avicidum; Bacillus Avisepticus; Bacillus of Rabbit Septicemia; Bacillus Cuniculicida

General Characteristics.—A non-motile, non-flagellated, non-sporogenous, non-liquefying, non-chromogenic, aërobic bacillus, pathogenic for birds and mammals, staining by the ordinary methods, but not by Gram's nethod, producing acids, indol, and phenol, and coagulating milk.

The barnyards of both Europe and America are occasionally visited by an epidemic disease known as "chicken-cholera," *Hühnercholera*, or *cholera de poule*, which rapidly destroys pigeons, turkeys, chickens, ducks, and geese. Rabbit-warrens are also at times affected and the rabbits killed.

The bacillus responsible for this disease was first observed by Perroncito* in 1878, and afterward thoroughly studied by Toussaint and Pasteur.†

Morphology.—The organisms are short and broad, with rounded ends, measuring 1×0.4 to $0.6\ \mu$, sometimes joined to produce chains. Pasteur at first regarded them as diplococci, because the poles stain intensely, a narrow space between them remaining almost uncolored. This peculiarity is very marked, and careful examination is required to detect the intermediate substance. The bacillus does not form spores, is not motile, and has no flagella.‡

Staining.—The organism stains with ordinary anilin dye solutions, but not by Gram's method.

Cultivation.—Colonies.—Colonies upon gelatin plates appear after about two days as small, irregular, white points. The deep colonies reach the surface slowly, and do not attain to any considerable size. The gelatin is not liquefied. The colonies appear under the microscope as irregularly rounded yellowish-brown disks with distinct smooth borders and granular contents. Sometimes there is a distinct concentric arrangement.

Gelatin.—In gelatin puncture cultures a delicate white line occurs along the entire path of the wire. Upon the surface the development is much more marked, so that the growth resembles a nail with a good-sized flat head. If the bacilli be planted upon the surface of obliquely solidified gelatin, a much more pronounced growth takes place, and along the line of inoculation a dry, granular coating is formed. There is no liquefaction of the medium.

Bouillon.—The growth in bouillon is accompanied by a slight cloudiness.

Agar.—This growth, like that upon agar-agar and blood-serum, is white, shining, rather luxuriant, and devoid of characteristics.

Potato.—Upon potato no growth occurs except at 37°C. It is a very insignificant, yellowish-gray, translucent film.

Milk is acidulated and slowly coagulated.

Vital Resistance.—The bacillus readily succumbs to the action of heat and dryness. The organism is an obligatory aërobe.

Metabolic Products.—Indol and phenol are formed. Acids are produced in sugar-containing media, without gas formation.

Pathogenesis.—The introduction of cultures of this bacillus into chickens, geese, pigeons, sparrows, mice, and rabbits is sufficient to produce fatal septicemia. Feeding chickens, pigeons, and rabbits with material infected with the bacillus is also sufficient to produce the disease. Guinea-pigs, cats, and dogs seem immune, though they may succumb to large doses if given intraperitoneally. The organism is probably harmless to man.

Fowls ill with the disease fall into a condition of weakness and apathy, which causes them to remain quiet, seemingly almost paralyzed, and the feathers ruffled up. The eyes are closed shortly after the illness begins, and the birds gradually fall into a stupor, from which they do not awaken. The disease is fatal in from twenty-four to forty-eight hours. During its course there is profuse diarrhea, with very frequent fluid, slimy, grayish-white discharges.

* "Archiv. f. wissenschaftliche und praktische Thierheilkunde," 1879.

† "Compte-rendu de l'Acad. de Sci. de Paris," vol. xc.

‡ Thoinot and Masselin assert that the organism is motile. "Précis de Microbie," 2d ed., 1893.

Lesions.—The autopsy shows that when the bacilli are introduced subcutaneously a true septicemia results, with the formation of a hemorrhagic exudate and gelatinous infiltration at the seat of inoculation. The liver and spleen are enlarged; circumscribed, hemorrhagic, and infiltrated areas occur in the lungs; the intestines show an intense inflammation with red and swollen mucosa, and occasional ulcers following small hemorrhages. Pericarditis is frequent. The bacilli are found in all the organs. If, on the other hand, the disease has been produced by feeding, the bacilli are chiefly to be found in the intestine. Pasteur found that when pigeons were inoculated into the pectoral muscles, if death did not come on rapidly, portions of the muscle (*sequestra*) underwent degeneration and appeared anemic, indurated, and of a yellowish color.

Immunity.—Pasteur* discovered that when cultures are allowed to remain undisturbed for several months, their virulence becomes greatly lessened, and new cultures transplanted from them are also attenuated. If chickens be inoculated with such attenuated cultures, no other change occurs than a local inflam-

Fig. 234.—Bacillus of chicken-cholera, from the heart's blood of a pigeon. × 1000 (Fränkel and Pfeiffer).

matory reaction that soon disappears and leaves the birds protected against future infection with virulent bacilli. From these observations Pasteur worked out a system of protective vaccination in which the fowls are first inoculated with attenuated, then with more active, and finally with virulent, cultures, with resulting protection and immunity.

Use has been made of this bacillus to kill rabbits in Australia, where they are pests. It is estimated that two gallons of bouillon culture will destroy 20,000 rabbits, irrespective of infection by contagion.

The bacillus of chicken-cholera may be identical with organisms found in various epidemic diseases of larger animals, and, indeed, no little confusion has arisen from the description of what is now pretty generally accepted to be the same organism as the bacillus of rabbit septicemia (Koch), Bacillus cuniculicida

* An interesting account of Pasteur's experiments upon chicken-cholera can be found in the "Life of Pasteur," by Vallery-Radot, translated by Mrs. R. S. Devonshire, 1909. Popular Edition, New York, Doubleday, Page and Co.

(Flügge), bacillus of "Wildseuche" (Hüppe), bacillus of "Büffelseuche" (Oriste-Armanni), etc.

Bacillus Suisepticus (Löffler and Schütz)

General Characteristics.—A non-motile, non-flagellated, non-sporogenous, non-liquefying, non-chromogenic, aërobic and optionally anaërobic bacillus, pathogenic for hogs and many other animals, staining by the ordinary methods, but not by Gram's method. It produces a slight acidity in milk, but does not coagulate it.

The bacillus of swine-plague, or Bacillus suisepticus of Löffler and Schütz* and Salmon and Smith,† but slightly resembles the bacillus of hog-cholera (*q.v.*), though it was formerly confounded with it and at one time thought to be identical with it. The species have sufficient well-marked characteristics, however, to make their differentiation easy.

Swine-plague is a rather common and exceedingly fatal epidemic disease. It not infrequently occurs in association with hog-cholera, and because of the lack of sufficiently well-characterized symptoms—sick hogs appearing more or less alike—is often mistaken for it. The confusion resulting from such faulty diagnosis makes it difficult to determine exactly how fatal either may be in uncomplicated cases.

Morphology.—The bacillus of swine-plague much resembles that of chicken-cholera. It is a short organism, rather more slender than the related species, not possessed of flagella, incapable of movement, and producing no spores.

It is an optional anaërobe.

Staining.—The bacillus stains by the ordinary methods, sometimes only at the poles, then closely resembling the bacillus of chicken-cholera. It is not colored by Gram's method.

Cultivation.—In general, the appearance in culture-media is very similar to that of the hog-cholera bacillus. Kruse,‡ however, points out that when the bacillus grows in bouillon the liquid remains clear, the bacteria gathering to form a flocculent, stringy sediment. The organism does not grow upon ordinary acid potato, but if the reaction of the medium be alkaline, a grayish-yellow patch is formed. In milk a slight acidity is produced, but the milk is not coagulated.

Vital Resistance.—The vitality of the organism is low, and it is easily destroyed. Salmon says that it soon dies in water or when dried, and that the temperature for its growth must be more constant and every condition of life more favorable than for the hog-cholera bacillus. The organism is said to be widely distributed in nature, and is probably present in every herd of swine, though not pathogenic except when its virulence becomes increased or the vital resistance of the animals diminished by some unusual condition.

Rabbits, mice, and small birds are very susceptible to the infection, usually dying of septicemia in twenty-four hours; guinea-pigs are less susceptible, except very young animals, which die without exception. Chickens are more immune, but usually succumb to large doses. Hogs die of septicemia after subcutaneous injection of the bacilli. There is a marked edema at the point of injection. If injected into the lung, a pleuropneumonia follows, with multiple necrotic areas in the lung. In these cases the spleen is not much swollen, there is slight gastro-intestinal catarrh, and the bacilli are present everywhere in the blood.

Animals can be infected only by subcutaneous, intravenous, and intraperitoneal inoculation, not by feeding.

As seen in hogs, the symptoms of swine-plague closely resemble those of hog-cholera, but differ in the occurrence of cough, swine-plague being prone to affect the lungs and oppress the breathing, which becomes frequent, labored, and painful, while hog-cholera is chiefly characterized by intestinal symptoms.

The course of the disease is usually rapid, and it may be fatal in a day or two.

Lesions.—At autopsy the lungs are found to be inflamed, and to contain numerous small, pale, necrotic areas, and sometimes large cheesy masses 1 or

* "Arbeiten aus dem kaiserlichen Gesundheitsamte," 1.

† "Zeitschrift f. Hygiene," x.

‡ Flügge's "Die Mikroörganismen, 1896," p. 419.

2 inches in diameter. Inflammations of the serous membranes affecting the pleura, pericardium, and peritoneum, and associated with fibrinous inflammatory deposits on the surfaces, are common. There may be congestion of the mucous membrane of the intestines, particularly of the large intestine, or the disease in this region may be an intense croupous inflammation with the formation of a fibrinous exudative deposit on the surface. A hemorrhagic form of the disease is said to be common in Europe, but, according to Salmon, is rare in the United States.

CHAPTER XXVI

ASIATIC CHOLERA

Spirillum Choleræ Asiaticæ (Koch*)

General Characteristics.—A motile, flagellated, non-sporogenous, liquefying, non-chromogenic, non-aërogenic, parasitic and saprophytic, pathogenic, aërobic and optionally anaërobic spirillum, staining by ordinary methods, but not by Gram's method.

Cholera is a disease endemic in certain parts of India and probably indigenous in that country. Though early mention of it was made in the letters of travelers, and though it appeared in medical literature and in governmental statistics more than a century ago, we find that little attention was paid to the disease, except in its disastrous effect upon the armies, native and European, of India and adjacent countries. The opening up of India by Great Britain in the last century has made scientific observation of the disease possible and has permitted us to determine the relation its epidemics bear to the manners and customs of the people.

The filthy habits of the Oriental people, their poverty, crowded condition, and peculiar religious customs, are all found to aid in the distribution of the disease. Thus, the city of Benares drains into the Ganges River by a most imperfect system, which distributes the greater part of the sewage immediately below the banks upon which the city is built and along which are the numerous "Ghats" or staircases by which the people reach the sacred waters. It is a matter of religious observance for every zealot who makes a pilgrimage to the "sacred city" to take a bath in and drink a quantity of this sacred but polluted water, and it may be imagined that the number of pious Hindoos who leave Benares with "comma bacilli" in their intestines or upon their clothes must be great, for there are few months in the year when the city is exempt from the disease.

The pilgrimages and great festivals of both Hindoos and Moslems, by bringing together enormous numbers of people to crowd in close quarters where filth and bad diet prevail, cause a rapid increase in the number of cases during these periods and facilitate the distribution of the disease when the festivals break up. Probably no more favorable conditions for the dissemination of a disease can be imagined than occurs with the return of the Moslem pilgrims from Mecca. The disease extends readily along the regular lines of travel, visiting town after town, until from Asia it has frequently extended into

* "Deutsche med. Wochenschrift," 1884–1885, Nos. 19, 20, 37, 38, and 39.

Europe, and by steamships plying foreign waters has several times been carried to our own continent. Many cases are on record which show conclusively how a single ship, having a few cholera cases on board, may be the starting-point of an outbreak of the disease in the port at which it arrives.

The most recent great epidemic of cholera began in 1883. From Asia it spread westward throughout Europe, extended by means of the steamship lines to numerous of the large ports, of which Hamburg in Germany suffered most acutely, and even extended to some of the ports of Africa and America. Russia probably suffered more than any other European country, and it is estimated that in that country there were no less than 800,000 deaths. During 1911 the disease again appeared in Europe and invaded the countries along the Mediterranean coasts.

Fig. 235.—Cholera spirilla.

Specific Organism.—The discovery of the spirillum of cholera was made by Koch while serving as a member of a German commission appointed to study the disease in Egypt and India in 1883–84. Since its discovery the spirillum has been subjected to much careful investigation, and an immense amount of literature, a large part of which was stimulated by the Hamburg epidemic of 1892, has accumulated.

Distribution.—The cholera spirilla can be found with great regularity in the intestinal evacuations of cholera cases, and can often be found in drinking-water and milk, and upon vegetables, etc., in cholera-infected districts. There can be little doubt that they find their way into the body with the food and drink. Cases in the literature show how cholera germs enter drinking-water and are thus distributed; how they are sometimes thoughtlessly sprinkled over green vegetables offered for sale in the streets, with infected

water from polluted gutters; how they enter milk with water used to dilute it; how they appear to be carried about in clothing and upon food-stuffs; how they can be brought to articles of food by flies that have preyed upon cholera excrement; and other interesting modes of infection. The literature is so vast that it is scarcely possible to mention even the most instructive examples. A bacteriologist became infected while experimenting with the cholera spirilla in Koch's laboratory. It is commonly supposed that the cholera organism may remain alive in water for an almost unlimited length of time, but experiments have not shown this to be the case. Thus, Wolffhügel and Riedel have shown that if the spirilla be planted in sterilized water they grow with great rapidity after a short time,

Fig. 236.—Spirillum of Asiatic cholera, from a bouillon culture three weeks old, showing long spirals. × 1000 (Fränkel and Pfeiffer).

and can be found alive after months have passed. Fränkel, however, points out that this ability to grow and remain vital for long periods in sterilized water does not guarantee the same power of growth in unsterilized water, for in the latter the simultaneous growth of other bacteria serves to extinguish the cholera spirilla in a few days.

Morphology.—The micro-organism described by Koch, and now generally accepted to be the cause of cholera, is a short rod 1 to 2 μ in length and 0.5 μ in breadth, with rounded ends, and a distinct curve, so that the original name by which it was known, the "comma bacillus," applies very well. One of the most common forms is that in which two short curved individuals are conjoined in an S-shape.

When the conditions of nutrition are good, multiplication by fission progresses with rapidity; but when adverse conditions arise, long

spiral threads—unmistakable spirilla—develop. Fränkel found that the exposure of the cultures to unusually high temperatures, the addition of small amounts of alcohol to the culture-media, and other unfavorable conditions lead to the production of spirals instead of "commas."

The cholera spirilla are actively motile, and in hanging-drop preparations can be seen to swim about with great rapidity. Both comma-shaped and spiral organisms move with a rapid rotary motion.

The presence of a single flagellum attached to one end can be demonstrated without difficulty.

Fig. 237.—Cover-glass preparation of a mucous floccule in Asiatic cholera. × 650 (Vierordt).

Involution-forms of bizarre appearance are common in old and sometimes in fresh cultures. Many individuals show by granular cytoplasm and irregular outline that they are degenerated. Cholera spirilla from various sources differ in the extent of involution.

In partially degenerated cultures containing long spirals, Hüppe observed, by examination in the "hanging-drop," certain large spheric bodies which he described as spores (arthrospores). Koch and, indeed, all other observers fail to find spores in the cholera organism, and the nature of the bodies described by Hüppe must be regarded as doubtful.

Staining.—The cholera spirillum stains well with the ordinary aqueous solutions of the anilin dyes, especially fuchsin. At times the staining must be continued for from five to ten minutes to secure homogeneity. The organism does not stain by Gram's method. It may be colored and examined while alive; thus, Cornil and

Babes, in demonstrating it in the rice-water discharges, "spread out one of the white mucous fragments upon a glass slide and allow it to dry partially; a small quantity of an exceedingly weak solution of methyl violet in distilled water is then applied to it, and it is flattened out by pressing down a cover-glass, over which is placed a fragment of filter-paper, which absorbs any excess of fluid at the margin of the cover-glass. The characteristics of comma bacilli so prepared and examined with an oil-immersion lens (× 700–800) are readily made out because, though they take up enough stain to color them, they still retain the power of vigorous movement, which would be entirely lost if the specimen were dried, stained, and mounted in the ordinary fashion."

Fig. 238.—Spirillum of Asiatic cholera; colonies two days old upon a gelatin plate. × 35 (Heim).

Isolation of the Organism.—One of the best methods of securing a pure culture of the cholera spirillum, and also of making a bacteriologic diagnosis of the disease in a suspected case, is probably that of Schottelius.

A small quantity of the fecal matter is mixed with bouillon and stood in an incubating oven for twenty-four hours. If the cholera spirilla are present they will grow most rapidly at the surface of the liquid where the supply of air is good. A pellicle will be formed, a drop from which, diluted in melted gelatin and poured upon plates, will show typical colonies.

Cultivation.—The cholera organism is easily cultivated, and grows luxuriantly upon the usual laboratory media.

Colonies.—The colonies grown upon gelatin plates are characteristic and appear in the lower strata of the gelatin as small white

dots, which gradually grow out to the surface, effect a slow liquefaction of the medium, and then appear to be situated in little pits with sloping sides. The appearance suggests that the plate is full of little holes or air-bubbles, and is due to the slow evaporation of the liquefied gelatin.

Under the microscope the colony of the cholera spirillum is fairly well characterized. The little colonies that have not yet reached the surface of the gelatin soon show a pale yellow color and an irregular contour. They are coarsely granular, the largest granules being in the center. As the colony increases in size the granules do the same and attain a peculiar transparent appearance suggestive of powdered glass. The slow liquefaction causes the

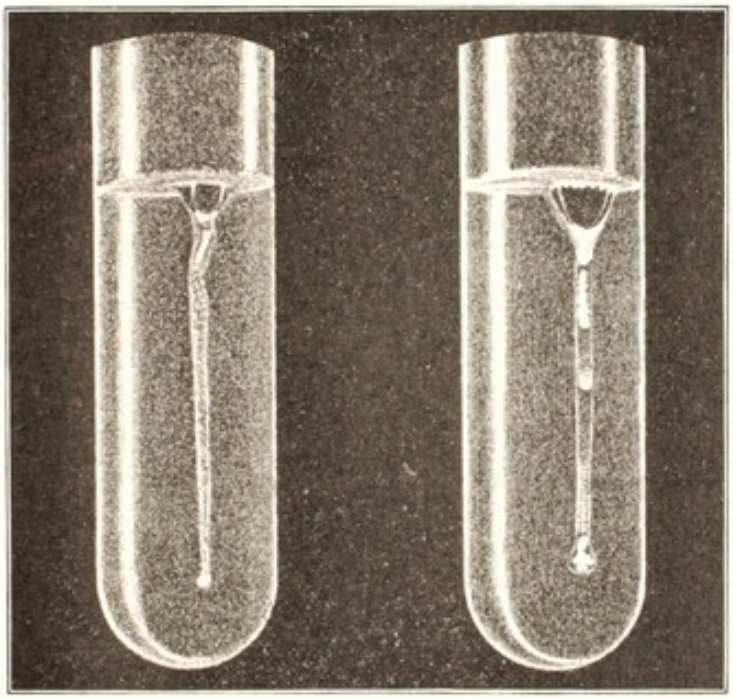


Fig. 239.—Spirillum choleræ asiaticæ; gelatin puncture cultures aged forty-eight and sixty hours (Shakespeare).

colony to be surrounded by a transparent halo. As the liquefied gelatin evaporates, the colony begins to sink, and also to take on a peculiar rosy color.

Gelatin.—In puncture cultures in gelatin the growth is also quite characteristic. It occurs along the entire puncture, but best at the surface, where it is in contact with the atmosphere. Liquefaction of the medium begins almost at once, keeps pace with the growth, but is always more marked at the surface than lower down. The result is the formation of a short, rather wide funnel at the top of the puncture. As the growth continues, evaporation of the medium takes place slowly, so that the liquefied gelatin is lower than the surrounding solid portions, and the growth appears to be surmounted by an air-bubble.

The luxuriant development of the spirilla in the liquefying gelatin is followed by the formation of considerable sediment in the lower third or half of the liquefied area. This solid material consists of masses of spirilla which have probably completed their life-cycle and become inactive. Under the microscope they exhibit the most varied involution-forms. The liquefaction reaches the sides of the tube in from five to seven days, but is not complete for several weeks.

Agar-agar.—When planted upon the surface of agar-agar the spirilla produce a grayish-white, shining, translucent growth along the entire line of inoculation. It is in no way peculiar or characteristic. The vitality of the organism is retained much better upon agar-agar than upon gelatin, and, according to Fränkel, the organism can be transplanted and grown when nine months old.

Blood-serum.—The growth upon blood-serum is also without distinct peculiarities; gradual liquefaction of the medium occurs.

Potato.—Upon potato the spirilla grow well, even when the reaction is acid. In the incubator, at a temperature of 37°C., a transparent, slightly brownish or yellowish-brown growth, somewhat resembling that of glanders, is produced. It contains large numbers of long spirals.

Bouillon.—In bouillon and in peptone solution the cholera organisms grow well, especially upon the surface, where a folded, wrinkled pellicle is formed, the culture fluid remaining clear.

Milk.—In milk the growth is luxuriant, but does not visibly alter its appearance. The existence of cholera organisms in milk is, however, rather short-lived, for the occurrence of acidity destroys them.

Vital Resistance.—Although an organism that multiplies with great rapidity under proper conditions, the cholera spirillum does not possess much resisting power. Sternberg found that it was killed by exposure of 52°C. for four minutes, but Kitasato found that ten or fifteen minutes' exposure to 55°C. was not always fatal to it. In a moist condition the organism may retain its vitality for months, but it is very quickly destroyed by desiccation, as was found by Koch, who observed that when dried in a thin film its power to grow disappeared in a few hours. Kitasato found that upon silk threads the vitality might be retained longer. Abel and Claussen* have shown that it does not live longer than twenty or thirty days in fecal matter, and often disappears in from one to three days. The organism is very susceptible to the influence of carbolic acid, bichlorid of mercury, and other germicides, and is also destroyed by acids. Hashimoto† found that it could not live longer than fifteen minutes in vinegar containing 2.2–3.2 per cent. of acetic acid.

* "Centralbl. f. Bakt. u. Parasitenk.," Jan. 31, 1895, vol. XVII, No. 4.
† "Kwai Med. Jour.," Tokyo, 1893.

According to Fränkel, the organisms in the liquefied cultures all die in eight weeks, and cannot be transplanted. Kitasato, however, has found them living and active on agar-agar after from ten to thirty days, and Koch occasionally found some alive after two years.

This low vital resistance of the microbe is very fortunate, for it enables us to establish satisfactory quarantine for the prevention of the spread of the disease. Excreta, soiled clothing, etc., are readily rendered harmless by the proper use of disinfectants. Water and food are rendered innocuous by boiling or cooking. Vessels may be disinfected by thorough washing with jets of boiling water discharged through a hose connected with a boiler, and baggage can be sterilized by superheated steam.

Metabolic Products.—Indol is one of the characteristic metabolic products of the cholera spirillum. As the cholera organisms also produce nitrites, all that is necessary to demonstrate its presence in a colorless solution is to add a drop or two of chemically pure sulphuric acid, when the well-known reddish color will appear.

The organism also produces acid in milk and other media. Bitter has also shown that the cholera organism produces a peptonizing and probably also a diastatic ferment.

Toxic Products.—Rietsch thinks the intestinal changes depend upon the action of the peptonizing ferment. Cantani, Nicati and Rietsch, Van Ermengem, Klebs, and others found toxic effects from cultures administered to dogs and other animals. Several toxic metabolic products of the spirilla have been isolated. Brieger,* Brieger and Fränkel,† Gamaléia,‡ Sobernheim,§ and Villiers have studied more or less similar toxic products. The real toxic substance is, however, not known.

Pathogenesis.—Through what activity the cholera organism provokes its pathogenic action is not yet determined. The organisms, however, abound in the intestinal contents, penetrate sparingly into the tissues, but slightly invade the lymphatics, and almost never enter the circulation; hence it is but natural to conclude that the first action must be an irritative one depending upon toxin-formation in the intestine.

In the beginning of the disease the small and large intestines are deeply congested, almost velvety in appearance, and contain liquid fecal matter. The patient suffers from diarrhea, by which the feces are hurried on and become extremely thin from the admixture of a copious watery exudate. As the feces are hurried out, more and more of the aqueous exudate accumulates, until the intestine seems to contain only watery fluid. The solitary glands and Peyer's patches are found enlarged and the mucosa becomes macer-

* "Berliner klin. Wochenschrift," 1887, p. 817.
† "Untersuchungen über die Bakteriengifte," etc., Berlin, 1890.
‡ "Archiv de méd. exp.," IV, No. 2.
§ "Zeitschrift für Hygiene," 1893, XIV, 145.

ated and necrotic, its epithelium separating in small shreds or flakes. The evacuations of watery exudate rich in these shreds constitute the characteristic "rice-water discharges" of the disease. As the disease progresses, the denudation of tissue results in the formation of good-sized ulcerations. Perforations and deep ulcerations are rare. Pseudo-membranous formations not infrequently occur upon the abraded and ulcerated surfaces. The other mucous membranes of the alimentary apparatus become congested and abraded; the parenchyma of the liver, kidneys, and other organs become markedly degenerated, so that the urine becomes highly albuminous and very scanty in consequence of the anhydremia. The cardio-vascular, nervous, and respiratory systems present no characteristic changes.

So far as is known, cholera is a disease of human beings only, and never occurs spontaneously in the lower animals.

Intraperitoneal injection of the virulent cultures produces fatal peritonitis in guinea-pigs.

Supposing that the lower animals were immune against cholera because of the acidity of the gastric juice, Nicati and Rietsch,* Van Ermengem, and Koch† have suggested methods by which the micro-organisms can be introduced directly into the intestine. The first-named investigators ligated the common bile-duct of guinea-pigs, and then injected the spirilla into the duodenum with a hypodermic needle, with the result that the animals usually died, sometimes with choleraic symptoms. The excessively grave nature of the operation upon such a small and delicately constituted animal as a guinea-pig, however, greatly lessens the value of the experiment. Koch's method of infection by the mouth is much more satisfactory. By injecting laudanum into the abdominal cavity of guinea-pigs the peristaltic movements of the intestine can be checked. The amount necessary for the purpose is large and amounts to about 1 gram for each 200 grams of body-weight. It completely narcotizes the animals for a short time (one to two hours), but they recover without injury. The contents of the stomach are neutralized after administering the opium, by introducing 5 cc. of a 5 per cent. aqueous solution of sodium carbonate through a pharyngeal catheter. With the gastric contents thus alkalinized and the peristalsis paralyzed, a bouillon culture of the cholera spirillum is introduced through the stomach-tube. The animal recovers from the manipulation, but shows an indisposition to eat, is soon observed to be weak in the posterior extremities, subsequently is paralyzed, and dies within forty-eight hours. The autopsy shows the intestine congested and filled with a watery fluid rich in spirilla—an appearance which Fränkel declares to be exactly that of cholera. In man, as well as in these artificially infected animals, *the spirilla are*

* "Deutsch. med. Wochenschrift," 1884.
† Ibid., 1885.

never found in the blood or tissues, but only in the intestine, where they frequently enter between the basement membrane and the epithelial cells, and aid in the detachment of the latter.

Issaëff and Kolle found that when virulent cholera spirilla are injected into the ear-veins of young rabbits the animals die on the following day with symptoms resembling the algid state of human cholera. The autopsy in these cases showed local lesions of the small intestine very similar to those observed in cholera in man.

Guinea-pigs are susceptible to intraperitoneal injections of the spirillum, and speedily succumb. The symptoms are rapid fall of temperature, tenderness over the abdomen, and collapse. The autopsy shows an abundant fluid exudate containing the micro-organisms, and injection and redness of the peritoneum and viscera.

Specificity.—The cholera spirillum is present in the dejecta of cholera with great regularity, and as regularly absent from the dejecta of healthy individuals and those suffering from other diseases. No satisfactory proof of the specific nature of the organisms can be obtained by experimentation upon animals. Animals are never affected by any disease similar to cholera during epidemics, nor do foods mixed with cholera discharges or with pure cultures of the cholera spirillum affect them. Subcutaneous inoculations do not produce cholera.

Detection of the Organism.—It often becomes a matter of importance to detect the cholera spirilla in drinking-water, and, as the number in which the bacteria exist in such a liquid may be very small, difficulty may be experienced in finding them by ordinary methods. One of the most expeditious methods is that recommended by Löffler, who adds 200 cc. of the water to be examined to 10 cc. of bouillon, allows the mixture to stand in an incubator for from twelve to twenty-four hours, and then makes plate cultures from the superficial layer of the liquid, where, if present, the development of the spirilla will be most rapid because of the free access of air.

Gordon* employs a medium composed of lemco 1 gram, peptone 1 gram, sodium bicarbonate 0.1 gram, starch 1 gram, and distilled water 100 cc. for the differentiation of the cholera and Finkler-Prior spirilla. If the medium be tinted with litmus and the cultures grown at 37°C., a strongly acid change is produced by the true cholera organism in twenty-four hours. The Finkler-Prior spirillum produces but slight acidity, which first appears about the third day.

The identification of the cholera spirillum, and its differentiation from spiral organisms of similar morphology obtained from feces or water in which no cholera organisms are expected, is becoming less and less easy as our knowledge of the organisms increases. The following points may be taken into consideration:

* "British Medical Journal," July 28, 1906.

(1) The typical morphology. The true cholera organism is short, has a single curve, is rounded at the ends, and possesses a *single* flagellum. (2) The infectivity. Freshly isolated cultures should be pathogenic for guinea-pigs and harmless to pigeons. (3) Vegetative: The organism should liquefy 10 per cent. gelatin and should not coagulate milk. (4) Metabolic: the indol reaction should be marked. (5) Immunity reactions: the organism when injected into guinea-pigs in ascending doses should occasion immunity against the typical cholera organism, and the serum of the immunized guinea-pig, when introduced into a new guinea-pig, should protect it from infection and produce Pfeiffer's phenomenon. The blood-serum of animals immunized against the cholera organism should agglutinate the doubtful organism in approximately the same dilution, and that of animals immunized to the doubtful organism should agglutinate the cholera organism reciprocally. Both organisms should have equal capacity for absorbing complements and amboceptors from blood-serum. (6) The true cholera organism should not be hemolytic. Too much reliance must not be placed upon the agglutination tests alone, as will be made clear by a perusal of the paper upon Bacteriological Diagnosis of Cholera by Ruffer.*

Pfeiffer and Vogedes† have applied the "immunity reaction" to the identification of cholera spirilla in cultures. A hanging drop of a 1 : 50 mixture of a powerful anticholera serum and a particle of cholera culture is made and examined under the microscope. The cholera spirilla at once become inactive, and are in a short time converted into little rolled-up masses. If the culture added be a spirillum other than the true cholera spirillum, instead of being destroyed the micro-organisms multiply and thrive in the mixture of serum and bouillon.

Immunity.—One attack of cholera usually leaves the victim immune from further attacks of the disease. Gruber and Wiener,‡ Haffkine,§ Pawlowsky,‖ and Pfeiffer** have immunized animals against toxic substances from cholera cultures and against living cultures.

Sobernheim†† found the Pfeiffer reaction specific against cholera alone, and thought the protection not due to the strongly bactericidal property of the serum, but to its stimulating effect upon the body-cells; for if the serum be heated to 60°–70°C., and its bactericidal power thus destroyed, it is still capable of producing immunity. This, of course, is in keeping with our present knowledge of the *immune body*, which is not destroyed by such temperatures.

* "British Medical Journal," March 30, 1907, 1, p. 735.
† "Centralbl. f. Bakt. u. Parasitenk.," March 21, 1896, Bd. XIX, No. 11.
‡ "Centralbl. f. Bakt.," 1892, XIV, p. 76.
§ "Le Bull. méd.," 1892, p. 1113, and "Brit. Med. Jour.," 1893, p. 278.
‖ "Deutsche med. Wochenschrift," 1893, No. 22.
** "Zeitschrift für Hygiene," Bd. XVIII and XX.
†† "Zeitschrift für Hygiene, XX, p. 438.

The immunity produced by the injection of the spirilla into guinea-pigs continues in some cases as long as four and a half months, but the power of the serum to confer immunity is lost much sooner.

Serum Therapy and Prophylaxis.—Of the numerous attempts to produce immunity against cholera in man, or to cure cholera when once established in the human organism, nothing very favorable can be said. Experiments in this field are not new. As early as 1885 Ferrán, in Spain, administered hypodermic injections of pure virulent cultures of the cholera spirillum, in the hope of bringing about immunity. The work of Haffkine,* however, is the chief important contribution, and his method seems to be followed by a positive diminution of mortality in protected individuals. Haffkine uses two vaccines—one mild, the other so virulent that it would bring about extensive tissue-necrosis and perhaps death if used alone. His studies embrace more than 40,000 inoculations performed in India. The following extract will show results obtained in 1895:

"1. In all those instances where cholera has made a large number of victims—that is to say, where it has spread sufficiently to make it probable that the whole population, inoculated and uninoculated, were equally exposed to the infection—in all these places the results appeared favorable to inoculation.

"2. The treatment applied after an epidemic actually breaks out tends to reduce the mortality even during the time which is claimed for producing the full effect of the operation. In the Goya Garl, where weak doses of a relatively weak vaccine had been applied, this reduction was to half the number of deaths; in the coolies of the Assam-Burmah survey party, where, as far as I can gather from my preliminary information, strong doses have been applied, the number of deaths was reduced to one-seventh. This fact would justify the application of the method independently of the question as to the exact length of time during which the effect of this vaccination lasts.

"3. In Lucknow, where the experiment was made on small doses of weak vaccines, a difference in cases and deaths was still noticeable in favor of the inoculated fourteen to fifteen months after vaccination in an epidemic of exceptional virulence. This makes it probable that a protective effect could be obtained even for long periods of time if larger doses of a stronger vaccine were used.

"4. The best results seem to be obtained from application of middle doses of both anticholera vaccines, the second one being kept at the highest possible degree of virulence obtainable.

"5. The most prolonged observations on the effect of middle doses were made in Calcutta, where the mortality from the eleventh up to the four hundred and fifty-ninth day after vaccination was, among the inoculated, 17.24 times smaller, and the number of cases 19.27 times smaller than among the not inoculated."

Pawlowsky and others have found the dog susceptible to cholera, and have utilized it in the preparation of an antitoxic serum. The dogs were first immunized against attenuated cultures, then against more and more virulent cultures, until a serum was obtained whose value was estimated at 1:130,000 upon experimental animals.

Freymuth† and others have endeavored to secure favorable

* "Le Bull. méd.," 1892, p. 1113; "Indian Med. Gazette," 1893, p. 97; "Brit. Med. Jour.," 1893, p. 278.

† "Deutsche med. Wochenschrift," 1893, No. 43.

results from the injection of blood-serum from convalescent patients into the diseased. One recovery out of three cases treated is recorded.

In all these preliminaries the foreshadowing of a future therapeusis must be evident, but as yet nothing satisfactory has been achieved.

One of the chief errors made in the experimental preparation of anticholera serums is that efforts have been directed toward endowing the blood with the power of resisting and destroying the bacteria that rarely, if ever, reach it. The two essentials to be aimed at are an *antitoxin* to neutralize the depressing effects of the toxalbumin, and some means of destroying the bacteria in the intestine.

Sanitation.—The first appearance of cholera may depend upon the introduction of the micro-organisms upon fomites, hence to avoid epidemics it is necessary to disinfect all such coming from cholera-infected localities.

So soon as cholera asserts itself, the chief danger lies in the probable contamination of the water-supply. To prevent this the utmost effort must be made to locate all cases and see that the dejecta are thoroughly disinfected, and as the micro-organisms persist in the intestinal discharges for some weeks after convalescence, the patients should not too soon be discharged from the hospital, but should be retained until a bacteriologic examination shows no more comma bacilli in the feces. During an epidemic the water consumed should all be boiled, raw milk should be avoided, and no green or uncooked vegetables or fruits eaten. Foods should be carefully defended from flies, which may carry the organisms to them and infect them. The intestinal evacuations and all the clothing, bedding, and other articles used by the patients should be carefully disinfected.

SPIRILLA RESEMBLING THE CHOLERA SPIRILLUM

The Finkler and Prior Spirillum (Spirillum Proteus)

Similar in morphology to the spirillum of cholera, and in other respects closely related to it, is the spirillum obtained from the feces of a case of *cholera nostras* by Finkler and Prior.*

Morphology.—It is shorter and stouter, with a more pronounced curve than the cholera spirillum, and rarely forms long spirals. The central portion is also somewhat thinner than the ends, which are a little pointed and give the organism a less uniform appearance. Involution forms are common in cultures, and appear as spheres, spindles, clubs, etc. Like the cholera spirillum, each organism is provided with a single flagellum situated at its end, and is actively motile.

Staining.—The organism stains readily with the ordinary solutions, but not by Gram's method.

Cultivation.—Colonies.—The growth upon gelatin plates is rapid, and leads to such extensive liquefaction that four or five dilutions must frequently be made to secure few enough organisms to enable one to observe the growth of a single

* "Centralbl. für allg. Gesundheitspflege," Bonn, 1885, Bd. 1; "Deutsche med. Wochenschrift," 1884, p. 632.

colony. To the naked eye the deep colonies appear as small white points. They rapidly reach the surface, begin liquefaction of the gelatin, and by the second day appear about the size of lentils, and are situated in little depressions. Under the

Fig. 240.—Spirillum of Finkler and Prior, from an agar-agar culture. × 1000 (Itzerott and Niemann).

microscope they are yellowish brown, finely granular, and are surrounded by a zone of sharply circumscribed liquefied gelatin. Careful examination with a high-power lens shows rapid movement of the granules in the colony.

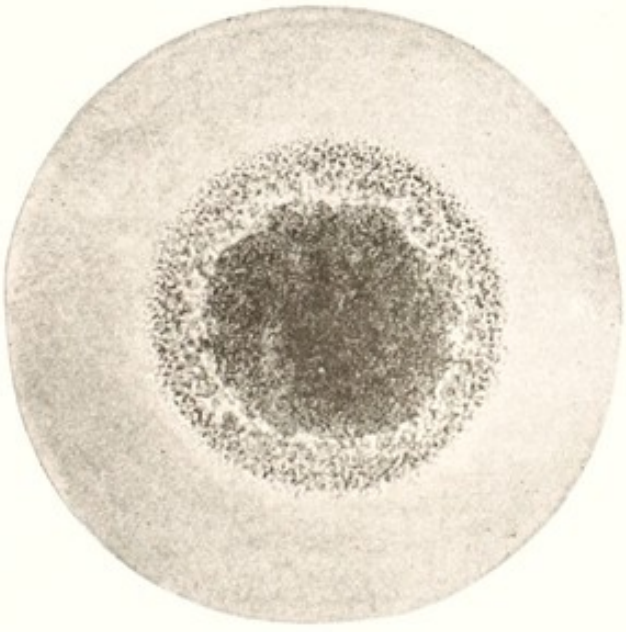


Fig. 241.—Spirillum of Finkler and Prior; colony twenty-four hours old, upon a gelatin plate. × 100 (Fränkel and Pfeiffer).

Gelatin Punctures.—In gelatin punctures the growth takes place rapidly along the whole length of the puncture, forming a stocking-shaped liquefaction filled with cloudy fluid which does not precipitate rapidly; a rather smeary, whitish

scum is usually formed upon the surface. The more extensive and more rapid liquefaction of the medium, the wider top to the funnel, the absence of the air-bubble, and the clouded nature of the liquefied material, all serve to differentiate the culture from the cholera spirillum.

Agar-agar.—Upon agar-agar the growth is also rapid, and in a short time the whole surface of the culture medium is covered with a moist, thick, slimy coating, which may have a slightly yellowish tinge.

Bouillon.—In bouillon the organism causes a diffuse turbidity with a more or less distinct pellicle on the surface. In sugar-containing culture-media it causes no fermentation and generates no gas.

Potato.—The cultures upon potato are also different from those of the cholera organism, for the Finkler and Prior spirilla grow rapidly at the room temperature, and produce a grayish-yellow, slimy shining layer, which may cover the whole of the culture-medium.

Blood-serum.—Blood-serum is rapidly liquefied by the organism.

Milk.—The spirillum does not grow well in milk, and speedily dies in water.

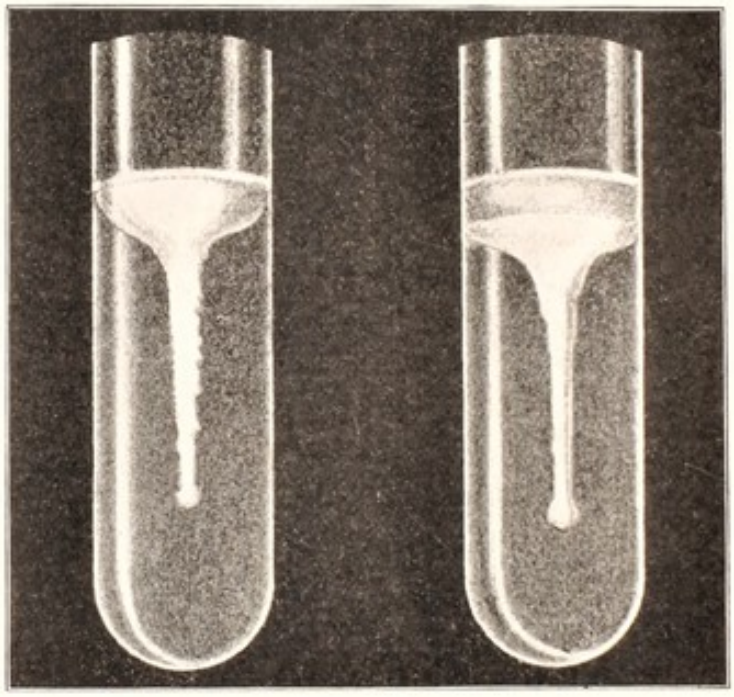


Fig. 242.—Spirillum of Finkler and Prior; gelatin puncture cultures aged forty-eight and sixty hours (Shakespeare).

Metabolic Products.—The organism does not produce indol. Buchner has shown that in media containing some glucose an acid reaction is produced. Proteolytic enzymes capable of dissolving gelatin, blood-serum, and casein are formed.

Pathogenesis.—It was at first supposed that if not the spirillum of cholera itself, this was a very closely allied organism. Later it was supposed to be the cause of cholera nostras. At present it is a question whether the organism has any pathologic significance. It was in one case secured by Knisl from the feces of a suicide, and has been found in carious teeth by Müller.

When injected into the stomach of guinea-pigs treated with tincture of opium according to the method of Koch, about 30 per cent. of the animals die, but the intestinal lesions produced are not identical with those produced by the cholera spirillum. The intestines in such cases are pale and filled with watery material having a strong putrefactive odor. This fluid teems with the spirilla.

It seems unlikely, from the evidence thus far collected, that the Finkler and Prior spirillum is pathogenic for the human species. As Fränkel points out, it is probably a frequent and harmless inhabitant of the human intestine.

The Spirillum of Denecke (Spirillum Tyrogenum)

Another organism with a partial resemblance to the cholera spirillum was found by Denecke* in old cheese.

Fig. 243.—Spirillum of Denecke, from an agar-agar culture. × 1000 (Itzerott and Niemann).

Morphology.—Its form is similar to that of the cholera spirillum, the shorter individuals being of equal diameter throughout. The spiral forms are longer

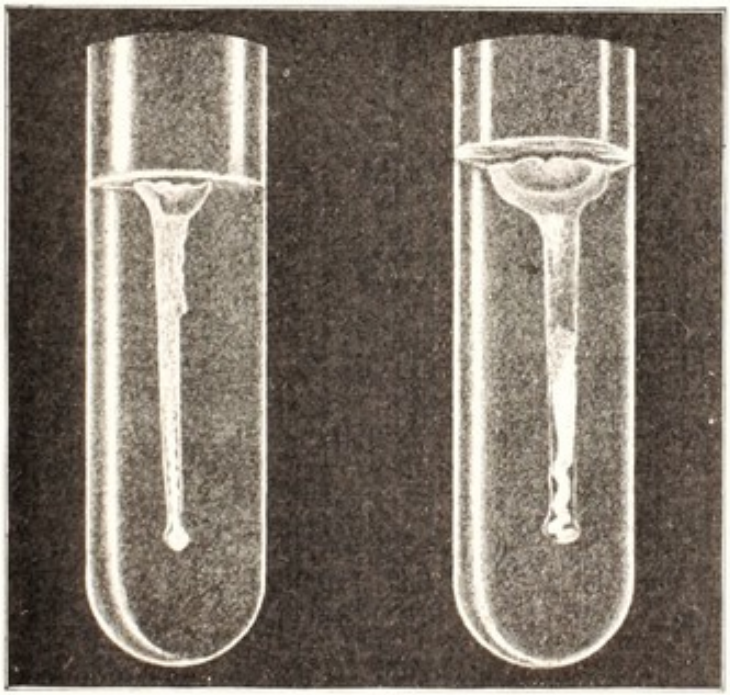


Fig. 244.—Spirillum of Denecke; gelatin puncture cultures aged forty-eight and sixty hours (Shakespeare).

than those of the Finkler and Prior spirillum, and are more tightly coiled than those of the cholera spirillum.

* "Deutsche med. Wochenschrift," 1885.

Like its related species, this micro-organism is actively motile and possesses a terminal flagellum.

Cultivation.—It grows at the room temperature, as well as at 37°C., in this respect, as in its reaction to stains, much resembling the other two.

Colonies.—Upon gelatin plates the growth of the colonies is much more rapid than that of the cholera spirillum, though slower than that of the Finkler and Prior spirillum. The colonies appear as small whitish, round points, which soon reach the surface of the gelatin and commence liquefaction. By the second day each is about the size of a pin's head, has a yellow color, and occupies the bottom of a conical depression. The appearance is much like that of colonies of the cholera spirillum.

The microscope shows the colonies to be of irregular shape and coarsely granular, pale yellow at the edges, gradually becoming intense toward the center, and at first circumscribed, but later surrounded by clear zones, resulting from the liquefaction of the gelatin. These, according to the illumination, appear pale or dark. The colonies differ from those of cholera in the prompt liquefaction of the gelatin, the rapid growth, yellow color, irregular form, and distinct line of circumscription.

Gelatin Punctures.—In gelatin punctures the growth takes place all along the track of the wire, and forms a cloudy liquid which precipitates at the apex in the form of a coiled mass. Upon the surface a delicate, imperfect, yellowish scum forms. Liquefaction of the entire gelatin generally requires about two weeks.

Agar-agar.—Upon agar-agar this spirillum forms a thin yellowish layer which spreads quickly over most of the surface.

Bouillon.—In bouillon the growth of the organism is characterized by a diffuse turbidity. No gas-formation occurs in sugar-containing media.

Potatoes.—The culture upon potato is luxuriant if grown in the incubating oven. It appears as a distinct yellowish, moist film, and when examined microscopically is seen to contain beautiful long spirals.

Metabolic Products.—The organism produces no indol.

Pathogenesis.—The spirillum of Denecke is mentioned only because of its morphologic resemblance to the cholera spirillum. It is not associated with any human disease. Experiments, however, have shown that when the spirilla are introduced into guinea-pigs whose gastric contents are alkalinized and whose peristalsis is paralyzed with opium, about 20 per cent. of the animals die.

The Spirillum of Gamaléia* (Spirillum Metchnikovi)

Resembling the cholera spirillum in morphology and vegetation, and possibly, as has been suggested, a descendant of the same original stock, is a spirillum which Gamaléia cultivated from the intestines of chickens affected with a disease similar to chicken-cholera.

Morphology.—This spirillum is a trifle shorter and thicker than the cholera spirillum. It is a little more curved, and has similar rounded ends. It forms long spirals in appropriate media, and is actively motile. Each spirillum is provided with a terminal flagellum. No spores have been demonstrated.

Staining.—The organism stains easily, the ends more deeply than the center. It is not stained by Gram's method.

Cultivation.—It grows well both at the temperature of the room and at that of incubation.

Colonies.—The colonies upon gelatin plates have a marked resemblance to those of the cholera spirillum, yet there is a difference; and as Pfeiffer says, "it is comparatively easy to differentiate between a plate of pure cholera spirillum and a plate of pure Spirillum metchnikovi, yet it is almost impossible to pick out a few colonies of the latter if mixed upon a plate with the former."

Fränkel regards this organism as a species intermediate between the cholera and the Finkler-Prior spirillum.

The colonies upon gelatin plates appear in about twelve hours as small whitish points, and rapidly develop, so that by the end of the third day large saucer-shaped liquefactions resembling colonies of the Finkler-Prior spirillum occur. The liquefaction of the gelatin is quite rapid, the resulting fluid being turbid. Usually, upon a plate of Vibrio metchnikovi some colonies are present which closely

* "Ann. de l'Inst. Pasteur," 1888.

resemble those of the cholera spirillum, being deeply situated in conical depressions in the gelatin. Under the microscope the contents of the colonies, which appear of a brownish color, are observed to be in rapid motion. The edges of the bacterial mass are fringed with radiating organisms.

Gelatin Punctures.—In gelatin tubes the growth closely resembles that of the cholera organism, but develops more slowly.

Agar-agar.—Upon the surface of agar-agar a yellowish-brown growth develops along the whole line of inoculation.

Potato.—On potato at the room temperature no growth occurs, but at the temperature of the incubator a luxuriant yellowish-brown growth takes place. Sometimes the color is quite dark, and chocolate-colored potato cultures are not uncommon.

Bouillon.—In bouillon the growth which occurs at the temperature of the incubator is quite characteristic, and very different from that of the cholera spirillum. The entire medium becomes clouded, of a grayish-white color, and opaque. A folded and wrinkled pellicle forms upon the surface.

Milk.—When grown in litmus milk, the original blue color is changed to pink in a day, and at the end of another day the color is all destroyed and the milk coagulated. Ultimately the clots of casein sediment in irregular masses, from the clear, colorless whey.

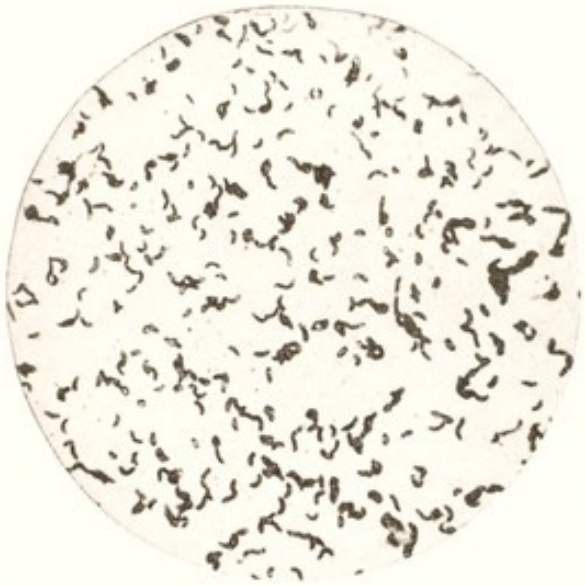


Fig. 245.—Spirillum metchnikovi, from an agar-agar culture. × 1000 (Itzerott and Niemann).

Vital Resistance.—The organism, like the cholera vibrio, is very susceptible to the influence of acids, high temperatures, and drying. The thermal death-point is 50°C., continued for five minutes.

Metabolic Products.—The addition of sulphuric acid to a culture grown in a medium rich in peptone produces the same rose color observed in cholera cultures and shows the presence of indol. When glucose is added to the bouillon no fermentation or gas-production results. The organism produces acids and curdling enzymes.

Pathogenesis.—The organism is pathogenic for animals, but not for man. Pfeiffer has shown that chickens and guinea-pigs are highly susceptible, and when inoculated under the skin usually die. The virulent organism is invariably fatal for pigeons. W. Rindfleisch has pointed out that this constant fatality for pigeons is a valuable criterion for the differentiation of this spirillum from that of cholera, as the subcutaneous injection of the most virulent cholera cultures is never fatal to pigeons, the birds only dying when the injections are made into the muscles in such a manner that the muscular tissue is injured and becomes a *locus minoris resistentiæ*. When guinea-pigs are treated by Koch's method of narcotization and cholera infection, the temperature of the animal rises for a short time, then abruptly falls to 33°C. or less. Death follows in from twenty to

twenty-four hours. A distinct inflammation of the intestine, with exudate and numerous spirilla, may be found. The spirilla can also be found in the heart's blood and in the organs of such guinea-pigs. When the bacilli are introduced by subcutaneous inoculation, the autopsy shows a bloody edema and a superficial necrosis of the tissues.

The organisms can be found in the blood and all the organs of pigeons and young chickens, in such large numbers that Pfeiffer has called the disease *Vibrionensepticæmia*. In the intestines very few alterations are noticeable, and very few spirilla can be found.

Immunity.—Gamaléia has shown that pigeons and guinea-pigs can be made immune by inoculating them with cultures sterilized for a time at a temperature of 100°C. Mice and rabbits are immune, except to very large doses.

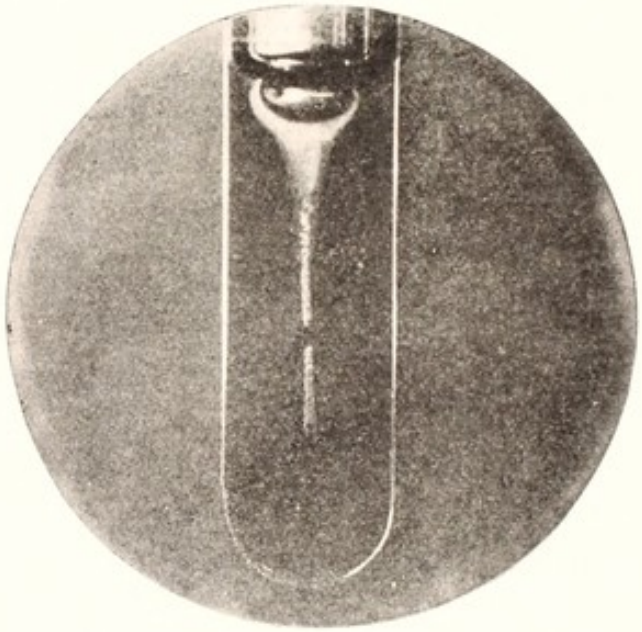


Fig. 246.—Spirillum metchnikovi; puncture culture in gelatin forty-eight hours old (Fränkel and Pfeiffer).

Spirillum Schuylkiliensis (Abbott)

Morphology.—This micro-organism, closely resembling the cholera spirillum, was found by Abbott* in sewage-polluted water from the Schuylkill River at Philadelphia.

Cultivation.—Colonies.—The colonies developed upon gelatin plates very closely resemble those of the Spirillum metschnikovi.

Gelatin punctures.—In gelatin puncture cultures the appearance is exactly like the true cholera spirillum. At times the growth is a little more rapid.

Agar-agar.—The growth on agar is luxuriant, and gives off a pronounced odor of indol.

Blood-serum.—Löffler's blood-serum is apparently not a perfectly adapted medium, but upon it the organisms grow, with resulting liquefaction.

Potato.—Upon potato, at the point of inoculation a thin, glazed, more or less dirty yellow growth, shading to brown and sometimes surrounded by a flat, dry, lusterless zone, is formed.

Milk.—In litmus milk a reddish tinge develops after the milk is kept twenty-four hours at body temperature. After forty-eight hours this color is increased and the milk coagulates.

Metabolic Products.—In peptone solutions indol is easily detected. No gas is produced in glucose-containing culture-media. Acids and coagulating enzymes are formed. The organism is a facultative anaërobe.

* "Journal of Experimental Medicine," July, 1896, vol. 1, No. 3, p. 419.

Vital Resistance.—The thermal death point is 50°C. maintained for five minutes.

Pathogenesis.—The organism is pathogenic for pigeons, guinea-pigs, and mice, behaving much like Spirillum metchnikovi. No Pfeiffer's phenomenon was observed with the use of serum from immunized animals.

Immunity.—Immunity could be produced in pigeons, and it was found that the serum was protective against both Spirillum schuylkiliensis and Spirillum metchnikovi, the immunity thus produced being of about ten days' duration.

In a second paper by Abbott and Bergey* it was shown that the spirilla occurred in the water during all four seasons of the year, and in all parts of the river within the city, both at low and at high tide. They were also found in the sewage emptying into the river, and in the water of the Delaware River as frequently as in that of the Schuylkill.

One hundred and ten pure cultures were isolated from the sources mentioned and subjected to routine tests. It was found that few or none of them were identical in all points. There seems to be, therefore, a family of river spirilla, closely related to one another, like the different colon bacilli.

The opinion expressed is that "the only trustworthy difference between many of these varieties and the true cholera spirillum is the specific reaction with serum from animals immune against cholera, or by Pfeiffer's method of intraperitoneal testing in such animals."

In discussing these spirilla of the Philadelphia water Bergey† says:

"The most important point with regard to the occurrence of these organisms in the river water around Philadelphia is the fact that similar organisms have been found in the surface waters of the European cities in which there had recently been an epidemic of Asiatic cholera, notably at Hamburg and Altona. . . . The foremost bacteriologists of Europe have been inclined to the opinion that the organisms which they found in the surface waters of the European cities were the remains of the true cholera organism, and that the deviations in the morphologic and biologic characters from those of the cholera organism were brought about by their prolonged existence in water. No such explanation of the occurrence of the organisms in Philadelphia waters can be given."

A number of interesting spirilla, more or less closely resembling that of Asiatic cholera, have been described from time to time. Their variation from the true cholera organism can best be determined by an examination of the following table, though for precise information the student will do well to look up the original descriptions, references to which are given in each case.

* "Journal of Experimental Medicine," vol. II, No. 5, p. 535.

† "Journal Amer. Med. Assoc.," Oct. 23, 1897.

DIFFERENTIAL TABLE FOR SEPARATING ORGANISMS RESEMBLING THE CHOLERA SPIRILLUM.

	Found in Intestinal Diseases.	Found in Water.	Stain by Gram's Method.	Comma Shape.	Thick Spirals.	Slender Spirals.	Spores.	Active Motility.	Terminal Flagella.	Bouillon. Scum and Slight Turbidity.	Bouillon. Scum and Marked Turbidity.	Gelatin. Very Slow Liquefaction.	Gelatin. Slow Liquefaction.	Gelatin. Rapid Liquefaction.	Agar-Agar. Yellowish.	Agar-Agar. Grayish.	Potato. Yellowish.	Potato. Brown.	Potato. Colorless.	Potato. No Growth.	Nitrites.	Indol.	Gelatin. Liquefaction.	Casein. Liquefaction.	Blood-serum. Liquefaction.	Fermentation.	Milk. Alkalinized.	Milk. Acidified.	Milk. Coagulated.	Milk. Phosphorescent.	Pigeons. Pathogenic.	Guinea-pigs. Pathogenic.	Rabbits. Pathogenic.	Chromogenic.	Fluorescent.
Intestinal Group.																																		
Spirillum choleræ asiaticæ (Koch*)	+	+	o	+	+	o	o	+	+	+	o	o	+	o	o	+		o	+	o	+	+	o	+	o	o	+	o	o	o		o	o
Spirillum choleræ nostras (Spirillum proteus) (Finkler and Prior†)	+	o	o	+	+	o	o	+	+	+	o	o	o	+	+	o	+	o	+	o	o	+			o	o	+	o	o	o	+	o	o
Spirillum tyrogenum (Denecke‡)	+	o	o	+	+	o	o	+	+	+	o	o	+	o	+	o	+	o	o	o	o	+			o				o			o	o
Spirillum metschnikovi (Gamaleia§)	+	o	o	+	+	o	o	+	+	o	+	+	o	o	+	o		+	o	o	+	+	o		o	o	+	+	o	o		o	o
Water Group.																																		
Spirillum dunbarensis (Dunbar‖)	o	+	o	+	o	o	o	+	+	+	o	o	o	+	o		+	o	o	o	+	+			o				o	+			o	o
Spirillum danubicus (Heider**)	o	+	o	+	o	+	o	+	+	+	o	o	+	o	o		+	o	o	o	+	+			o		+		o				o	o
Spirillum I (Wernicke††)	o	+	o	+	+	o	o	+	+	+	o	o	+	o	o			o	o	o	+	+			o	o	+	+	o	+			o	o
Spirillum II (Wernicke‡‡)	o	+	o	+	+	o	o	+	+	+	o	+	o	o	o			o	o	o	+	+			o				o				o	o
Spirillum liquefaciens (Bonhoff§§)	o	+	o	+	+	o	o	+	+	+	o	o	+	o	o			o	o	o	+	+			o				o				o	o
Spirillum weibeli (Weibel‖‖)	o	+	o	+	+	o	o	+	+	+	o	o	+	o	o			o	o	o	+	+			o				o				o	o
Spirillum milleri (Miller***)	o	o	o	+	+	o	o	+	+	+	o	o	+	o	o			o	o	o	+	+			o				o				o	o
Spirillum terrigenus (Günther†††)	o	o	o	+	+	o	o	+	+	+	o	o	+	o	o			o	o	o	+	+			o				o				o	o
Spirillum berolinensis (Neisser‡‡‡)	o	+	o	+	+	o	o	+	+	?	?	o	o	o	o					o	o	o							o				o	o
Spirillum aquatilis (Günther§§§)	o	+	o	+	+	o	o	+	+	+	o	o	+	o	o					o	+	+			o				o				o	o
Spirillum schuylkiliensis (Abbott and Bergey‖‖‖)	o	+	o	+	+	o	o	+	+	+	o	o	+	o	o			o	o	o	+	+			o				o				o	o

* "Berliner klin. Wochenschrift," 1884, Nos. 31 and 32.
§ "Ann. de l'Inst. Pasteur," 1888, II, p. 482.
†† "Archiv für Hygiene," XXI, 1894, p. 172.
‖‖ "Centralbl. f. Bakt.," etc., Bd. II, 1887, p. 469.
‡‡‡ "Hygienische Rundschau," 1893.
† "Deutsche med. Wochenschrift," 1884, p. 632.
‖ "Deutsche med. Wochenschrift," 1893, p. 799.
‡‡ "Archiv für Hygiene," XXI, 1894, p. 179.
*** "Deutsche med. Wochenschrift," 1885, p. 138.
‡ "Deutsche med. Wochenschrift," 1885.
** "Centralbl. f. Bakt.," etc., XIV, p. 341.
§§ "Archiv für Hygiene," XIX, 1893, p. 248.
††† "Hygienische Rundschau," IV, 1894, p. 721.
§§§ "Deutsche med. Wochenschrift," 1892, p. 1124.
‖‖‖ "Journal of Experimental Medicine," July, 1896, p. 419, vol. I, No. 3.

CHAPTER XXVII

TYPHOID FEVER

Bacillus Typhosus (Eberth-Gaffky)

General Characteristics.—A motile, flagellated, non-sporogenous, non-liquefying, non-chromogenic, non-aërogenic, aërobic and optionally anaërobic, pathogenic bacillus, staining by ordinary methods, but not by Gram's method, not forming indol, acids from sugars, or coagulating milk.

Typhoid fever, "typhus abdominalis," enteric fever, "la fievre typhique," is a disease so well known and of such universal distribution, that no introductory remarks concerning it are necessary.

The bacillus of typhoid fever (*Bacillus typhosus*) was discovered

Fig. 247.—Bacillus typhosus, from twenty-four-hour culture on agar. (From Hiss and Zinsser, "Text-book of Bacteriology," D. Appleton & Co., publishers.)

in 1880 by Eberth* and Koch,† and was first secured in pure culture from the spleen and lymphatic glands four years later by Gaffky.‡

Distribution.—The bacillus is both saprophytic and parasitic. It finds abundant opportunity in nature for growth and development, and, enjoying strong resisting powers, can accommodate itself to its environment much better than the majority of pathogenic bacteria, and can be found in water, soiled clothing, dust, sewage, milk, etc., contaminated directly or indirectly with the intestinal discharges of diseased persons.

* "Virchow's Archiv," 1881 and 1883.
† "Mittheilungen aus dem kaiserl. Gesundheitsamte," 1, 45.
‡ *Ibid.*, 2.

Morphology.—The typhoid bacillus measures about 1 to 3 μ (2 to 4 μ—Chantemesse, Widal) in length and 0.5 to 0.8 μ in breadth (Sternberg). The ends are rounded, and it is exceptional for the bacilli to be united in chains. The size and morphology vary with the nature of the culture-medium and the age of the culture. Thoinot and Masselin,* in describing these morphologic variations, point out that when grown in bouillon the typhoid bacillus is very slender; in milk it is stouter; upon agar-agar and potato it is thick and short; and in old gelatin cultures it forms long filaments. It produces no spores.

Flagella.—The organisms are actively motile and are provided with numerous flagella, which arise from all parts of the bacillus (peritricha), and are 10 to 20 in number. They stain well by

Fig. 248.—Bacillus typhosus.

Löffler's method. The movements of the short bacilli are oscillating; those of the longer bacilli, serpentine and undulating.

Staining.—The organism stains quite well by the ordinary methods, but not by Gram's method. As it gives up its color in the presence of almost any solvent, it is difficult to stain in tissue.

When sections of tissue are to be stained for the demonstration of the typhoid bacilli, the best method is to allow them to remain in Loffler's alkaline methylene blue for from fifteen minutes to twenty-four hours, then wash in water, dehydrate rapidly in alcohol, clear up in xylol, and mount in Canada balsam. Ziehl's method also gives good results: The sections are stained for fifteen minutes in a solution of distilled water, 100, fuchsin 1, and phenol 5. After staining they are washed in distilled water containing 1 per cent. of acetic acid, dehydrated in alcohol, cleared, and mounted. In such

* "Précis de Microbie," Paris, 1893.

preparations the bacilli are always found in scattered groups, which are easily discovered, under a low power of the microscope, as reddish specks, and readily resolved into bacilli with the oil-immersion lens.

In bacilli stained with the alkaline methylene-blue solution, dark-colored dots (Babes-Ernst or metachromatic granules) may sometimes be observed near the ends of the rods.

Isolation.—The bacillus can be secured in pure culture from an enlarged lymphatic gland or from the splenic pulp of a case of typhoid.

As the groups of bacilli are sometimes widely scattered throughout the spleen, E. Fränkel recommends that as soon as the organ is removed from the body it be wrapped in cloths wet with a solution of bichlorid of mercury and kept for three days in a warm room, in order that a considerable and massive development of the bacilli

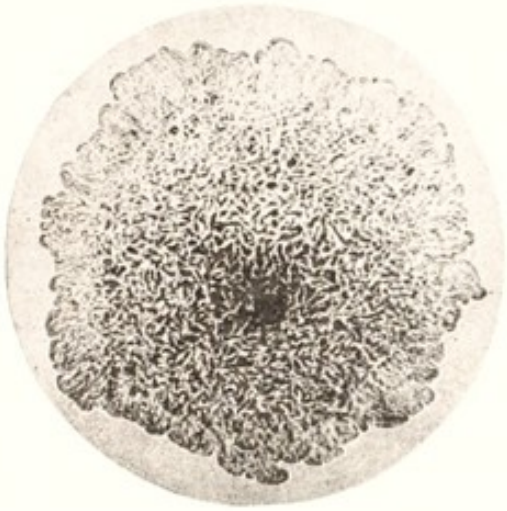


Fig. 249.—Bacillus typhi abdominalis; superficial colony two days old, as seen upon the surface of a gelatin plate. × 20 (Heim).

may take place. The surface is then seared with a hot iron and material for cultures obtained by introducing a platinum loop into the substance of the organ through the sterilized surface.

Cultures may be more easily obtained from the blood of the living patients. (See "Blood culture," under the section "Bacteriologic Diagnosis.")

The bacilli can also be secured from the alvine discharges of typhoid patients during the second and third weeks of the disease.

Cultivation.—The bacillus grows well upon all culture-media under both aërobic and anaërobic conditions.

Colonies.—The deep colonies upon gelatin plates appear under the microscope of a brownish-yellow color and spindle-shape, and are sharply circumscribed. When superficial, however, they become larger and form a thin, bluish, iridescent layer with notched edges. The superficial colonies are often described as resembling grapevine leaves in shape. The center of the superficial colonies is the only

portion which shows the yellowish-brown color. The gelatin is not liquefied.

Gelatin Punctures.—When transferred to gelatin puncture cultures, the typhoid bacilli develop along the entire track of the wire, with the formation of minute, confluent, spheric colonies. A small, thin, whitish layer develops upon the surface near the center. The gelatin is not liquefied, but is sometimes slightly clouded in the neighborhood of the growth.

Agar-agar.—The growth upon the surface of obliquely solidified gelatin, agar-agar, or blood-serum is not luxuriant. It forms a thin, moist, shining, translucent band with smooth edges and a grayish-yellow color.

Potato.—When potato is inoculated and stood in the incubating oven, no growth can be seen even at the end of the second day, but if the surface of the medium be touched with a platinum wire, it is found entirely covered with a rather thick, invisible layer of sticky vegetation which the microscope shows to be made up of bacilli. This is described as the *invisible growth*. Unfortunately, it is not a constant characteristic, for occasionally a typhoid bacillus will show a distinct yellowish or brownish color. The typical growth seems to take place only when the reaction of the potato is acid.

Bouillon.—In bouillon the only change produced by the growth of the bacillus is a diffuse cloudiness. Rarely a pellicle is formed. When sugars are added to the bouillon the typhoid bacillus is found to form acid from dextrose, levulose, galactose, mannite, maltose, and dextrin, but not to form acid from lactose or saccharose. No gas is formed in the fermentation tube with any of the sugars. No indol is formed.

Milk.—In milk containing litmus a very slight and slow acidity is produced, which later gives place to distinct alkalinity. The milk is not coagulated.

Vital Resistance.—The organisms grow well at all ordinary temperatures. The thermal death-point is given by Sternberg at 56°C., destruction being effected in ten minutes. Upon ordinary culture-media, the organisms remain alive for several months if drying is prevented. In carefully sealed agar-agar tubes Hiss found the organism still living after thirteen years. According to Klemperer and Levy,* the bacilli can remain vital for three months in distilled water, though in ordinary water the commoner and more vigorous saprophytes outgrow them and cause their disappearance in a few days. There seems to be some doubt, however, on this point, as Tavel† found that it lived for six months in the blind terminal of a water-supply pipe, and Hofmann,‡ after planting it in an aquarium con-

* "Clinical Bacteriology." Translated by A. A. Eshner, Phila., W. B. Saunders Co., 1900.

† "Centralbl. f. Bakt. u. Parasitenk.," 1903, XXXIII, p. 166.

‡ "Archiv. f. Hyg.," 1905, LII, 2, 208.

taining fish, snails, water-plants, and protozoa, was able to recover it from the water after thirty-six days, and from the mud in the bottom after two months. In elaborate experimental studies of this question Jordan, Russel, and Zeit* found its longevity to be only three or four days under conditions resembling as nearly as possible those found in nature. When buried in the upper layers of the soil the bacilli retain their vitality for nearly six months. Robertson† found that when planted in soil and occasionally fed by pouring bouillon upon the surface, the typhoid bacillus maintained its vitality for twelve months. He suggests that it may do the same in the soil about leaky drains.

Cold has little effect upon typhoid bacilli, for some can withstand freezing and thawing several times. Observing that epidemics of typhoid fever had never been traced to polluted ice, Sedgwick and Winslow‡ made some investigations to determine what quantitative reduction might be brought about by freezing, and accordingly experimentally froze a large number of samples of water intentionally infected with large numbers of typhoid bacilli from different sources. It was found that the bacilli disappeared in proportion to the length of time the water was frozen, and that the reduction averaged 99 per cent. in two weeks. The last two or three bacilli per thousand appeared very resistant and sometimes remained alive after twelve weeks.

They have been found to remain alive upon linen from sixty to seventy-two days, and upon buckskin from eighty to eighty-five days.

The typhoid bacillus resists the action of chemic agents rather better than most non-sporogenous organisms. The addition of from 0.1 to 0.2 per cent. of carbolic acid to the culture-media is without effect upon its growth. At one time the tolerance to carbolic acid was thought to be characteristic, but it is now known to be shared by other bacteria (colon bacillus). It is killed by 1 : 500 bichlorid of mercury solutions and 5 per cent. carbolic acid solutions in five minutes.

Metabolic Products.—The typhoid bacillus does not produce indol. It produces a small amount of acid when grown in sugar-containing media, but its regular tendency is to form alkalies, as is shown by the reactions in litmus milk. It forms no coagulating or proteolytic enzymes.

Toxic Products.—The disproportion of local to constitutional disturbance in typhoid fever and the irritative and necrotic character of its lesions suggest that we have to do with a toxic organism. Brieger and Fränkel have, indeed, separated a toxalbumin, which they thought to be the specific poison, from bouillon cultures. When

* "Journal of Infectious Diseases," 1904, I, p. 641.
† "Brit. Med. Jour.," Jan. 8, 1898.
‡ "Jour. Boston Soc. of Med. Sci.," March 20, 1900, vol. IV, No. 7, p. 181.

injected into guinea-pigs the typhotoxin of Brieger causes salivation, accelerated respiration, diarrhea, mydriasis, and death in from twenty-four to forty-eight hours. Klemperer and Levy also point out, as affording clinical proof of the presence of toxin, the occasional fatal cases in which the typical picture of typhoid has been without the characteristic postmortem lesions, the diagnosis being made by the discovery of the bacilli in the spleen.

Pfeiffer and Kolle* found toxic substance in the bodies of the bacilli only. It was not, like the toxins of diphtheria and tetanus, dissolved in the culture-medium. This was an obstacle to the immunization experiments of both Pfeiffer and Kolle and Löffler and Abel,† for the only method of immunizing animals was to make massive agar-agar cultures, scrape the bacilli from the surface, and distribute them through an indifferent fluid before injecting them into animals.

If the bacilli grown upon ordinary culture-media are several times washed in distilled water, and then allowed to macerate in normal salt solution, autolysis takes place and a toxin is liberated, showing that the toxin is intracellular. Macfadyen and Rowland‡ liberated an intracellular toxin from cultures of the typhoid bacilli by freezing them with liquid air and grinding them in an agate mortar. Animals immunized with this poison produced an antiserum active against it, but useless against infection with typhoid bacilli. Wright, of Netley§, observes that Macfadyen's method of securing this intracellular toxin was unnecessarily cumbersome, as the body juices of animals injected with dead cultures of the bacilli dissolve them at once and thus liberate the same toxic product.

Besredka‖ and Macfadyen** think that exotoxin is also formed. Vaughan†† has obtained poisonous and non-poisonous fractions by extracting massive cultures of typhoid bacilli with 2 per cent. solutions of sodium hydrate in absolute alcohol at 78°C.

Mode of Infection.—The typhoid bacillus enters the body by way of the alimentary tract with infected foods and water.

Rosenau, Lumsden, and Kastle‡‡ were able to connect 10 per cent. of the cases of typhoid fever occurring in the District of Columbia with infection through milk. Interesting additional facts upon the subject can be found in Bulletin No. 41 of the Hygienic Laboratory upon "Milk in its Relation to the Public Health." The bacillus occasionally enters milk in water used to dilute it or to wash the cans.

The occurrence of typhoid fever among the soldiers of the United States Army during the Spanish-American War in 1898 was shown by

* "Deutsche med. Wochenschrift," Nov. 12, 1896.
† "Centralbl. f. Bakt. u. Parasitenk.," Jan. 23, 1896, Bd. XIX, No. 23, p. 51.
‡ "Brit. Med. Jour.," 1903.
§ Ibid., April, 4, 1903, 1, p. 786.
‖ "Ann. de l'Inst. Pasteur," 1895, X, 1896, XI.
** "Centralbl. f. Bakt.," etc., 1906, 1.
†† "Amer. Jour. Med. Sci.," 1908, CXXXVI.
‡‡ "Hygienic Laboratory Bulletin No. 33," Washington, D. C., 1907.

Reed, Vaughan, and Shakespeare* to be largely the result of the pollution of the food of the soldiers by flies that shortly before had visited infected latrines.

The bacillus is also occasionally present upon green vegetables grown in soil fertilized with infected human excrement or sprinkled with polluted water, and epidemics are reported in which the occurrence of the disease was traced to oysters infected through sewage. Newsholme† found that in 56 cases of typhoid fever about one-third were attributable to eating raw shell-fish from sewage-polluted beds.

Pathogenesis.—The primary activities of the typhoid bacillus are unknown. It is supposed that it passes uninjured through the acid secretions of the stomach to enter the intestine, where local disturbances are set up. Whether during an early residence in the intestine its metabolism is accompanied by the formation of a toxic product, irritating to the mucosa, and affording the bacilli means of entrance to the lymph-vessels, through diminutive breaches of continuity, is not known. We usually find it well established in the intestinal and mesenteric lymphatics at the time we are able to recognize the disease.

It is quite certain that the chief operations of the typhoid bacillus are in the tissues and not in the intestine, as seems to be a widely prevalent error. It is contrary to most of our knowledge of the organism that it should easily adapt itself to saprophytic existence among the more vigorous intestinal organisms. Those who look for it in the feces are usually surprised at the difficulty of finding it, or at the small numbers present. It is far more easy to isolate the organism from the blood than from the feces, and much greater numbers occur in the urine than in the feces. It probably escapes from the blood into the bile, where it grows luxuriantly, and entering the gall-bladder may take up permanent residence there, escaping into the intestine each time the gall-bladder is emptied. Many bacilli thus discharged probably meet with destruction in the intestine, though some convalescents from typhoid fever for years have a periodic appearance of bacilli in the feces. Such individuals have become known as "typhoid carriers" and are a menace to the public.

In a case studied by Miller‡ bacilli were found in the gall-bladder *seven years* after recovery from typhoid fever; in a case studied by Droba§ they were found in both the gall-bladder and a gall-stone *seventeen years* after recovery from the disease; Humer,‖ found them in the gall-bladder of a patient suffering from cholecystitis, *eighteen years* after recovery from an attack of typhoid fever, and in a

* "Report on Typhoid Fever in the U. S. Military Camps in the Spanish War," vol. 1.

† "Brit. Med. Jour.," Jan., 1895.

‡ "Bull. of the Johns Hopkins Hospital," May, 1898.

§ "Wiener klin. Wochenschrift," 1899, XII, p. 1141.

‖ "Bull. of the Johns Hopkins Hospital," Aug. and Sept., 1899.

case studied by Dean,* they were present in the stools of a man *twenty-nine years* after he had had an attack of typhoid fever.

Cushing† invariably found the bacilli in the bile in clumps resembling the agglutinations of the Widal reaction. He thinks it probable that these clumps form nuclei upon which bile salts can be precipitated and calculous formation begun. The presence of gall-stones, together with the long-lived infective agents, may at any subsequent time provoke cholecystitis. Cushing collected 6 cases of operation for cholecystitis with calculi in which typhoid bacilli were present, and 5 in which Bacillus coli was present in the gall-bladder.

Fig. 250.—Intestinal perforation in typhoid fever. Observe the threads of tissue obstructing the opening. (Museum of the Pennsylvania Hospital.) (Keen, "Surgical Complications and Sequels of Typhoid Fever.")

With the most approved methods yet devised, Peabody and Pratt‡ were unable to recover the micro-organism from the intestinal contents in more than 21 per cent. of febrile cases, and only in small numbers as a rule. The greatest number was obtained when there was much blood in the stool.

There is always well-marked blood-infection during the first weeks of the disease, and upon this depends the occurrence of the rose-colored spots.

* "British Medical Journal," March 7, 1908, 1, p. 562.
† "Bull. of the Johns Hopkins Hospital," IX, No. 86.
‡ "Journal of the American Medical Association," Sept. 7, 1907, XLIX, p. 846.

The bacilli enter the solitary glands and Peyer's patches, and multiply slowly during the incubation period of the disease—one to three weeks. The immediate result of their activity in the lymphatic structures is an increase in the number of cells, the ultimate effect is necrosis and sloughing of the Peyer's patches and solitary glands. From the intestinal lymphatics the bacilli pass, in all probability, to the mesenteric nodes, which become enlarged, softened, and sometimes rupture. They also invade the spleen, liver and sometimes the kidneys, and other organs where they may be found in small clusters in properly stained specimens.

Mallory* found the histologic lesions of typhoid fever to be widespread throughout the body and not limited to the Peyer's patches of the intestine, where they are most evident. His conclusions regarding the pathology of the disease are briefly: "The typhoid bacillus produces a mild diffusible toxin, partly within the intestinal tract, partly within the blood and organs of the body. This toxin produces proliferation of the endothelial cells, which acquire for a certain length of time malignant properties. The new-formed cells are epithelioid in character, have irregular, lightly staining, eccentrically situated nuclei, abundant, sharply defined, acidophilic protoplasm, and are characterized by marked phagocytic properties. These phagocytic cells are produced most abundantly along the line of absorption from the intestinal tract, both in the lymphatic apparatus and in the blood-vessels. They are also produced by distribution of the toxin through the general circulation, in greatest numbers where the circulation is slowest. Finally, they are produced all over the body in the lymphatic spaces and vessels by absorption of the toxin eliminated from the blood-vessels. The swelling of the intestinal lymphoid tissue of the mesenteric lymph nodes and of the spleen is due almost entirely to the formation of phagocytic cells. The necrosis of the intestinal lymphoid tissue is accidental in nature and is caused through occlusion of the veins and capillaries by fibrinous thrombi, which owe their origin to degeneration of phagocytic cells beneath the lining endothelium of the vessels. Two varieties of focal lesions occur in the liver: one consists of the formation of phagocytic cells in the lymph-spaces and vessels around the portal vessels under the action of the toxin absorbed by the lymphatics; the other is due to obstruction of liver capillaries by phagocytic cells derived in small part from the lining endothelium of the liver capillaries, but chiefly by embolism through the portal circulation of cells originating from the endothelium of the blood-vessels of the intestine and spleen. The liver-cells lying between the occluded capillaries undergo necrosis and disappear. Later the foci of cells degenerate and fibrin forms between them. Invasion by polymorphonuclear leukocytes is rare."

* "Journal of Experimental Medicine," 1898, vol. III, p. 611.

". . . Histologically the typhoid process is proliferative and stands in close relationship to tuberculosis, but the lesions are diffuse and bear no intimate relation to the typhoid bacillus, while the tubercular process is focal and stands in the closest relation to the tubercle bacillus."

The growth of the bacilli in the kidneys causes albuminuria, and the bacilli can be found in the urine in about 25 per cent. of the cases. Smith* found them in the urine in 3 out of 7 cases which he investigated; Richardson,† in 9 out of 38 cases. They did not occur before the third week, and remained in one case twenty-two days after cessation of the fever. Sometimes they were present in immense numbers, the urine being actually clouded by their presence. Petruschky‡ found that albuminuria sometimes occurs without the presence of the bacilli; that their presence in the urine is infrequent; that the bacilli never appear in the urine in the early part of the disease, and hence are of little importance for diagnostic purposes. Gwyn§ has found as many as 50,000,000 typhoid bacilli per cubic centimeter of urine, and mentions a case of Cushing's in which the *bacilli persisted in the urine for six years after the primary attack of typhoid fever.* Their occurrence, no doubt, depends primarily upon a typhoid bacteremia, by which they are brought to the kidney. Their persistence in the urine after recovery from typhoid fever, depends upon continued growth in the bladder and not upon continuous escape from the blood. It is of importance from a sanitary point of view to remember that the urine as well as the feces is infectious.

The bacilli pass from the lymphatics to the general circulation, so that all cases of typhoid fever are true *bacteremias* during part or all of their course.

Bacilli can be found in the circulating blood. The eruption may be regarded as one of the local irritative manifestations of the bacillus, as the blood from the roseolæ always contains them, and Richardson‖ found it necessary to examine a number of spots in each case. He carefully disinfected the skin, freezing it with chlorid of ethyl, making a crucial incision, and cultivating from the blood thus obtained. He was able to secure the typhoid bacillus in 13 out of 14 cases examined.

As a means of diagnosis the matter is of some importance, as the rose spots may precede the occurrence of the Widal reaction by a number of days.

In rare instances the bacillus may be found in the expectoration, especially when pulmonary complications arise in the course of the

* "Brit. Med. Jour.," Feb. 13, 1897.
† "Journal of Experimental Medicine," May, 1898.
‡ "Centralbl. f. Bakt. u. Parasitenk.," May 13, 1898, No. 13, p. 577.
§ "Phila. Med. Jour.," March 3, 1900.
‖ "Phila. Med. Jour.," March 3, 1900.

disease. Cases of this kind have been reported by Chantemesse and Widal* and Fränkel.†

The pyogenic power of the typhoid bacillus was first pointed out by A. Fränkel, who observed it in a suppuration that occurred four months after convalescence. Low‡ found virulent typhoid bacilli in the pus of abscesses occurring from one to six years after convalescence.

Weichselbaum has seen general peritonitis from rupture of the spleen in typhoid fever, with escape of the bacilli. Otitis media, ostitis, periostitis, and osteomyelitis are common results of the lodgment of the bacilli in bony tissue. Ohlmacher§ has found the bacilli in suppurations of the membranes of the brain. The bacilli are also encountered in other local suppurations occurring in or following typhoid fever. Flexner and Harris‖ have seen a case in which the distribution of the bacilli was sufficiently widespread to constitute a real septicemia.

Lower Animals.—Typhoid fever is communicable to animals with difficulty. They are not infected by bacilli contained in fecal matter or by the pure cultures mixed with the food, and are not injured by the injection of blood from typhoid patients. Gaffky failed completely to produce any symptoms suggestive of typhoid fever in rabbits, guinea-pigs, white rats, mice, pigeons, chickens, and calves, and found that Java apes could feed daily upon food polluted with typhoid bacilli for a considerable time, yet without symptoms. Grünbaum** produced typhoid fever in chimpanzees by inoculating them with the bacillus. The introduction of virulent cultures into the abdominal cavity of animals is followed by peritonitis.

Germano and Maurea†† found that mice succumbed in from one to three days after intraperitoneal injection of 1 or 2 cc. of a twenty-four-hour-old bouillon culture. Subcutaneous injections in rabbits and dogs caused abscesses.

Lösener found the introduction of 3 mg. of an agar-agar culture into the abdominal cavity of guinea-pigs to be fatal.

Petruschky‡‡ found that mice convalescent from subcutaneous injections of typhoid cultures frequently suffered from a more or less widespread necrosis of the skin at the point of injection.

Prophylaxis.—One of the most important and practical points for the physician to grasp in relation to the subject of typhoid fever is the highly infective character of the discharges, *both feces and urine*.

* "Archiv. de physiol. norm. et. path.," 1887.
† "Deutsche med. Wochenschrift," 1899, XV, XVI.
‡ "Sitz. der k. k. Gesellschaft d. Aerzt. in Wien," "Aerztl. Central-Anz.," 1898, No. 3.
§ "Jour. Amer. Med. Assoc.," Aug. 28, 1897.
‖ "Bull. Johns Hopkins Hospital," Dec., 1897.
** "Brit. Med. Jour.," April 9, 1904.
†† "Ziegler's Beiträge," Bd. XII, Heft 3, p. 494.
‡‡ "Zeitschrift für Hygiene," 1892, Bd. XII, p. 261.

In every case the greatest care should be taken for their proper disinfection, a rigid attention paid to all the details of cleanliness in the sick-room, and the careful sterilization of all articles which are soiled by the patient. If country practitioners were as careful in this particular as they should be, the disease would be much less frequent in regions remote from the filth and squalor of large cities with their unmanageable slums, and the distribution of the bacilli to villages and towns, by milk, and by watercourses polluted in their infancy, might be checked.

In large cities where typhoid fever has been endemic the incidence of the disease has been enormously reduced by purification of the water-supply. Where this measure is not possible, the safety of the individual citizens can be promoted by using bottled pure waters for drinking purposes or by boiling the water for domestic consumption.

In military camps, etc., the fly as a carrier of the infection must first be excluded from the latrines and then as well from the kitchens and mess tents. When epidemics are in progress, green vegetables and oysters that may be polluted by infected water must be guarded against.

Prophylactic Vaccination.—Following the principle of Haffkine's anticholera inoculations, Pfeiffer and Kolle,* Wright,† and Wright and Semple‡ have used subcutaneous injections of sterilized cultures as a prophylactic measure. One cubic centimeter of a bouillon culture sterilized by heat was used.

The "Indian Medical Gazette" gives the following important figures showing what was accomplished in 1899: Among the British troops in India there were 1312 cases of typhoid fever, with 348 deaths (25 per cent.). The ratio of admissions to the total strength was 20.6 per 1000. There were 4502 inoculations, and among them there were only 9 deaths from typhoid fever—0.2 per cent. of the strength. There were 44 admissions, giving 0.98 per cent. of the strength. Among the non-inoculated men of the same corps and at the same stations, of 25,851 men there were 675 cases and 146 deaths, giving the relative percentages of admissions and deaths as 2.54 and 0.56.§

In a later contribution, Wright‖ showed that the prophylactic vaccination against typhoid fever reduced the number of cases and diminished the death-rate among the inoculated, and also called attention to the slight risk the inoculated run of being injured in case their vital resistance is below normal, or they are already in the early stages of the disease, or where the dose administered is too large, or the second vaccination given too soon after the first.

* "Deutsche med. Wochenschrift," 1896, XXII; 1898, XXIV.
† "Lancet," Sept., 1896.
‡ "Brit. Med. Jour.," 1897, I, p. 256.
§ "Phila. Med. Jour.," Oct. 13, 1900, p. 688.
‖ "The Lancet," Sept. 6, 1902.

In 1903 Wright* published new statistics on the subject, and between 1903 and 1908 numerous references to the subject appear in the "British Medical Journal," in the "Lancet," and in the "Journal of the Royal Army Medical Corps," all favorable in their general attitude.

During the Mexican Revolution of 1911, the United States Government began, on March 10, 1911, the mobilization of regiments of the United States Army on the Mexican frontier near San Antonio, Texas. In order to prevent repetition of the sad experiences of the Spanish-American War, in which the troops suffered terribly from typhoid fever, the Secretary of War determined that the entire command should be immunized against the disease. Many of the soldiers arriving on the ground had already been immunized, the remainder were at once given the necessary injection upon arrival. The mean strength of the command at San Antonio was 12,000 up to June 30, 1911, a period approximating four months. During all that time there were only 2 cases of typhoid fever in the encampment, 1 in an uninoculated civilian teamster and 1 in an inoculated soldier. Both cases recovered. The soldier suffered from so mild an attack that it would not have been diagnosed had not a blood-culture been made. During the four months from March 10th to June 30th the typhoid fever was prevalent among the civilians of San Antonio, there being 40 cases with 19 deaths.†

The prophylactic used was prepared from a specially selected strain of Bacillus typhosus grown on agar-agar in Kolle flasks for twenty-four hours. The growth was washed off with normal salt solution, killed by heating at 55° to 56°C. in a water-bath, standardized by counting the bacteria according to the method of Wright, and after being diluted with salt solution, 0.25 per cent. of trikresol was added. One cubic centimeter of the finished prophylactic contained 1,000,000,000 bacilli. The first dose injected contained 500,000,000 bacilli, the second and third, given after ten and twenty days, contained 1,000,000,000 each. The injections caused little inconvenience either locally or constitutionally. Only 1 case had fever, chills, and sweats, and this was the only case requiring treatment in the hospital. It subsequently developed that this soldier was suffering from early tuberculosis, which may explain the untoward symptoms from which he suffered.

Specific Therapy.—Animals can be immunized to this bacillus, and then, according to Chantemesse and Widal, develop antitoxic blood capable of protecting other animals. Stern‡ found in the blood of human convalescents a substance thought to have a protective effect upon infected guinea-pigs. His observation is in accordance with a

* "Brit. Med. Jour.," Oct. 10, 1903.

† "Report of the Surgeon-General of the United States Army to the Secretary of War," 1911, Washington, D. C.

‡ "Zeitschrift für Hygiene," 1894, XVI, p. 458.

previous one by Chantemesse and Widal, and has recently been abundantly confirmed.

The immunization of dogs and goats by the introduction of increasing doses of virulent cultures has been achieved by Pfeiffer and Kolle* and by Löffler and Abel.† From these animals immune serums were secured.

Walger‡ reported 4 cases treated successfully with a serum obtained from convalescent patients. Ten cubic centimeters were given at a dose, and the injection was repeated in 1 case with relapse.

Rumpf§ and Kraus and Buswell‖ report a number of cases of typhoid favorably influenced by hypodermic injections of small doses of sterilized cultures of Bacillus pyocyaneus.

Jez** believes that the antitoxic principle in typhoid fever is contained in some of the internal organs instead of the blood, and claims

Fig. 251.—Typhoid bacilli, unagglutinated (Jordan).

Fig. 252.—Typhoid bacilli, showing typical clumping by typhoid serum (Jordan).

to have obtained remarkable results in 18 cases treated with extracts of the bone-marrow, spleen, and thymus of rabbits previously injected with the typhoid bacillus.

Chantemesse,†† Pope,‡‡ and Steele§§ have all used serums from animals immunized against typhoid cultures for the treatment of typhoid fever, with more or less success but an analysis of the results shows them to be very inconclusive.

The serum prepared by Macfadyen,‖‖ by crushing cultures

* "Centralbl. f. Bakt. u. Parasitenk.," Jan. 23, 1896, Bd. XIX, No. 23, p. 51.
† Ibid, 1896.
‡ "Münchener med. Wochenschrift," Sept. 27, 1898.
§ "Deutsche med. Wochenschrift," 1893, No. 41.
‖ "Wiener klin. Wochenschrift," July 12, 1894.
** "Méd. moderne," March 25, 1899.
†† "Gaz. des Hôpitaux," 1898, LXXI, p. 397.
‡‡ "Brit. Med. Jour.," 1897, 1, 259.
§§ Ibid., April 17, 1897.
‖‖ "Brit. Med. Jour.," April 3, 1903.

frozen with liquid air and injecting animals with the thus liberated intracellular toxin, seems to be no improvement upon others.

Meyer and Bergell* prepared a serum by injecting horses with a new typhoid toxin. After two years' treatment they were able to demonstrate its value in curing infection in laboratory animals. von Leyden† speaks in favorable terms of this serum.

The typhoid immune (bacteriolytic) serum is specific, but its action requires the presence of additional complementary substance, and by itself it is useless. Indeed, it may do harm by causing the formation of anti-immune bodies.

So far no serum has been produced that is of any certain value in therapeutics.

Bacteriologic Diagnosis.—There are four bacteriologic methods that may assist the clinician in completing the diagnosis of typhoid fever. In the order of their general usefulness and practicability these are:

1. The Widal reaction of agglutination.
2. The blood-culture.
3. The isolation of the bacillus from the feces.
4. The conjunctival and dermal reactions.

Widal Reaction of Agglutination.—This very valuable adjunct to our means of making the diagnosis of atypical and obscure cases of typhoidal infection was discovered in 1896 when Widal and Grünbaum,‡ working independently, observed that when blood-serum from typhoid fever patients is added to cultures of the typhoid bacillus a definite reactive phenomenon occurs. The phenomenon, now familiarly known as the "Widal reaction," consists of complete loss of the motion so characteristic of the typhoid bacillus, and the collection of the micro-organisms into clusters or groups—agglutination. The bacteria frequently appear shrunken and partly dissolved.

The technic of the test is outlined in the section upon Agglutination (*q.v.*). For the use of the practising physician, commercial houses now furnish various outfits known as "agglutometers," in which are found such simple apparatus and directions as will enable those inexpert in laboratory manipulations to arrive at fairly accurate results.

The Blood-culture.—The technic of this operation is simple. The skin of the fold of the elbow is thoroughly cleansed, a fillet put about the arm, and as the veins become prominent, a sterile hypodermic needle is introduced into one and about 10 cc. of blood drawn into a Keidel tube or into a syringe. Before clotting can take place, this is discharged into a small flask containing 100 cc. of bouillon, mixed, and stood away to incubate. After twenty-four hours the bacilli can usually be found in pure culture.

* "Med. Klinik," III, No. 31, p. 917, Aug. 4, 1907.
† "Berl. klin. Wochenschrift," 1907, No. 18.
‡ "La Semaine Médicale," 1896, p. 295.

In case the culture is not pure, the typhoid bacillus can be separated from contaminating organisms by plating.

The Isolation of the Bacillus from the Feces.—This method of making the diagnosis has practically been abandoned because of its uncertainty, its cumbersomeness, its tediousness, and because the preceding methods suffice in all cases.

An excellent résumé of the many methods employed for isolating the bacillus from the stools has been published by Peabody and Pratt,* and is appropriate reading for those interested in this subject.

The Conjunctival Reaction.—An additional aid to the diagnosis of typhoid in doubtful cases based upon the Wolff-Eisner-Calmette reaction in tuberculosis is the "ocular typhoid reaction" of Chantemesse.† This test consists in the instillation into the eye of a solution made by extracting the typhoid bacillus as follows: "Gelatin plates covered with an eighteen- to twenty-hour-old culture of virulent typhoid bacilli were washed with 4 to 5 cc. of sterile water. The suspension thus obtained was heated to 60°C., centrifugated, and the supernatant fluid withdrawn. The centrifugated organisms were then dried and triturated. A second suspension of these broken up bacillary bodies was then made, and allowed to stand for from two to three days at 60°C. The extract thus obtained, after removing the disintegrated and digested remnants, was precipitated with alcohol, forming a fine coagulum. This was subsequently dried, powdered and dissolved in sterile water in the proportion of 0.02 mg. to a drop."‡

When one drop of this is placed upon the conjunctiva of a patient in the early days of typhoid fever, diffuse redness increases and becomes marked in two or three hours. There is also some feeling of heat in the eye. Tears flow freely, and there is a slight mucopurulent exudate in some cases. The reaction persists about ten hours and then declines, usually disappearing in twenty-four hours. Hamburger§ confirmed the results of Chantemesse. It is too early to say how useful the reaction is, but it seems to promise aid in diagnosing difficult cases.

Differential Diagnosis of the Typhoid and Colon Bacilli.—This constitutes the chief perplexity of bacteriologic work with the typhoid bacillus, and is the great bugbear of beginners. A great deal of energy has been expended upon it, a considerable literature has been written about it, and much still remains to be learned by which it may be simplified.

Two chief methods are in vogue at present:

1. The serum differentiation.
2. The culture differentiation.

* "Boston Medical and Surgical Journal," 1907.
† "Deutsche med. Wochenschrift," 1907, No. 31, p. 1264.
‡ See Hamburger, "Jour. Amer. Med. Assoc.," L, 17, p. 1344, April 25, 1908.
§ Loc. cit.

Serum Differentiation.—The specific agglutinating action of experimentally prepared serums can be used to differentiate cultures of the colon, paracolon, typhoid, and paratyphoid bacilli, the typhoid bacilli alone exhibiting the specific effect of the typhoid serum. This is a very reliable means of differentiation when the cultures have already been isolated. The method is described under the heading "Agglutination," in the section devoted to the "Special Phenomenon of Infection and Immunity."

Richardson* has found it very convenient to saturate filter-paper with typhoid serum, dry it, cut into 0.5 cm. squares, and keep it on hand in the laboratory for the purpose of making this differentiation. To make a test, one of these little squares is dropped in 0.5 cc. of a twenty-four-hour-old bouillon culture of the suspected bacillus and allowed to stand for five minutes. A drop of the fluid placed upon a slide and covered will then show typical agglutinations if the culture be one of the typhoid fever bacillus. In a second mention of this method† he has found its use satisfactory in practice and the paper serviceable after fourteen months' keeping.

The Cultural Differentiation.—When the typhoid bacilli are to be isolated from the blood of living patients, they are so likely to be obtained in pure culture that little trouble is experienced. If they are to be isolated from the pus of a posttyphoidal abscess, or from viscera at autopsy, from water suspected of pollution, and especially when they are to be isolated from the intestinal contents, with its rich bacterial flora, the matter becomes progressively complicated.

As the colonies of the typhoid bacilli closely resemble those of Bacillus coli, etc., special media have, from time to time, been devised for the purpose of emphasizing such differences as rapidity of growth, acid production, etc. Thus, Elsner‡ has suggested the employment of a special medium made as follows:

One kilogram of grated potatoes (the small red German potatoes are best) is permitted to macerate over night in 1 liter of water. The juice is carefully pressed out and filtered cold, to get rid of as much starch as possible. The filtrate is boiled and again filtered. The next step is a neutralization, for which Elsner used litmus as an indicator, and added 2.5 to 3 cc. of a $\frac{1}{10}$ normal sodium hydrate solution to each 10 cc. of the juice. Abbott prefers to use phenolphthalein as an indicator. The final reaction should be slightly acid. Ten per cent. of gelatin (no peptone or sodium chlorid) is dissolved in the solution, which is boiled, and must then be again neutralized to the same point as before. After filtration the medium receives the addition of 1 per cent. of potassium iodid; then it is filled into tubes and sterilized like the ordinary culture-media.

When water or feces suspected to contain the typhoid bacillus are mixed in this medium and poured upon plates, no bacteria develop well except the typhoid and colon bacilli.

These, however, differ markedly in appearance, for the colon colonies appear of the usual size in twenty-four hours, at which time the typhoid bacillus, if present, will have produced no colonies discoverable by the microscope.

It is only after forty-eight hours—long after the colon colonies have become conspicuous—that little colonies of the typhoid bacillus appear as finely granular, small, round, shining, dew-like points, in marked contrast to their large, coarsely granular predecessors.

* "Centralbl. f. Bakt. u. Parasitenk.," 1897, p. 445.
† "Journal of Experimental Medicine," May, 1898, p. 353, note.
‡ "Zeitschrift für Hygiene," 1895, XXII, Heft 1; Dec. 6, 1896.

Unfortunately, many of the small colonies that develop in Elsner's medium subsequently prove to be those of the colon bacillus, and the method is thus rendered unreliable.

Rémy* prefers to make an artificial medium approximating a potato in composition, but without dextrin or glucose. The composition is as follows:

Distilled water	1000.0	grams
Asparagin	6.0	"
Oxalic acid	0.5	"
Lactic acid	0.15	"
Citric acid	0.15	"
Disodic phosphate	5.0	"
Magnesium sulphate	2.5	"
Potassium sulphate	1.25	"
Sodium chlorid	2.0	"

All the salts excepting the magnesium sulphate are powdered in a mortar and introduced into a flask with the distilled water. Thirty grams of Witte's or Grubler's peptone are then added and the mixture heated in the autoclave under pressure for one-quarter hour. As soon as removed, the contents are poured into another flask into which 120 to 150 grams of gelatin had previously been placed. The flask is shaken to dissolve the gelatin, and the contents then made slightly alkaline with soda solution. The mixture is again heated in the autoclave at 110°C., for one-quarter hour, then acidified with a one-half normal solution of sulphuric acid, so that 10 cc. have an acidity neutralized by 0.2 cc. of one-half normal soda solution. This acidity is equal to 0.5 cc. sulphuric acid per liter. After shaking, place the flask in a steam sterilizer for ten minutes, then filter. When filtered, verify the acidity of the medium, correcting if necessary. Finally, add the magnesium sulphate, dissolve, dispense in tubes, and sterilize by the intermittent method.

At the moment of using, put into each tube 1 cc. of a 35 per cent. solution of lactose and 0.1 cc. of a 2.5 per cent. solution of carbolic acid.

Upon this medium the colonies of the typhoid and colon bacilli show marked differences. The colon colonies are yellowish brown, the typhoid colonies bluish white and small. Fine bubbles of gas from the fermentation of the lactose often occur about the colon colonies.

By this method Rémy was able to isolate the typhoid bacillus from the stools in 23 cases which he studied. He believes that the constant presence of the typhoid bacillus in the stools of typhoid fever, and its absence from them under all other conditions, is a far more important and valuable method of diagnosis than even the Widal reaction.

Würtz† and Kashida‡ make the differential diagnosis by observing the acid production of Bacillus coli in a medium consisting of bouillon containing 1.5 per cent. of agar, 2 per cent. of milk-sugar, 1 per cent. of urea, and 30 per cent. of tincture of litmus. This is the so-called *litmus-lactose-agar-agar*. The culture-medium should be blue. When liquefied, inoculated with the colon bacillus, poured into Petri dishes, and stood for from sixteen to eighteen hours in the incubator, the blue color passes off and the culture-medium becomes

* "Ann. de l'Inst. Pasteur," Aug., 1900.

† "Archiv. de med. Experimentale," 1892, IV, p. 85.

‡ "Centralbl. f. Bkt. u. Parasitenk, June 24, 1897," Bd. XXI, Nos. 20 and 21.

red. If a glass rod dipped in hydrochloric acid be held over the dish, vapor of ammonium chlorid is given off. The typhoid bacillus produces no acid in this medium, and there is consequently no change in its color. Upon plates with colonies of both bacilli, the typhoid colonies produce no change of color, while the colon colonies at once redden the surrounding medium.

Rothberger* first employed *neutral red* for the differentiation of the typhoid and colon bacilli. When grown in fluid media containing it, the colon bacillus produces a yellowish fluorescence, while the typhoid bacillus does not destroy the port-wine color. Savage† and Irons‡ have made use of the color reaction for the routine detection of the colon bacillus in water. The best adaptation of the method is by Stokes,§ who adds it to the various sugar bouillons in the proportion of 0.1 gram per liter, and uses the medium in the fermentation tube. The colon bacillus always ferments the sugars and produces a typical color reaction.

Hiss‖ recommends the use of two special media.

The first consists of 5 grams of agar-agar, 80 grams of gelatin, 5 grams of Liebig's beef-extract, 5 grams of sodium chlorid, and 10 grams of glucose to the liter. The agar is dissolved in the 1000 cc. of water, to which have been added the beef-extract and sodium chlorid. When the agar is completely melted, the gelatin is added and thoroughly dissolved by a few minutes' boiling. The medium is then titrated to determine its reaction, phenolphthalein being used as the indicator, and enough HCl or NaOH added to bring it to the desired reaction—*i.e.*, a reaction indicating 1.5 per cent. of normal acid. To the clear medium add one or two eggs, well beaten in 25 cc. of water; boil for forty-five minutes, and filter through a thin layer of absorbent cotton. Add the glucose after clearing.

This medium is used in tubes, in which the culture is planted by the ordinary puncture. *The typhoid bacillus alone has the power of uniformly clouding this medium* without showing streaks or gas-bubbles.

The second medium is used for *plating*. It contains 10 grams of agar, 25 grams of gelatin, 5 grams of beef-extract, 5 grams of sodium chlorid, and 10 grams of glucose. The method of preparation is the same as for the tube-medium, care always being taken to add the gelatin after the agar is thoroughly melted, so as not to alter this ingredient by prolonged exposure to high temperature. The preparation should never contain less than 2 per cent. of normal acid. Of all the organisms upon which Hiss experimented with this medium, *Bacillus typhosus alone displayed the power of producing thread-forming colonies.*

The colonies of the typhoid bacillus when deep in Hiss' medium appear small, generally spheric, with a rough, irregular outline, and, by transmitted light, of a vitreous greenish or yellowish-green color. The most characteristic feature consists of well-defined filamentous outgrowths, ranging from a single thread to a complete fringe about the colony. The young colonies are, at times, composed solely of threads. The fringing threads generally grow out nearly at right angles to the periphery of the colony.

The colonies of the colon bacillus appear, on the average, larger than those of the typhoid bacillus; they are spheric or of a whetstone form, and by transmitted light are darker, more opaque, and less refractive than the typhoid colonies. By reflected light they are pale yellow to the unaided eye.

Surface colonies are large, round, irregularly spreading, and are brown or yellowish-brown in color. Hiss claims that by the use of these media the typhoid bacillus can readily be detected in typhoid stools.

* "Centralbl. f. Bakt.," 1893, p. 187.
† "Journal of Hygiene," 1901, I, p. 437.
‡ Ibid., 1902, II, p. 437.
§ "Jour. of Infectious Diseases," 1904, I, p. 341.
‖ "Jour. of Experimental Medicine," Nov., 1897, vol. II, No. 6.

Piorkowski* recommends a culture-medium composed of urine two days old, to which 0.5 per cent. of peptone and 3.3 per cent. of gelatin have been added. Colonies of the typhoid bacillus appear radiated and filamentous; those of the colon bacillus, round, yellowish, and sharply defined at the edges. The cultures should be kept at 22°C., and the colonies should appear in twenty-four hours.

Adami and Chapin* have suggested a method for the isolation of typhoid bacilli from water, in which use is made of the agglutination of the bacilli by immune serum.

Two quart bottles (Winchester quarts) are carefully sterilized and filled with the suspected water with an addition of 25 cc. of nutrient broth and incubated for eighteen to twenty-four hours at 37°C. By this time the typhoid bacillus grows abundantly in spite of the small amount of nourishment the water contains. At the end of the incubation, 10 cc. of the fluid is filled into each of a number of long narrow (7 mm.) test-tubes made by sealing a glass tube one-half meter long at one end. About 1 inch from the bottom the tube is filed completely round so as to break easily at that point. The different tubes next receive additions of typhoid immune serum sufficient to make the dilutions 1:60, 1:100, 1:150, and 1:200. If typhoid bacilli are present, within a quarter of an hour beginning agglutination can be seen, and by the end of two to five hours flocculent masses collect at the bottom of the tube, forming a flocculent precipitate. The next procedure should be with the tube showing agglutination with the greatest dilution, as the more concentrated preparations carry down not only the typhoid bacilli, but also closely related organisms. After the sedimentation of the agglutinated bacilli is complete, the tube is broken at the file mark, and the sediment contained in the short tube washed with two or three changes of distilled water, being allowed to settle each time. This removes many of the organisms not agglutinated. A loopful of the washed sediment is transferred to a tube of nutrient broth, and finally from this tube plate cultures are made upon Elsner's or Hiss' media.

A culture-medium for isolating the typhoid bacillus from feces is recommended by Drigalski-Conradi† and by Petkowitsch.‡ It is made as follows:

Horse-meat infusion (3 pounds of horse meat to 2 liters of water)	2 liters
Witte's peptone	20 grams
Nutrose	20 grams
Sodium chlorid	10 grams
Agar-agar	60 grams
Litmus solution (Kubel and Tiemann)	260 cc.
Lactose	30 grams
Crystal-violet solution (0.01 per cent.)	20 cc.

Before adding the crystal-violet solution render feebly alkaline to litmus (about 0.04 per cent. of pure soda).

Colon colonies upon this medium appear in fourteen to sixteen hours to be red and opaque. Typhoid colonies blue or violet, transparent and drop-like.

Beckman§ modifies the preparation, making it as follows:

* "Berliner klin. Wochenschrift," Feb. 13, 1899.

† "Zeitschrift f. Hygiene," Bd. XXIX.

‡ "Centralbl. f. Bakt.," etc., May 28, 1904, Bd. XXXVI, No. 2, p. 304.

§ See F. F. Wesbrook, "Jour. Infectious Diseases," May, 1905, Supplement, No. 1, p. 319.

(*a*) Add 1 liter of water to 680 grams of finely chopped lean beef and place in the cold for twenty-four hours. Express the juice and make up to 1 liter. Coagulate the albumin, either by boiling for ten minutes or by heating to 120°C. in the autoclave. Filter. Add 10 grams of Witte's peptone, 10 grams of nutrose, and 5 grams of sodium chlorid. Heat in the autoclave at a temperature of 120°C. for thirty minutes, or boil vigorously for fifteen minutes. Render slightly alkaline to litmus paper. Filter. Add 30 grams of agar. Heat in the autoclave at a temperature of 120°C. for one-half hour, or heat over the gas-flame until the agar is dissolved. Render slightly alkaline to litmus paper while hot, if necessary. Filter through glass wool into a sterile vessel.

(*b*) To 130 cc. of litmus solution (Kubel and Tiemann's) add 15 grams of chemically pure lactose. Boil for ten minutes.

(*c*) Mix (*a*) and (*b*) while hot. Render slightly alkaline to litmus, if necessary. To the mixture add 2 cc. of hot sterile solution of 10 per cent. sodium hydrate in distilled water and 10 cc. of a fresh solution of Höchst's crystal violet (0.1 gram of crystal violet to 100 cc. of sterile water).

The medium is now poured into Petri dishes and is of a deep purple color. So much water of condensation forms on the solidified surface that it is an advantage to use porous clay covers (Hill) for the Petri dishes instead of the ordinary glass covers. The medium keeps well but dries up rapidly.

A very ingenious method of isolating the typhoid and colon bacilli from drinking water has been suggested by Starkey,* who uses a tubular labyrinth of glass filled with ordinary bouillon containing 0.05 per cent. of carbolic acid, or, as recommended by Somers,† Pariette's bouillon. The original formula for the latter medium is as follows:

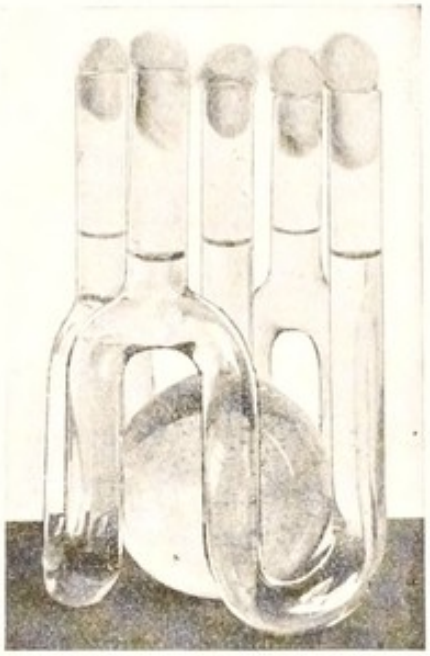


Fig. 253.—Starkey's labyrinth as modified by Somers.

1. Measure out pure hydrochloric acid, 4 cc., and add it to carbolic acid solution (5 per cent.), 100 cc. Allow the solution to stand at least a few days before use.
2. This solution is added in quantities of 0.1, 0.2, and 0.3 cc. (delivered by means of a sterile graduated pipette to tubes, each containing 10 cc. of previously sterilized nutrient bouillon).
3. Incubate at 37°C. for forty-eight hours to eliminate contaminated tubes.

The restraining medium prevents the ready growth of most organisms except colon and typhoid bacilli. The anaërobic conditions prevent the development of aërobic organisms which form the majority of bacteria with which one comes in contact in ordinary bacteriological examinations. The typhoid bacillus, being more motile than the colon, travels more quickly through the coils of the labyrinth and first arrives at its end, where it can be found in pure or nearly pure culture after about forty-eight hours.

Somers has improved the labyrinth by bending it in a circular

* "Amer. Jour. Med. Sci.," July, 1906, CXXXII, No. 1, No. 412, p. 109.
† "Trans. Phila. Path. Soc.," 1906.

form, so that it can stand alone, and by adapting its size to the Novy jar, so that satisfactory anaërobic conditions can easily be attained.

Hesse* has recommended the following medium:

Agar-agar	5 grams	(4.5 grams absolutely dry).
Witte's peptone	10	"
Liebig's beef-extract	5	"
Sodium chlorid	8.5	"
Distilled water	1000	"

Dissolve the agar-agar in 500 cc. of the water over a free flame, making up the loss by evaporation. Dissolve the other ingredient, in the remaining 500 cc. of water, heat until dissolved, replacing the loss by evaporation. Pour the two solutions together, heat for thirty minutes and add distilled water to replace loss by evaporation. Filter through cotton until clear. Adjust reaction to 1 per cent. acidity. Tube—10 cc. to a tube. Sterilize in the autoclave.

The medium is used for plating. The material containing the micro-organisms must be so dilute that only a few colonies will develop upon the plates. The typhoid colonies greatly outgrow the colon colonies and may attain to a diameter of several centimeters. They show a small opaque center and an opalescent body and appear circular.

Capaldi† recommends the following medium for plating typhoid and colon colonies:

Witte's peptone	20	grams
Gelatin	10	"
Agar-agar	20	"
Dextrose or mannite	10	"
Sodium chlorid	5	"
Potassium chlorid	5	"
Distilled water	1000	"

Dissolve the agar in 500 cc. of water, the other ingredients in the other 500 cc. of water. Pour together, add 10 cc. of NaOH, filter, and tube.

Upon this medium the typhoid colonies are small, glistening, bluish, and translucent. Colon colonies are larger, opaque, and brownish.

Endo‡ recommends the employment of the following medium upon which colonies of the typhoid bacillus grow large and remain colorless while those of the colon bacillus remain small and red:

1000 cc. of meat infusion.
30 grams of agar-agar.
10 grams of peptone (Witte's).
5 grams of sodium chlorid.

Neutralize and clear by filtration, then add 10 cc. of a 10 per cent. solution of NaOH to alkalinize, 10 grams of chemically pure lactose and 5 cc. of a filtered, saturated, alcoholic solution of fuchsin. Next add 25 cc. of a 10 per cent. sodium sulphite solution, by which the intense red given by the fuchsin is entirely bleached by the time the agar-agar is cold. After adding the necessary reagents and while still warm and perhaps red, tube the medium. The tubes should be kept in the dark.

* "Zeitschrift für Hygiene," 1908, LVIII, 441.
† "Zeitschrift für Hygiene," 1896, XXIII, 475.
‡ "Centralbl. f. Bakt.," etc., 1904, XXXV.

Löffler* has found malachite green a very useful adjunct to our means of differentiating the typhoid from other similar bacilli.

For the purpose, 2½ to 3 per cent. of a 2 per cent. solution of malachite green are added to the culture-medium. The preparation given the preference consists of 1 pound of meat macerated in 1 liter of water, neutralized with potassium, with the addition of 2 per cent. of peptone, 5 per cent. of lactose, 1 per cent. of glucose, 0.5 per cent. of sodium sulphate, 2 per cent. of nitrate of potassium, and 3 per cent. of a 2 per cent. solution of malachite green.

In the medium the ordinary cocci and bacilli do not grow, Gärtner's bacillus and the paratyphoid bacillus *b* leave the medium clear, but grow as a deposit at the bottom of the tube; the typhoid bacillus destroys the green. If agar-agar be added, the colonies are surrounded by a clear yellow zone. The colon and other organisms grow slowly if at all.

Not many workers were satisfied with the results obtained by malachite green, nor were the results obtained uniform. A careful study of the subject was made by Peabody and Pratt,† who found great differences in the quality and reactions of different malachite greens in the market. That with which Löffler worked was commercially known as "120." They obtained three samples of this dye, which varied in acidity between wide margins (0.2–1.0). Experimenting with the different preparations, they found that the least acid was the most useful preparation. The success of the method, therefore, depends upon the adjustment of the concentration of the dye to the reaction of the medium. When this is done, malachite green becomes a valuable adjunct to specific differentiation. Their studies of the media led Peabody and Pratt to the invention of a new method of isolating typhoid bacilli from the feces. Instead of employing malachite green agar-agar directly for this purpose, they first employ malachite green bouillon as an "enriching" culture, and after eighteen to twenty-four hours' growth in the incubator inoculate one or two large (20 cm. diameter) Drigalski-Conradi plates, from which the colonies can subsequently be picked out.

Bile salts were first employed in culture-media by Limbourg‡ and have been more or less popular ever since, though for differentiation of typhoid and colon bacilli they cause occasional disappointment.

Buxton and Coleman§ prepare a medium composed of:

Ox-bile	900 cc.
Glycerin	100 cc.
Peptone	20 grams

This was placed in a number of 100 cc. flasks, sterilized in the Arnold sterilizer, and employed chiefly for blood-culture. The typhoid bacillus grows well in it.

* "Boston Med. and Surg. Journal," Feb. 13, 1908, CLVIII, p. 213.

† "Zeitschrift f. physiol. Chemie," 1889, III, p. 196.

‡ "Inst. hyg. Univers. Griefswald," see "Bull. Inst. Past.," IV, No. 9, May 15, 1906, p. 393.

§ "Journal of Infectious Diseases," 1909, VI, No. 2, p. 194.

Jackson* prepares a medium for water examination when typhoid and colon bacilli are suspected. It consists of undiluted ox-bile to which 1 per cent. of peptone and 1 per cent. of lactose are added. It is filled into fermentation-tubes of 40 cc. capacity and sterilized in the Arnold apparatus. If fresh ox-bile cannot be secured, an 11 per cent. solution of dry ox-bile can be made; 10 cc. of suspected water or milk are planted in the tubes of this medium. The contained micro-organisms grow rapidly, typhoid bacilli outgrowing all others, and not fermenting the sugar; rapid fermentation and copious gas-formation take place if colon bacilli are present.

An excellent medium suggested by MacConkey† has the following composition:

Agar	1.5 grams
Sodium taurocholate	0.5 gram
Peptone	2.0 grams
Water	100.0 cc.

It is boiled, clarified, and filtered as usual, then receives an addition of 1.0 gram of lactose, is tubed, and then sterilized three times on successive days.

For determining fermentation by colon bacilli the same investigator advises a broth composed of:

Sodium taurocholate (pure)	0.5 gram
Peptone	2.0 grams
Glucose	0.5 gram
Water	100.0 cc.

Boil, filter, add sufficient neutral litmus, fill into fermentation-tubes, and sterilize at 100°C. Colon colonies appear red; typhoid, blue.

In a careful study of the bile-salt media MacConkey‡ points out an error, first discovered by Theobald Smith, that depends upon the alkali production of the colon bacillus in the absence of sugar. If too little sugar be added to the medium, the alkali production masks the acid production unless the oxygen be removed, and red colonies of the colon bacillus grown upon the medium may in time turn distinctly blue. It becomes obvious, therefore, that the medium should be as neutral as possible to the indicator used. After trial he found neutral red preferable to litmus, and makes the medium as follows:

1. A stock solution is made:

Sodium taurocholate (commercial from ox-bile and *neutral to neutral red*)	0.5 per cent.
Peptone (Witte's)	2.0 per cent.
Water (distilled or tap)	100.0 cc.

(As calcium 0.03 per cent. is favorable to the growth of the organisms, it should be added if distilled water is used.)

The ingredients should be mixed, steamed in a steam sterilizer for one to two hours, filtered while hot, allowed to stand twenty-four to forty-eight hours, then filtered cold through paper. A clear solution should then result, which will keep indefinitely under proper conditions. The various bile-salt media are prepared

* "Biological Studies of the Pupils of W. T. Sedgwick," 1906, University of Chicago Press.

† "The Thompson-Yates Laboratory Reports," III, p. 151.

‡ "Journal of Hygiene," 1908, VIII, p. 322.

from this stock solution by adding glucose, 0.5 per cent.; lactose, 1 per cent.; cane-sugar, 1 per cent.; dulcit, 0.5 per cent.; adonit, 0.5 per cent., or inulin, 1 per cent.; and neutral red (1 per cent. solution), 0.25 per cent., distributing into fermentation-tubes and sterilizing in the steamer for fifteen minutes on each of three successive days.

Bile-salt agar-agar is made by dissolving 2 per cent. of agar-agar in the stock fluid, either in the steamer or in the autoclave. The mixture is cleared with an egg, filtered, neutral red added in the same proportion as for the broth, and distributed into flasks in quantities of 80 cc. When required for use, the fermentable substance is added to the agar in the flask, and the whole placed in a water-bath or steamer (care must be taken not to heat either the fluid or solid medium beyond 100°C.). When melted, the agar preparation is poured into Petri dishes, allowed to solidify, and then dried in an incubator or warm room, the plate being placed upside down with the bottom detached and propped up on the edge of the cover. It is necessary that the surface of the agar-agar should not be too wet, lest the colonies become confluent, nor too dry, lest the growth be stunted. Inoculations are made by placing a loopful of the material to be examined on the center of one plate, and rubbed over the surface with a bent glass rod; the same rod, without recharging, being used to inoculate the surface of two other plates. The plates are then incubated upside down. The colonies of the colon bacillus appear yellow.

Bacilli Resembling the Typhoid Bacillus

Bacillus typhosus is one of a group of organisms possessing a considerable number of common characteristics, each member of which, however, can be differentiated by some one fairly well-marked peculiarity. At one end of the series is the typhoid bacillus, which we conceive to be devoid of the power to liquefy gelatin, ferment sugars, form indol, coagulate milk, or progressively form acids. At the other extreme stands Bacillus coli, an organism whose typical representatives coagulate milk, form indol, ferment dextrose, lactose, saccharose, and maltose with the formation of hydrogen and carbon dioxid in the proportion of $\frac{H}{CO_2} = \frac{2}{1}$.

Between these extremes are numerous organisms known as "intermediates." It is usually a simple matter to differentiate these forms from the typical species at the two ends of the series, but it is quite difficult to differentiate them from one another. Whether they are of sufficient importance to make it worth while to pay much attention to them is, as yet, uncertain; and, indeed, we do not know whether they are to be regarded as variations from the type species or separate and distinct organisms. The fact that some of them are associated with serious and fatal disorders—paracolon bacillus and bacillus of psittacosis—proves them, at least, to be important. Buxton* summarizes the main points of difference as follows:

	B. coli communis.	Intermediates.	B. typhosus
Coagulation of milk	+	−	−
Production of indol	+	−	−
Fermentation of lactose with gas	+	−	−
Fermentation of glucose with gas	+	+	−
Agglutination by typhoid serum.	−	−	+

* "Journal of Medical Research," vol. VIII, No. 1, June, 1902, p. 201.

The characteristics of the three groups as shown by the fermentation-test stand thus:*

	Gas upon dextrose.	Gas upon lactose.	Gas upon saccharose.
Bacillus typhosus	−	−	−
Intermediates	+	−	−
Bacillus coli communis	+	+	−
Bacillus coli communior	+	+	+

Buxton finds those pathogenic for man clinically divisible into three groups, as follows:

(*a*) *The Meat-poisoning Group.*—This includes Bacillus enteritidis of Gärtner and others. The symptoms begin soon after eating the poisonous meat, and are toxic. Bacilli quickly invade the body. The illness continues four or five days, after which recovery is quick. In a few cases death has occurred on the second or third day.

(*b*) *The Pneumonic or Psittacosis Group.*—Psittacosis is an epidemic infectious disease with pneumonic symptoms and a high mortality. Its origin has been traced to diseased parrots, and from them Nocard isolated Bacillus psittacosis, supposed to be the cause of the disease in man. Later epidemics were studied by Achard and Bensaude.

(*c*) *The Typhoidal Group.*—The organisms to be included in this group occasion symptoms closely resembling typhoid fever, though they differ biologically from the typhoid bacillus, and do not agglutinate with typhoid serums.

It is thus evident that some of the intermediates occasion symptoms resembling typhoid fever, while others occasion symptoms widely differing from it. It is suggested that to the former the term *paratyphoid bacilli* be applied, while the latter are known as *paracolon bacilli.*

Although Achard and Bensaude,† and Johnson, Hewlett, and Longcope‡ have studied the paratyphoid infections, Gwyn,§ Libman,‖ and others the paracolon bacilli, and Cushing** and Durham†† have made comparative studies of the members of the group, it is still too soon to regard the knowledge attained sufficient to warrant particular mention of the various intermediate and related organisms in a work of this kind. In the following pages, therefore, attention will be devoted only to the more important organisms of the group.

* Hiss and Zinsser, "Text-book of Bacteriology," 1910, p. 429.
† "Soc. Med.," Nov., 1896.
‡ "Amer. Jour. Med. Sci.," Aug., 1902.
§ "Johns Hopkins Bulletin," 1898, vol. IX.
‖ "Journal of Medical Research," 1902, p. 168.
** "Johns Hopkins Bulletin," 1900, vol. XI.
†† "Journal of Experimental Medicine," 1901, vol. V, p. 353.

TABLE FOR THE DIFFERENTIATION OF CERTAIN BACTERIA RESEMBLING THE TYPHOID BACILLUS

| Group | Organism | Discoverer | Morphology | | | | | Cultural Peculiarities | | | | | | | Biochemic Peculiarities | | | | | | |

Bacillus Coli Communis (Escherich)

General Characteristics.—A motile, flagellated, non-sporogenous, aërobic and optionally anaërobic, non-chromogenic, non-liquefying, aërogenic, saprophytic, occasionally pathogenic bacillus, staining by the ordinary methods, but not by Gram's method. It produces indol, coagulates milk, and produces acids and gases from dextrose, lactose, and sucrose.

This micro-organism was first isolated from human feces by Emmerich,* in 1885, who thought it to be the specific cause of Asiatic cholera, and called it Bacillus neapolitanus. Many have since studied it until it has now become one of the best known bacteria.

Distribution.—It is habitually present in the feces of animals, and in water and soil contaminated by them. Soon after birth the organism finds its way into the alimentary canal and permanently

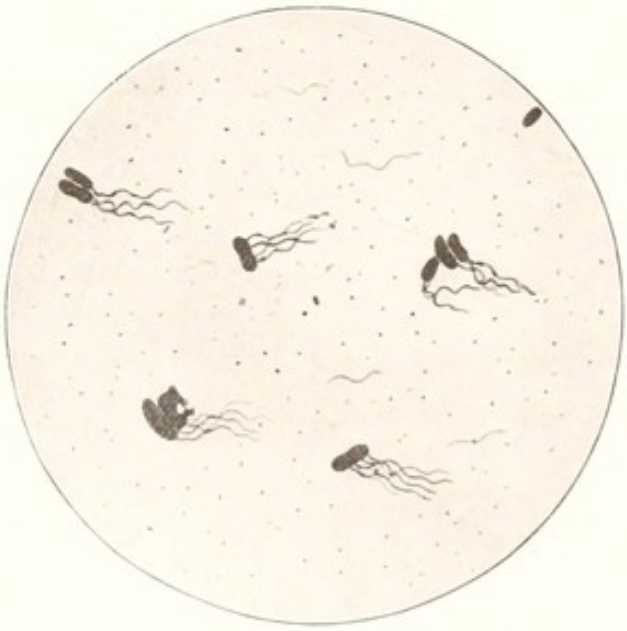


Fig. 254.—Bacillus coli (Migula).

establishes itself in the intestine, where it can be found in great numbers throughout the entire life of the individual. It is almost certainly identical with Bacillus pyogenes fœtidus of Passet, and so closely resembles B. acidi lactici that Prescott† believes them to be identical. It may also be identical with Bacillus lactis aerogenes, Bacillus cavicida, and other separately described species.

Morphology.—The bacillus is rather variable, both size and form depending to a certain extent upon the culture medium on which it grows. It measures about 1–3 × 0.4–0.7 μ. It usually occurs in the form of short rods, but coccus-like and elongate individuals may be found in the same culture. The bacilli are usually separate from one another, though occasionally joined in pairs, are actively

* "Deutsche med. Wochenschrift," 1885, No. 2.
† Society of American Bacteriologists, Dec. 31, 1902.

motile, and provided with flagella, which are variable in number, usually from four to a dozen. The organisms from some cultures swim actively, even when the culture is some days old; others are sluggish even when young and actively growing, and still other cultures consist of bacilli that scarcely move at all. It forms no endospores.

Staining.—The bacillus stains well with the aqueous solutions of the anilin dyes, but not by Gram's method.

Cultivation.—It is readily cultivated upon the ordinary media.

Colonies.—Upon gelatin plates the colonies are visible in twenty-four hours. Those situated below the surface appear round, yellow-brown, and homogeneous. As they increase in size they become opaque. The superficial colonies are larger and spread out upon the

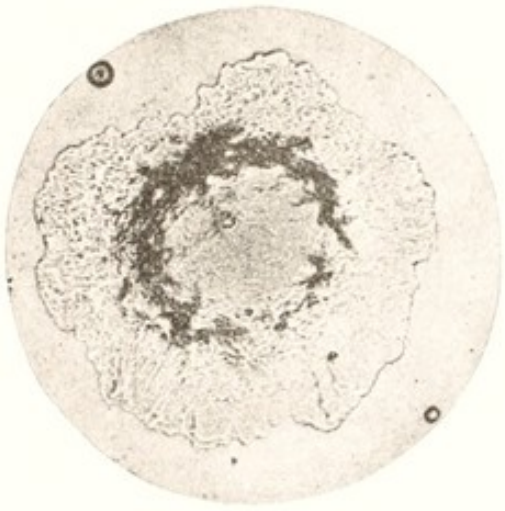


Fig. 255.—Bacillus coli communis; superficial colony two days old upon a gelatin plate. × 21 (Heim).

surface. The edges are dentate and slightly resemble grape-vine leaves, often showing radiating ridges suggestive of the veins of a leaf. They may have a slightly concentric appearance. The colonies rapidly increase in size and become more and more opaque. The gelatin is not liquefied.

Gelatin Punctures.—Development in gelatin punctures occurs upon the surface, and also in the needle's track, causing the formation of a nail-like growth. The head of the nail may reach the walls of the test-tube. No gas is formed in ordinary gelatin, but should any dextrose be present, sufficient gas-production may occur to break up the medium. The gelatin may become slightly clouded but is not liquefied.

Agar-agar.—Upon agar-agar, along the line of inoculation, a grayish-white, translucent, smeary growth, devoid of any characteristics, takes place. The entire surface of the culture-medium is never covered, the growth remaining confined to the inoculation line, except

where the moisture of condensation allows it to spread out at the bottom. Kruse says that crystals may form in old cultures.

Bouillon.—Bouillon is densely clouded by the growth of the bacteria, a delicate pellicle at times forming upon the surface. There is usually considerable sediment in the culture.

Potato.—Upon potato the growth is luxuriant. The bacillus forms a yellowish-brown, glistening layer spreading from the line of inoculation over about one-half to two-thirds of the potato. The color varies considerably, sometimes being pale, sometimes quite brown, sometimes greenish. It cannot, therefore, be taken as a characteristic of much importance. The growth on potato may be almost invisible.

Milk.—In milk rapid coagulation and acidulation occur, with the evolution of gas. The culture gives off a fecal odor. Litmus added to the culture-media is first reddened, then decolorized by the bacilli.

Vital Resistance.—It is quite resistant to antiseptics and germicides, and grows in culture-media containing from 0.1–0.2 per cent. of carbolic acid. It is, however, easily killed by heat, and is destroyed by exposure to 60°C. for ten minutes.

Metabolic Products.—Würtz found that Bacillus coli produced ammonia in culture-media free from sugar, and thus caused an intense alkaline reaction in the culture-media. The cultures usually give off an odor that varies somewhat, but is, as a rule, unpleasant.

Nitrates are reduced to nitrites by the growth of the bacillus.

In bouillon containing 1 per cent. of dextrose, lactose, levulose, galactose, and mannite, the colon bacillus splits up the sugar, liberating CO_2 and H, the gas formula being $\frac{H}{CO_2} = \frac{2}{1}$. This gas formula is very constant for the micro-organisms of the colon group and forms one of their most important differential characteristics. In calculating the gas formula Winslow has shown that some care ought to be taken to do it at the appropriate time. According to his observations the $\frac{H}{CO_2} : \frac{2}{1}$ formula only obtains between the twenty-fourth and forty-eighth hours. Before this period the H, which is first formed, preponderates; after it the CO_2 may preponderate. In sugar-containing bouillon, acetic, lactic, and formic acids are produced. It does not ferment saccharose. When a similar bacillus is found to ferment saccharose it is best regarded as a subspecies or separate type, for which Dunham has introduced the name *Bacillus coli communior*.

The bacillus requires very little nutriment. It grows in Uschinsky's asparagin solution, and is frequently found living in river and well waters.

Indol is formed in both bouillon and peptone solutions, but phenol is not produced. The presence of indol is best determined by Salkowski's method (*q.v.*).

Toxic Products.—Vaughan and Cooley* have shown that the toxin of the colon bacillus is contained in the germ-cell and under ordinary conditions does not diffuse from it into the culture-medium. The toxin may be heated in water to a very high temperature without injuring its poisonous nature. They have devised an apparatus in which enormous cultures can be prepared and the bacteria pulverized.† Of such a preparation 0.0002 gram will kill a 200-gram guinea-pig.

Pathogenesis.—The bacillus begins to penetrate the intestinal tissues almost immediately after death, and is the most frequent contaminating micro-organism met with in cultures made at autopsy. It may spread by direct continuity of tissue, or *via* the blood-vessels.

Although under normal conditions a saprophyte, the colon bacillus is not infrequently found in the pus in suppurations connected with the intestines—as, for example, appendicitis—and sometimes in suppurations remote from them.

In intestinal diseases, such as typhoid, cholera, and dysentery, the bacillus not only seems to acquire an unusual degree of virulence, but because of the existing denudation of mucous surfaces, etc., finds it easy to enter the general system, with the formation of remote secondary suppurative lesions in which it is the essential factor. When absorbed from the intestine, it frequently enters the kidney and is excreted with the urine, causing, incidentally, local inflammatory areas in the kidney, and occasionally cystitis. A case of urethritis is reported to have been caused by it.

In infants cholera infantum may not infrequently be caused by the colon bacillus, though sometimes in this disease other bacteria play an important rôle (B. dysenteriæ?).

The bile-ducts are sometimes invaded by the bacillus, which may lead to inflammation, obstruction, suppuration, or calculus formation.

The colon bacillus has also been met with in puerperal fever, Winckel's disease of the newborn,‡ endocarditis, meningitis, liver-abscess, bronchopneumonia, pleuritis, chronic tonsillitis, urethritis, and arthritis.

An interesting summary of the pathogenic effect of Bacillus coli can be found in Rolleston's paper in the "British Medical Journal" for Nov. 4, 1911, p. 1186.

In a certain number of cases general hemic infection may be caused by Bacillus coli. In 1909 Jacob§ published an analysis of 39 such cases, and in 1910 Draper‖ increased the number to 43. Wiens** also reported 6 cases and Maher†† 1 case, so that the total now stands 50.

* "Jour. Amer. Med. Assoc.," 1901; "American Medicine," 1901.
† "Trans. Assoc. Amer. Phys.," 1901.
‡ "Kamen-Ziegler's Beiträge," 1896, 14.
§ "Deutsch. Archiv. f. Klin. Med.," 1909, XCVII, 303.
‖ "Bull. of the Ayer Clin. Lab. of the Penna. Hosp.," 1910, No. 6, p. 21.
** "Munch. med. Woch.," 1909, LVI, 962.
†† "Med. Record," 1909, LXXV, 482.

Virulence.—It is a question whether the colon bacillus is always virulent, or whether it becomes so under abnormal conditions. Klencki* found it very virulent in the ileum, and less so in the colon and jejunum of dogs. He also found that the virulence was greatly increased in a strangulated portion of intestine. Dreyfus† found that the colon bacillus as it occurs in normal feces is not virulent. Most experimenters believe that pathologic conditions, such as disease of the intestine, strangulation of the intestine, etc., increase its virulence.

Frequent transplantation lessens the virulence of the bacillus; passage through animals increases it.

It has been observed that cultures of the bacillus obtained from cases of cholera, cholera nostras, and other intestinal diseases are more pathogenic than those obtained from normal feces or from pus.

Adelaide Ward Peckham,‡ in an elaborate study of the "Influence of Environment on the Colon Bacillus," concludes that while the conditions of nutrition and development in the intestine seem to be most favorable, the colon bacillus is ordinarily not virulent. She says:

"Its first force is spent upon the process of fermentation, and as long as opportunities exist for the exercise of this function the affinities of this organism appear to be strongest in this direction.

"Moreover, the contents of the intestine remain acid until they reach the neighborhood of the colon, and by that time the tryptic peptones have been formed and absorbed to a great extent.

"During the process of inflammation in the digestive tract a very different condition may exist. The peptic and tryptic enzymes may be partially suppressed. Fermentation of carbohydrates and proteid foods then begins in the stomach, and continues after the mass of food is passed on into the intestine. The colon bacillus cannot, therefore, spend its force upon fermentation of sugars, because they are already broken up and an alkaline fermentation of the proteids is in progress. It also cannot form peptones from the original proteids, for it does not possess this property, and unless trypsin is present it must be dependent upon the proteolytic activity of other bacteria for a suitable form of proteid food. Perhaps these bacteria form an albuminate molecule which, like leucin and tyrosin, cannot be broken up into indol, and thus there might be caused an important modification of the metabolism of the colon bacillus, which might have either an immediate or remote influence upon its acquisition of disease-producing properties, for our own experiments indicate that the power to form indol, and the actual forming of it, are to some extent an indication of the possession of pathogenesis."

For the laboratory animals the colon bacillus is pathogenic in varying degree. Intraperitoneal injections into mice cause death in from one to eight days if the culture be virulent. Guinea-pigs and rabbits also succumb to intraperitoneal and intravenous injection. Subcutaneous injections are of less effect, and in rabbits produce abscesses only.

When injected into the abdominal cavity, the bacilli set up a serofibrinous or purulent peritonitis, and are very numerous in the abdominal fluids.

* "Ann. de l'Inst. Pasteur," 1895, No. 9.

† "Centralbl. f. Bakt.," etc., XVI, p. 581.

‡ "Journal of Experimental Medicine," Sept., 1897, vol. II, No. 4, p. 549.

Cumston,* from a careful study of 13 cases of summer infantile diarrheas, comes to the following conclusions:

Bacterium coli seems to be the pathogenic agent of the greater number of summer infantile diarrheas.

The organism is often associated with Streptococcus pyogenes.

The virulence, more considerable than in the intestine of a healthy child, is almost always in direct relation to the condition of the child at the time the culture is taken, and does not appear to be proportionate to the ulterior gravity of the case.

The mobility of Bacterium coli is, in general, proportionate to its virulence. The jumping movement, nevertheless, does not correspond to an exalted virulence in comparison with the cases in which the mobility was very considerable, without presenting these jumping movements.

The virulence of Bacterium coli found in the blood and other organs is identical with that of Bacterium coli taken from the intestines of the same individual.

Lesage,† in studying the enteritis of infants, found that in 40 out of 50 cases depending upon Bacillus coli the blood of the patient agglutinated the cultures obtained, not only from his own stools, but from those of all the other cases. From this uniformity of action Lesage suggests that the colon bacilli in these cases are all of the same species.

The agglutinating reaction occurs only in the early stages and acute forms of the disease.

Immunization.—It is not difficult to immunize an animal against the colon bacillus. Löffler and Abel immunized dogs by progressively increased subcutaneous doses of live bacteria, grown in solid culture and suspended in water. The injections at first produced hard swellings. The blood of the immunized animals possessed an active bactericidal effect upon the colon bacteria. The serum was not in the correct sense antitoxic.

Differential Diagnosis.—This problem is considered at greater length under the heading "Cultural Differentiation of the Bacillus Typhosus" (*q.v.*). For the recognition of the colon bacillus the most important points are the motility, the indol-formation, the milk-coagulation, and the active gas-production. As, however, most of these features are shared by other bacteria to a greater or less degree, the most accurate differential point is the immunity reaction with the serum of an immunized animal, which protects susceptible animals from the effects of inoculation, and produces a similar agglutinative reaction to that observed in connection with the blood and serum of typhoid patients, convalescents, and immunized animals.

The fact that, with rare exceptions, the typhoid serum produces a specific reaction with the typhoid bacillus, and the colon serum with the colon bacillus, should be the most important evidence that they are entirely different species.

What is commonly known as Bacillus coli communis is, no doubt,

* "International Medical Magazine," Feb., 1897.

† "La Semaine Médicale," Oct. 20, 1897.

not a single species, but a group of bacilli too similar to be differentiated into groups, types, or families by our present methods.

In order to establish a *type species* of Bacillus coli communis, Smith* says:

"I would suggest that those forms be regarded as true to this species which grow on gelatin in the form of delicate bluish or more opaque, whitish expansions with irregular margin, which are actively motile when examined in the hanging drop from young surface colonies taken from gelatin plates, which coagulate milk within a few days; grow upon potato, either as a rich pale or brownish-yellow deposit, or merely as a glistening, barely recgonizable layer, and which give a distinct indol reaction. Their behavior in the fermentation-tube must conform to the following scheme:

"*Variety α:*

"One per cent. dextrose-bouillon (at 37°C.). Total gas approximately ½; H : CO_2 = approximately 2:1; reaction strongly acid.

"One per cent. lactose-bouillon: as in dextrose-bouillon (with slight variations).

"One per cent. saccharose-bouillon; gas-production slower than the preceding, lasting from seven to fourteen days. Total gas about ⅔; H : CO_2 = nearly 3:2. The final reaction in the bulb may be slightly acid or alkaline, according to the rate of gas-production (B. coli communior, Dunham).

"*Variety β:*

"The same in all respects, excepting as to its behavior in saccharose-bouillon; neither gas nor acids are formed in it."

DIFFERENTIAL CHARACTERISTICS

Typhoid Bacillus	Colon Bacillus
Bacilli usually slender.	Bacilli a little thicker and shorter.
Flagella numerous (10–20), long, and wavy (peritricha).	Flagella fewer (8–10) (peritricha).
Growth not very rapid, not particularly luxuriant.	Growth rapid and luxuriant. This character is by no means constant.
Upon Elsner's, Hiss', Piorkowski's, and other media gives characteristic appearances.	Upon Elsner's, Hiss', Piorkowski's, and other media gives characteristic appearances.
Upon fresh acid potato the so-called "invisible growth" formerly thought to be differential.	Upon potato a brownish-yellow distinct pellicle.
Acid-production in whey not exceeding 3 per cent. Sometimes slight in ordinary media, and succeeded by alkali-production.	Acid-production well marked throughout.
Grows in media containing sugars without producing any gas.	Fermentation with gas-production well marked in solutions containing dextrose, lactose, etc., the usual formula being H : CO_2 = 2:1.
Produces no indol.	Indol-production marked.
Growth in milk unaccompanied by coagulation.	Milk coagulated.
Gives the Widal reaction with the serum of typhoid blood.	Does not react with typhoid blood.

Colon Bacillus in Drinking Water.—Much importance attaches to the presence or absence of colon bacilli in judging the potability of drinking waters.

* "Amer. Jour. Med. Sci.," 1895, 110, p. 287.

It is a speculation whether the colon bacilli were originally micro-organisms of the soil that accidentally found their way into the congenial environment of the intestine and there took up permanent residence, or whether they have always been intestinal parasites and have been discharged with the excrement of animals until the soil has become generally infected with them. However this may be, they are at present found in the intestinal canals of all animals, and in pretty much all soils, their number being greatest in manured soils. From the soil it is inevitable that the organisms shall pass into the surface waters, which with few exceptions will be found to contain them. The numbers, however, can be made use of to indicate the quality of the water, a few organisms indicating that the water is pure, many that it is freely mixed with surface washings.

As sewage contains as many as 1,000,000 colon bacilli per cubic centimeter and pure water very often 0 per cubic centimeter (only 1 cc. being examined at a time), the number of bacilli per cubic centimeter can be expressed as indicating the amount of sewage pollution. The number of colon bacilli in the water is, therefore, of importance in determining its potability, and in cases in which the quality of the water is doubtful, should always be employed. There is no infallible criterion for judging the quality of water, but most American bacteriologists are in accord in concluding that when the repeated examination of 1 cc. samples shows the presence of numerous colon bacilli, the water is seriously polluted and doubtfully potable, but when samples of 1 cc. are without colon bacilli or contain very few, the water is safe.

Another important matter in regard to the colon bacillus in water is the presence or absence of certain characters by which one can judge how recently it has ended its intestinal parasitism and taken up a saprophytic life. The chief of these characters is the ability to ferment lactose. Only recently isolated organisms manifest this fermentative power in the laboratory, so that when organisms capable of fermenting lactose are found, one can suppose that they result from recent sewage pollution.

Many media have been recommended for the rapid detection of the colon bacilli in water, the favorite at the present time probably being the litmus-lactose-agar plate (*q.v.*) of Würtz.* This depends upon the fermentative and acid-producing power of the bacillus, which is shown through the presence of red colonies (acid producers) on the elsewhere blue plate. These red colonies are then fished up and transplanted to appropriate media for further study.

Kline† substitutes lactose for the glucose in this medium, pointing out that by so doing one at once differentiates between typical colon bacilli which ferment lactose and atypical varieties which do not.

* "Archiv. de méd. Experimentale," 1892, iv, p. 85.
† "British Medical Journal," Oct. 27, 1906, p. 1090.

Other media and methods useful in studying the colon bacilli are also discussed in the chapter upon Typhoid Fever (*q.v.*).

Bacillus Enteritidis (Gärtner)

General Characteristics.—A motile, flagellated, non-sporogenous, non-chromogenic, non-liquefying, aërogenic, aërobic and optionally anaërobic, pathogenic bacillus staining by the ordinary methods, but not by Gram's method.

This bacillus was first cultivated by A. Gärtner* from the flesh of a cow slaughtered because of an intestinal disease, and from the spleen of a man poisoned by eating meat obtained from it. The bacillus was subsequently found by Karlinski and Lubarsch in other cases of meat-poisoning.

Morphology.—The bacillus closely resembles Bacillus coli communis. It is short and thick, is surrounded by a slight capsule, is actively motile, and has flagella.

Staining.—It stains irregularly with the ordinary solutions, but not by Gram's method. It has no spores.

Cultivation.—Upon gelatin plates it forms round, pale gray, translucent colonies. It does not liquefy the gelatin. The deep colonies are brown and spheric. The growth on agar-agar is similar to that of the colon bacillus. The organism produces no indol, coagulates milk in a few days, and reduces litmus. Its fermentative powers have not been sufficiently studied, but it is known to ferment dextrose media. Upon potato it forms a yellowish-white, shining layer.

Pathogenesis.—The bacillus is pathogenic for mice, guinea-pigs, pigeons, lambs, and kids, but not for dogs, cats, rats, or sparrows. The infection may be fatal for mice and guinea-pigs, whether given subcutaneously, intraperitoneally, or by the mouth.

Lesions.—The bacilli are found scattered throughout the organs in small groups, resembling those of the typhoid bacillus.

At the autopsy a marked enteritis and swelling of the lymphatic follicles and patches, with occasional hemorrhages, are found. The bacilli occur in the intestinal contents. The spleen is somewhat enlarged.

The bacillus is differentiated from the colon bacillus chiefly by the absence of indol-production, by its ability to produce infection when ingested, and by the fact that it elaborates a toxic substance capable of producing symptoms similar to those seen in the infection.

It may be distinguished from Bacillus lactis aërogenes by its motility. It closely resembles certain water bacteria; but its pathogenesis can be made use of for assisting in its differentiation in doubtful cases.

Bacillus Fæcalis Alkaligenes (Petruschky)

General Characteristics.—A motile, flagellated, non-sporogenous, non-liquefying, non-chromogenic, non-aërogenic, aërobic and optionally anaërobic, non-pathogenic bacillus of the intestine, staining by ordinary methods, but not by Gram's method.

This bacillus has occasionally been isolated by Petruschky† and others from feces. It closely resembles the typhoid bacillus, being short, stout, with round ends, forming no spores, staining with the usual dyes, but not by Gram's method, being actively motile, and having numerous flagella. It does not liquefy gelatin, does not coagulate milk, produce gas, or form indol. Its pathogenic powers for the lower animals are similar to those of the typhoid bacillus.

It grows more luxuriantly than the typhoid bacillus upon potato, producing a brown color, and generates a strong alkali when grown in litmus-whey. Its cultures are not agglutinated by the typhoid serums.

* "Korrespond. d. allg. ärztl. Ver. von Thüring," 1888, 9.

† "Centralbl. f. Bakt. u. Parasitenk.," XIX, 187.

Bacillus Psittacosis (Nocard)

General Characteristics.—A motile, flagellated, non-sporogenous, aërobic, optionally anaërobic, non-chromogenic, aërogenic, pathogenic, non-liquefying bacillus, staining by the ordinary methods, but not by Gram's method.

This micro-organism was discovered by Nocard,* who first observed it in 1892 in certain cases of psittacosis, or epidemic pneumonia, traceable to infection from diseased parrots. The original paper contained an excellent account of the specific organism.

The subsequent work of Gilbert and Fournier† shows the specificity of the micro-organism to be quite well established and Nocard's characterizations accurate.

Morphology.—The bacillus is short, stout, rounded at the ends, and actively motile. It is provided with flagella, but forms no spores. It resembles the typhoid and the colon bacilli and is evidently a form intermediate between the two.

Isolation.—Gilbert and Fournier succeeded in isolating it from the blood of a patient dead of psittacosis, and from parrots, by the use of lactose-litmus agar. The organism does not alter the litmus, and if a small percentage of carbolic acid be added to the culture-media, it grows as does the typhoid bacillus.

Cultivation.—The colonies, agar-agar and gelatin cultures, closely resemble those of the typhoid fever organism. Upon potato it more closely resembles the colon bacillus. Bouillon becomes clouded.

Metabolic Products.—In bouillon containing sugars the micro-organism is found to ferment dextrose, but not lactose. Milk is not coagulated and not acidulated. No indol is formed.

Pathogenesis.—Bacillus psittacosis can be immediately differentiated from the typhoid and colon bacilli by its peculiar pathogenesis. It is extremely virulent for parrots, producing a fatal infection in a short time. White and gray mice and pigeons are equally susceptible. Ten drops of a bouillon culture injected in the ear-vein of a rabbit kill it in from twelve to eighteen hours. Guinea-pigs are more resistant. Subcutaneous injection of dogs produces a hard, painful swelling, which persists for a short time and then disappears without suppuration. It is also infectious for man, a number of epidemics of peculiar pneumonia, characterized by the presence of the bacillus in the blood, traceable to diseased parrots, having been reported.

Differentiation.—Bacillus psittacosis can best be differentiated from the typhoid and the colon bacilli and others of the same group by its pathogenesis and by the reaction of agglutination. Typhoid immune serum produces some small agglutinations, but a comparison between these and the agglutinations formed by cultures of the typhoid bacillus shows immediately that the micro-organisms are dissimilar. Differentiation is best made out when the prepared hanging-drop specimens of serums and cultures are kept for some hours in an incubating oven. It is not known whether the bacillus is peculiar to the intestines of parrots, invading their tissues when they become ill, or whether it is a purely pathogenic micro-organism found only in psittacosis.

Bacillus Suipestifer (Salmon and Smith)

General Characteristics.—An actively motile, flagellated, non-sporogenous, non-chromogenic, non-liquefying, aërobic and optionally anaërobic, aërogenic bacillus pathogenic for hogs and other animals. It stains by the ordinary methods, but not by Gram's method. It ferments dextrose, lactose, and sucrose, but does not form indol or coagulate or acidulate milk.

Hog-cholera, or "pig typhoid," as the English call it, is a common epidemic disease of swine, which at times kills 90 per cent. of the infected animals, and thus causes immense losses to breeders. Salmon estimates that the annual losses

* "Séance du Conseil d'hygiene publique et Salubrite du Departement de la Seine," March 24, 1893.

† "Comptes rendu de la Sociétè de Biologie," 1896; "La Presse médicale," Jan. 16, 1897.

from this disease in the United States range from $10,000,000 to $25,000,000. For years it was thought to be caused by the Bacillus suipestifer, but DeSchweinitz and Dorset* were able to transmit the disease from one hog to another in certain of the body fluids that had been passed through the finest porcelain filters and were shown by inoculation and cultivation to be free of bacilli. It therefore depends upon a filterable and unknown virus.

This observation was received with approval by those who had any experience with the effect of hog-cholera bacilli upon hogs, all of whom must have observed that though infection with the bacilli *occasionally* caused the death of an animal, the dead animal usually did not show the typical lesions of the disease and never infected other animals with which it was kept. The papers upon the subject by Dorset, Bolton, and McBryde† and by Dorset, McBryde, and Niles‡ are worth reading.

These investigations entirely changed our ideas of the importance of the hog-cholera bacillus, whose relation to the disease now comes to resemble that of Bacillus icteroides to yellow fever.

The bacillus of hog-cholera was first found by Salmon and Smith,§ but was for a long time confused with the bacillus of "swine-plague," which it closely resembles, and in association with which it frequently occurs. It is a member of the group of bacteria to which Bacillus icteroides and B. typhi murium belong. The organism was secured by Smith from the spleens of more than 500 hogs. It occurs in the blood and in all the organs, and has also been cultivated from the urine.

Morphology.—The organisms appear as short rods with rounded ends, 1.2 to 1.5 μ long and 0.6 to 0.7 μ in breadth. They are actively motile and possess long flagella (peritrichia), easily demonstrable by the usual methods of staining. No spore production has been observed. In general the bacillus resembles that of typhoid fever. It stains readily by the ordinary methods, but not by Gram's method.

Cultivation.—No trouble is experienced in cultivating the bacilli, which grow well in all the media under aërobic and anaërobic conditions.

Colonies.—Upon gelatin plates the colonies become visible in from twenty four to forty-eight hours, the deeper ones appearing spheric with sharply defineborders. The surfaces are brown by reflected light, and without markingsd They are rarely larger than 0.5 mm. in diameter and are homogeneous throughout. The superficial colonies have little tendency to spread upon the gelatin. They rarely reach a greater diameter than 2 mm. The gelatin is not liquefied.

Upon agar-agar they attain a diameter of 4 mm. and have a gray, translucent appearance with polished surface. They are round and slightly arched.

Gelatin.—In gelatin punctures the growth takes the form of a nail with a flat head. There is nothing characteristic about it. The medium is not liquefied.

Agar-agar.—Linear cultures upon agar-agar present a translucent, circumscribed, grayish, smeary layer without characteristic appearances.

Potato.—Upon potato a yellowish coating is formed, especially when the culture is kept in the thermostat.

Bouillon.—Bouillon made with or without peptone is clouded in twenty-four hours. When the culture is allowed to stand for a couple of weeks without being disturbed, a thin surface growth can be observed.

Milk is an excellent culture-medium, but is not visibly changed by the growth of these bacteria. Its reaction remains alkaline.

Vital Resistance.—The bacillus is hardy. Smith found it vital after being dry for four months. It ordinarily dies sooner, however, and difficulty may be experienced in keeping it in the laboratory for any length of time unless frequently transplanted. The thermal death-point is 54°C., maintained for sixty minutes.

Metabolic Products.—Gas Production.—The hog-cholera bacillus is a copious gas-producer, capable of breaking up dextrose and lactose into CO_2, H, and an

* "Circular No. 41 of Bureau of Animal Industry," U. S. Dept. of Agriculture, Washington, D. C.

† "Bull. No. 72 of Bureau of Animal Industry," U. S. Dept. Agriculture, Washington, D. C., 1905.

‡ "Bull. No. 102 of Bureau of Animal Industry," U. S. Dept. Agriculture, Washington, D. C., Jan. 18, 1908.

§ "Reports of the Bureau of Animal Industry," 1885–91; and "Centralbl. f. Bakt. u. Parasitenk.," March 2, 1891, Bd. ix, Nos. 8, 9, and 10.

acid, which, formed late, eventually checks its further development. It does not ferment saccharose.

Indol.—No indol and no phenol are formed in the culture-media.

Toxin.—In pure cultures of the hog-cholera bacillus Novy* found a poisonous base with the probable composition $C_{16}H_{26}N_2$, which he gave the provisional name "susotoxin." In doses of 100 mg. the hydrochlorid of this base causes convulsive tremors and death within one and one-half hours in white rats. He has also obtained a poisonous protein of which 50 mg. were fatal for white rats, and which immunized them against highly virulent hog-cholera organisms when administered by repeated subcutaneous injection.

De Schweinitz† has also separated a slightly poisonous base which he calls "sucholotoxin," and a poisonous protein that crystallizes in white, translucent plates when dried over sulphuric acid *in vacuo*, forms needle-like crystals with platinic chlorid, and was classed among the albumoses.

Pathogenesis.—The bacillus is disappointing in its effects upon hogs. When it is subcutaneously or intravenously introduced into such animals or fed to them, they sometimes show no signs of disease; sometimes show fever and depression, but rarely sicken enough to die. Animals thus made ill do not communicate hog cholera to others.

Smith found that 0.75 cc. of a bouillon culture injected into the breast muscles of pigeons would kill them.

In Smith's experiments one four-millionth of a cubic centimeter of a bouillon culture injected subcutaneously into a rabbit was sufficient to cause its death. The temperature abruptly rises 2° to 3°C., and remains high until death. Subcutaneous injection of larger quantities may kill in five days. Injected intravenously in small doses the bacillus may kill rabbits in forty-eight hours.

Agglutination.—Pitfield‡ found that after a single injection of a killed bouillon culture of the bacillus into a horse, the serum, which originally had very slight agglutinative power, showed a decided increase. If the horse be immunized to large doses of such sterile cultures, the serum becomes so active that with a dilution of 1:10,000 a typical agglutination occurs in sixty minutes.

McClintock, Boxmeyer and Siffer§ found that the serum of normal hogs agglutinates strains of ordinary hog-cholera bacilli in dilutions occasionally as high as 1:250 and consider reaction in a dilution of less than 1:300 without diagnostic value.

Bacillus Icteroides (Sanarelli)

General Characteristics.—An actively motile, flagellated, non-sporogenous, non-liquefying, non-chromogenic, aërogenic, aërobic and optionally anaërobic, pathogenic bacillus which stains by the ordinary method, but not by Gram's method. It produces indol, but does not coagulate milk.

Sanarelli regarded this bacillus as the specific organism of yellow fever. He found it in 11 autopsies upon yellow fever cases, but always in association with streptococci, colon bacilli, proteus, and other organisms. It is found in the blood and tissues, and not in the gastro-intestinal tract, and isolation of the organism was possible in only 58 per cent. of the cases, and only in rare instances was accomplished during life.

Distribution.—By suitable methods it can be found in the organs of yellow fever cadavers, usually aggregated in small groups, in the capillaries of the liver, kidneys, and other organs. The best method of demonstration is to keep a fragment of liver, obtained from a body soon after death, in the incubator at 37°C. for twelve hours, and allow the bacteria to multiply in the tissue before examination.

Morphology.—The bacillus presents nothing morphologically characteristic. It is a small pleomorphous bacillus with rounded ends, usually joined in pairs. It is 2 to 4 μ in length, and, as a rule, two or three times longer than broad. It is actively motile and has flagella. It does not form spores.

Staining.—It stains by the usual methods, but not by Gram's method.

* "Medical News," 1900, p. 231.
† "Medical News," 1900, p. 237.
‡ "Microscopical Bulletin," 1897, p. 35.
§ "Jour. of Infectious Diseases," March 1, 1905, vol. II, No. 2, p. 351.

Cultivation.—The bacillus can be grown upon the usual media. It grows readily at ordinary room temperatures, but best at 37°C.

Colonies.—Upon gelatin plates it forms rounded, transparent, granular colonies, which during the first three or four days somewhat resemble leukocytes. The granular appearance becomes continuously more marked, and usually an opaque central or peripheral nucleus is seen. In time the entire colony becomes opaque, but does not liquefy gelatin.

Gelatin.—Stroke cultures on obliquely solidified gelatin show brilliant, opaque, white colonies resembling drops of milk. The medium is not liquefied.

Bouillon.—In bouillon it develops slowly, without either pellicle or flocculi.

Agar-agar.—The culture upon agar-agar is said to be characteristic.

Fig. 256.—Culture of Bacillus icteroides on agar (Sanarelli).

The peculiar and characteristic appearances of the colonies do not develop if grown at 37°C.; but at 20° to 22°C. the colonies appear rounded, whitish, opaque, and prominent, like drops of milk. This appearance of the colonies also shows well if the cultures are kept for the first twelve to sixteen hours at 37°C., and afterward at the room temperature, when the colonies will show a flat central nucleus transparent and bluish, surrounded by a prominent and opaque zone, the whole resembling a drop of sealing-wax. Sanarelli refers to this appearance as constituting the chief diagnostic feature of Bacillus icteroides. It can be observed in twenty-four hours.

Blood-serum.—Upon blood-serum the growth is very meager.

Potato.—The growth upon potato corresponds with that of the bacillus of typhoid fever.

Vital Resistance.—It strongly resists drying, but dies when exposed in cultures to a temperature of 60°C. for a few minutes, and is killed in seven hours by the solar rays. It can live for a considerable time in sea-water.

Metabolism.—The bacillus is an optional anaërobe. It slowly ferments dextrose, lactose, and saccharose, forming gas only in dextrose solutions in which there are no other sugars. It does not coagulate milk. In the cultures a small amount of indol is formed.

Pathogenesis.—The bacillus is pathogenic for the domestic animals, all mammals seeming to be more or less sensitive to it. Birds are often immune. White mice are killed in five days, guinea-pigs in from eight to twelve days, rabbits in from four to five days, by virulent cultures. The morbid changes present include splenic tumor, hypertrophy of the thymus, and adenitis. In the rabbit there are, in addition, nephritis, enteritis, albuminuria, hemoglobinuria, and hemorrhages into the body cavities.

Sanarelli states that the *dog is the most susceptible animal.* When this animal is injected intravenously, symptoms appear almost immediately and recall the clinical and anatomic features of yellow fever in man. The most prominent symptom in the dog is vomiting, which begins directly after the penetration of the virus into the blood, and continues for a long time. Hemorrhages appear after the vomiting, the urine is scanty and albuminous, or is suppressed shortly before death. Grave jaundice was once observed.

Bacillus Typhi Murium (Löffler)

General Characteristics.—A motile, flagellated, non-sporogenous, non-liquefying, non-chromogenic, non-aërogenic, aërobic and optionally anaërobic bacillus, pathogenic for mice and other small animals, staining by the ordinary methods, but not by Gram's method. It acidulates but does not coagulate milk.

Bacillus typhi murium was discovered by Löffler* in 1889, when it created havoc among the mice in his laboratory at Greifswald.

* "Centralbl. f. Bakt. u. Parasitenk.," XI, p. 129.

Morphology.—The organism bears a close resemblance to that of typhoid fever, sometimes appearing short, sometimes long and flexible. There are many long and curly flagella with peritrichial arrangement, and the organism is actively motile. It does not produce spores.

Staining.—It stains with the ordinary dyes, but rather better with Löffler's alkaline methylene blue, not by Gram's method.

Isolation.—The bacilli were first isolated from the blood of dead mice.

Cultivation.—Their cultivation presents no difficulties.

Colonies.—Upon gelatin plates the deep colonies are at first round, slightly granular, transparent, and grayish. Later they become yellowish brown and granular. Superficial colonies are similar to those of the typhoid bacillus.

Gelatin.—In gelatin punctures there is no liquefaction. The growth takes place principally upon the surface, where a grayish-white mass slowly forms, and together with the growth in the puncture suggests a large flat-headed nail.

Agar-agar.—Upon agar-agar a grayish-white growth devoid of peculiarities occurs.

Potato.—Upon potato a rather thin whitish growth may be observed after a few days.

Milk.—The bacillus grows well in milk, causing acid reaction, without coagulation.

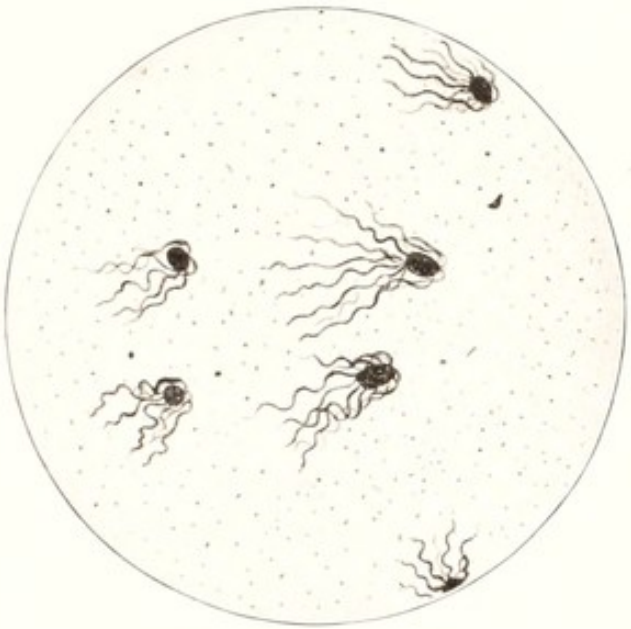


Fig. 257.—Bacillus typhi murium (Migula).

Bouillon.—In bouillon it produces clouding. There is no fermentation of saccharose, dextrose, lactose, or levulose.

Pathogenesis.—The organism is pathogenic for mice of all kinds, which succumb in from one to two days when inoculated subcutaneously, and in from eight to twelve days when fed upon material containing the bacillus. The bacilli multiply rapidly in the blood- and lymph-channels, and cause death from septicemia.

Löffler expressed the opinion that this bacillus might be of use in ridding infested premises of mice, and its use for this purpose has been satisfactory in many places. He has succeeded in ridding fields so infested with mice as to be useless for agricultural purposes, by saturating bread with bouillon cultures of the bacillus and distributing it near their holes. The bacilli not only killed the mice that had eaten the bread, but also infected others that ate their dead bodies, the extermination progressing until scarcely a mouse remained.

In discussing the practical employment of this bacillus for the satisfactory destruction of field-mice, Brunner* calls attention to certain conditions that are requisite: (1) It is necessary, first of all, to attack extensive areas of the invaded territory, and not to attempt to destroy the mice of a small field into which an

* "Centralbl. f. Bakt.," etc., Jan. 19, 1898, Bd. XXIII, No. 2, p. 68.

indefinite number of fresh animals may immediately come from surrounding fields. The country people, who are the sufferers, should combine their efforts so as to extend the benefits widely. (2) The preparation of the cultures is a matter of importance. Agar-agar cultures are most readily transportable. They are broken up in water, well stirred, and the liquid poured upon a large number of small pieces of broken bread. These are then distributed over the ground with care, being dropped into the fresh mouse-holes, and pushed sufficiently far in to escape the effects of sunlight upon the bacilli. Attention should be paid to holes in walls, under railway tracks, etc., and other places where mice live in greater freedom from disturbance than in the fields. (3) The destruction of the mice should be attempted only at a time of the year when their natural food is not plenty. By observing these precautions the mice can be eradicated in from eight to twelve days. In the course of two years no less than 250,000 cultures were distributed from the Bacteriological Laboratory of the Tierarznei Institut in Vienna, for the purpose of destroying field-mice.

The bacilli are not pathogenic for animals, such as the fox, weasel, ferret, etc., that feed upon the mice, do not affect man in any way, and so seem to occupy a useful place in agriculture by destroying the little but almost invincible enemies of the grain.

A similar organism, secured from an epidemic among field-mice and greatly increased in virulence by artificial manipulation, has been recommended by Danysz* for the destruction of rats. When subjected to a thorough study by Rosenau† this organism was found to be identical with Bacillus typhi murium. It is, however, too uncertain in action to be relied upon for the destruction of rats in plague-threatened cities for which it was suggested.

* "Ann. de l'Inst. Pasteur," April, 1900.

† "Bulletin No. 5 of the Hygienic Laboratory of the U. S. Marine Hospital Service," Washington, D. C., 1901.

CHAPTER XXVIII

DYSENTERY

DYSENTERY is an acute, subacute, or chronic, infectious colitis, usually characterized by an acute onset, mild fever, pain in the abdomen, rectal tenesmus, and the passage of frequent, usually small, mucous and bloody evacuations from the rectum.

The disease was known to the ancients. It was probably dysentery that is meant by "emerods" in describing an epidemic that took place among the people of Israel during the time of the Judges. Hippocrates differentiated between diarrhea and dysentery.

Sporadic cases of the disease occur in almost all countries, the number of such increasing as the equator is approached. In addition to these sporadic cases epidemics not infrequently appear. Though such may break out at any time in towns or cities, they are more apt to occur when unusual activities of the people are in progress. The most frequent of these is military, and armies are apt to be the greatest sufferers. The incidence of dysentery in the Federal Army during the War of the Rebellion was appalling. Woodward* states that there were 259,071 cases of acute and 28,451 cases of chronic dysentery.

Endemics also occur from time to time and assume devastating proportions, as in Japan, where between 1878 and 1899 there were 1,136,096 cases, with 275,308 deaths—a mortality of 25.23 per cent.† Osler quotes Macgregor as saying: "In the tropics dysentery is a destructive giant compared to which strong drink is a mere phantom. It is one of the great camp diseases and has been more destructive to armies than powder and shot."

The disease early came under the observation of the bacteriologists, and Klebs, Ziegler, Ogata, Grigorjeff, de Silvestri, Maggiora, Arnaud, Celli and Fiocca, Galli-Valerio, Valagussa, Deycke, and others published descriptions of various micro-organisms isolated from dysenteric stools, and looked upon by their discoverers as its cause. The results were, however, so discordant that none of the described micro-organisms could be agreed upon as the excitant of the disease.

In 1860 Lambl‡ published a description of an ameba found in the human intestine. No one seemed inclined to believe that it might have any significance until much later.

In 1875 Lösch§ described an ameba which he found in great num-

* "Medical and Surgical History of the War of the Rebellion," Medical, II.
† "Public Health Reports," Jan. 5, 1900, XV, No. 1.
‡ "Aus. d. Franz Joseph Kinderspital zur Prague," 1860, I, 326.
§ "Virchow's Archives," 1875, Bd. LXV.

bers in the colon of a case of dysentery occurring in St. Petersburg. Not much notice was taken of his paper or much made of his observation until eight years later, when Koch and Gaffky,* in studying the cholera in Egypt, also observed amebas in the intestinal discharges in certain cases, and Kartulis† wrote upon the "Etiology of the Dysentery in Egypt," which he referred to them. In America the study of these amebas was quickly taken up. Osler‡ discovered the organisms in the evacuations of a case of dysentery contracted by a patient during a visit to Panama. Councilman and Lafleur§ wrote a fine monograph upon "Amebic Dysentery," while Quincke and Roos‖ and Kruse and Pasquale** confirmed the observations and results in Europe.

Thus it came to be recognized that an ameba might be the cause of dysentery. It was soon pointed out, however, that there were cases of dysentery in which no amebas could be found in the intestinal discharges, or in which they were so few that it seemed impossible that they could be the cause of the disease. This was particularly impressive throughout the years of the endemic dysentery in Japan, already referred to. Great numbers of cases occurred, great numbers of people died, no amebas were found to account for the disease. It therefore occurred to Kitasato that some other causal agent must be looked for, and Shiga took up the problem, which was a difficult one, and might not have been solved had he not made use of a then new means of investigation, viz., the phenomenon of agglutination. By studying such bacteria as could be cultivated from the intestinal discharges, with particular reference to the agglutinating effect of the blood of dysenteric patients upon them, Shiga†† succeeded in discovering a new micro-organism which he called Bacillus dysenteriæ. Two years afterward Kruse‡‡ investigated an outbreak of dysentery in an industrial section of Westphalia and found the same bacillus and Flexner§§ showed it to be present in the epidemic dysentery of the Philippine Islands.

Thus through the discovery of Shiga it became evident that there are two forms of dysentery, one *amebic* the other *bacillary*. Both occur sporadically and endemically in the tropics and in temperate climates, and both may occur epidemically, though of the two the bacillary form is the more liable to do so. Of the chronic cases of dysentery 90 per cent. are amebic.

* "Bericht über die Erforschung der Cholera," 1883; "Arbeiten aus dem Kaiserl. Gesundheitsamte.," III, 65.
† "Virchow's Archives," 1886, CV.
‡ "Centralbl. f. Bakt. u. Parasitenk.," 1890, VII, 736.
§ "Johns Hopkins Hospital Reports," 1891, II.
‖ "Berliner klin. Wochenschrift," 1893.
** "Zeitschrift f. Hygiene," etc., 1894, XVI.
†† "Centralbl. f. Bakt. u. Parasitenk.," 1898, XXIV, 817.
‡‡ "Deutsche med. Wochenschrift," 1900, No. 40.
§§ "Centralbl. f. Bakt. u. Parasitenk.," 1900, XXVIII, No. 19.

I. AMEBIC DYSENTERY

Amœba Coli (Lösch, 1875); Amœba Dysenteriæ (Councilman and Lafleur, 1893); Entamœba Histolytica (Schaudinn, 1903)

As has been shown, amebas were first found in the human intestine by Lambl; in dysentery, by Lösch, Koch, Gaffky, Kartulis, Osler, Councilman and Lafleur, and many others. The welcome finally accorded to the organisms as excitants of dysentery was sufficiently enthusiastic to compensate for the neglect of a quarter of a century.

Celli and Fiocca* were the first to study the amebas systematically and to cultivate them upon artificial media. Councilman and Lafleur pointed out that there were two varieties of amebas which they called Amœba coli and Amœba dysenteriæ. The former was supposed to be a harmless commensal, the latter a pathogenic organism and the cause of dysentery. As, however,

Fig. 258.—Amœba coli in intestinal mucus, with blood-corpuscles and bacteria (Lösch).

Lösch had called the organism found in dysentery the Amœba coli, Stiles declared the nomenclature faulty, and pointed out that Amœba coli, variety dysenteriæ, must be the name of the pathogenic form. Schaudinn† reviewed the subject and grouped all of the intestinal amebas under the following:

I. Chlamydophrys stercorea (Cienkowsky).

II. Amœba coli rhizopodia.

1. Entamœba coli (Lösch) (Schaudinn).
2. Entamœba histolytica (Schaudinn).

To these has been since added in 1907:

Entamœba tetragena (Viereck).

* "Centralbl. f. Bakt. u. Parasitenk.," 1894, XV, 470.

† "Arbeiten aus d. Kaiserl. Gesundheitsamte.," 1903, XIX, No. 3.

1. **Entamœba Coli** (Lösch, 1875).—This organism seems to be a harmless commensal, living in the intestines of man, many domestic, and many wild animals. It may be abundant when the reaction of the intestinal contents is neutral or alkaline. It usually measures between 10 and 20 μ in diameter when free, but when encysted from 15 to 50 μ. It is spheroidal when not in motion, and under these conditions it is difficult to differentiate endoplasm and ectoplasm. The ameboid movement is sluggish and the pseudopods are rather short, broad, and blunt. As they are protruded the clear ectoplasm becomes visible. The organism has a grayish color, a finely granular cytoplasm, and usually only a single vacuole. The nucleus is usually fairly well defined and spherical, and, in addition to the chromatin, contains several nucleoli. When stained with polychrome methylene-blue the ectoplasm stains blue; the endoplasm, violet; and the nucleus, red.

Reproduction usually takes place by simple division, but a form of autogamous sporulation also takes place, the organism first becoming encysted, the nucleus dividing into eight segments, and the whole process eventuating in the formation of eight young organisms.

This ameba is easily cultivated upon artificial media according to methods to be described below.

It is not pathogenic, and all attempts to make it damage the intestines of experiment animals have failed.

2. **Entamœba Histolytica** (Schaudinn*).—This is now recognized as the organism seen by Lösch, Koch, Kartulis, Councilman and Lafleur, and accepted as the cause of the amebic form of dysentery. It is found in all parts of the world, but more frequently in tropical than colder climates, and is present only in the intestines of those suffering from dysentery. It is usually present in great numbers so that its discovery in the evacuations is easy.

Morphology.—It is usually considerably larger than Entamœba coli and varies in diameter up to 50 μ. When at rest it is spherical, when active it is very irregular. Its movement is active and the pseudopodia are larger and more numerous than in the other species. The differentiation of ectoplasm and endoplasm is usually distinct. The former is hyaline, the latter granular. The protoplasm has a greenish or yellowish color. The nucleus is small, not very distinct. There are numerous vacuoles. In the intestinal evacuations of dysentery its protoplasm commonly contains many red blood-corpuscles, upon which the organism seems to feed.

Staining.—When stained with polychrome methylene-blue the ectoplasm stains more deeply than the endoplasm. The nucleus contains relatively little chromatin.

Reproduction.—Multiplication takes place by binary division after karyokinesis and by encystment and sporulation. The sporula-

* "Arbeiten a. d. k. k. Gesundheitsamt.," 1903, XIX, 547.

tion is quite different from that seen in Entamœba coli, and only takes place when conditions are unfavorable to continued division. It is accomplished by a peculiar nuclear budding, by which chromatin granules or chronidia are pushed out from the nucleus toward the ectoplasm, where they develop into new nuclei, about which the cytoplasm collects until a distinct bud is formed and cast off as a small but distinct new organism—a spore or bud. These when separated are round or oval, measure 3 to 6 μ in diameter, and are

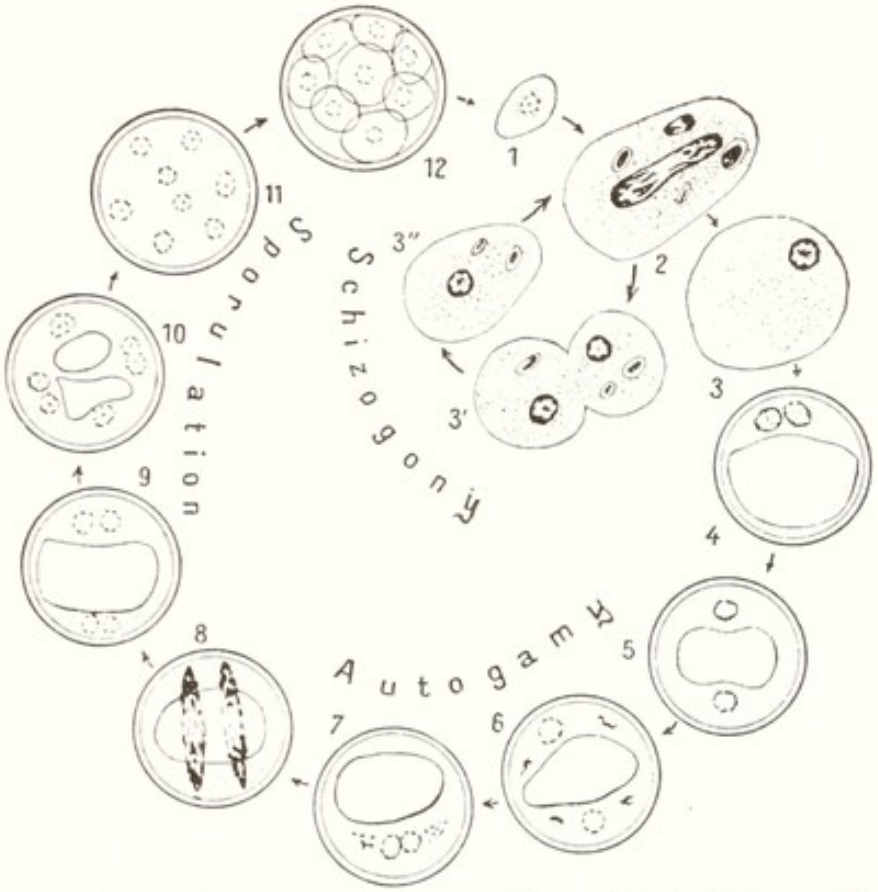


Fig. 259.—Reproductive cycle of parasitic ameba (Wenyon). The small circle indicated by 1, 2, 3, 3′ and 3″ indicated multiplication by schizogony or binary division. The large circle indicated by 1–12, the sporogeny or sexual cycle. The ameba having arrived at its full size (3) becomes encysted (4). The nucleus then divides into two (5), each half expels a small fragment of nuclear material (6), and when this has been effected, they conjugate (7) forming a synkaryon. The synkaryon then divides into two, into four, and then generally into eight (8–9–10–11–12) when the cyst ruptures, the spores are liberated (1) and both cycles are again started.

surrounded by a yellowish envelope, which resists drying and the penetration of stains and chemicals.

Craig gives a tabulation of the differential features of Entamœba coli, Entamœba histolytica, and Entamœba tetragena (*vide infra*).

3. **Entamœba Tetragena** (Viereck*).—This organism resembles Entamœba histolytica more than Amœba coli, but differs from it in the mode of reproduction, the sporocysts containing four instead of eight spores.

* "Archiv. f. Schiffs. u. Tropenhygiene," 1907, II, 1.

Relationship of the Organisms.—In recent years (1910–1915) much morphological and experimental study of these amœbas has been conducted with results that are given in full, together with the literature, in a paper "The Identity of Entamœba Histolytica and Entamœba Tetragena, with Observations upon the Morphology and Life Cycle of Entamœba Histolytica" by Charles F. Craig.* The results of his studies, as set forth in the paper, go to show that Schaudinn was in error in regard to the developmental cycle of Entamœba histolytica, that what he supposed to be its sole method of reproduction, is only that means that preponderates during the period of its greatest activity; that as the acme of the dysenteric disease is passed and the process of repair sets in, the other mode of reproduction characteristics of Entamœba tetragena is observed, and that the two species Entamœba histolytica and Entamœba tetragena are one. There is, therefore, to all appearances, and according to the best information available at present, only one pathogenic intestinal amœba, the Entamœba histolytica. The same conclusions have also been arrived at by Darling.†

With regard to Entamœba coli, opinion as to its non-pathogenic disposition is much less certain than a few years ago. Williams and Calkins‡ close their excellent paper upon "Cultural Amœba; a Study in Variation" with the statement that "it is unwise for anyone at present to be too positive in regard to the distinctive features of Entamœba coli, E. tetragena and E. histolytica, or any of the Entamœba groups. There may be in man, three or more, or two (as Hartmann, Whitman, Walker and Craig now think) or possibly only one species of ameba manifesting different forms under different conditions."

Isolation and Cultivation.—Many experimenters have made more or less successful attempts to cultivate amebas. Musgrave and Clegg,§ whose interesting paper the student will do well to read, and in which he will find a complete review of all antecedent work, were able to cultivate a considerable variety of amebas upon agar-agar made of:

Agar	20.0	grams
Sodium chlorid	0.3–0.5	"
Extract of beef	0.3–0.5	"
Water	1000.0	cc.

Prepare as ordinary culture agar, and render 1 per cent. alkaline to phenolphthalein. The finished medium is poured into Petri dishes. To obtain the greatest number of most active amebas the patient should be given a dose of a saline purgative, and the fluid evacuation resulting from its action employed for inoculating the media. The cultures are, naturally, not pure; they contain various amebas and numerous bacteria.

* "Jour. Infectious Diseases," 1913, XIII, 30.

† "Trans. of the Fifteenth International Congress on Hygiene and Dermography," Washington, D. C., Sept., 1912.

‡ "Jour. Med. Research," 1913–1914, XXIV, 43.

§ "Department of the Interior, Bureau of Government Laboratories, Biological Laboratory," Manila, Oct., 1904, No. 8.

To isolate and cultivate a single kind of ameba Musgrave and Clegg have recommended an ingenious technic.

A plate is selected upon which the desired amebas are so widely separated from one another that not more than one is in a microscopic field of a low-power objective. The microscope used should have a double or triple nose-piece. With a low-power (Zeiss A A) objective, a well-isolated organism is brought to the center of the field. The lens is then swung out and a perfectly clean higher-power lens (Zeiss D D) swung in and racked down until it touches the surface of the agar-agar, when it is quickly elevated again. In three out of five cases the ameba adheres to the objective and is so picked up. Whether it has done so or not can be determined by swinging in the low-power lens again and looking for the organism. If it has disappeared, it is attached to the objective. It is now planted upon a fresh plate by depressing the high-power lens until it touches the surface of the culture-medium, when, upon elevating it again, it usually leaves the ameba behind. Observation with the low power will enable one to determine whether it be successfully planted or not.

Naturally the organisms cannot be thus transplanted without some bacteria falling upon the plate, but this is not very material, for in the first place they do not grow very rapidly upon the medium used for culture, and in the second, they are useful for the nourishment of the ameba, which is holophagous, and cannot live by the absorption of nutritious fluids.

Later it was shown by Tsugitani* that killed cultures of bacteria could supply the necessary nourishment. All cultures of amebas must contain some kind of cells upon which the amebas can feed. When planted as above suggested a variety of organisms grow, and as the amebas multiply and gradually extend over the plate, their preference for one or other of the associated bacteria may be determined in part by placing a drop of the ameba culture upon a plate of sterile media, and then with the platinum wire, dipped in a culture of the bacteria, and drawing concentric circles about the drop further and further apart. As the amebas move about over the plate, passing through the growing circles of bacteria, they soon lose the miscellaneous bacteria and come to contain the one variety planted with them, or if several have been used in drawing different circles, that one which they prefer to feed upon. By transplanting amebas from plate to plate with suitable bacteria or other cells for them to feed upon, the cultures may be kept growing almost indefinitely.

Anna Williams† has been able to grow ameba in pure culture without bacteria, either dead or alive, by smearing the surface of a freshly prepared agar-agar plate with a fragment of freshly removed rabbit's or guinea-pig's brain, kidney, or liver, held in a pair of forceps. The ameba gladly take up and live upon the cells left behind upon the surface of the agar.

Vital Resistance.—The free amebas in the intestinal discharges are easily destroyed by dilute germicides and by drying. Encysted amebas are, however, more difficult to kill. They resist drying well and also resist the penetration of germicides. Direct sunlight inhibits the activities of the organisms, but does not kill them.

* "Centralbl. f. Bakt. u. Parasitenk.," Abt. I, XXIV, 666.

† "Journal of Medical Research," Dec., 1911, XXV, No. 2, p. 263.

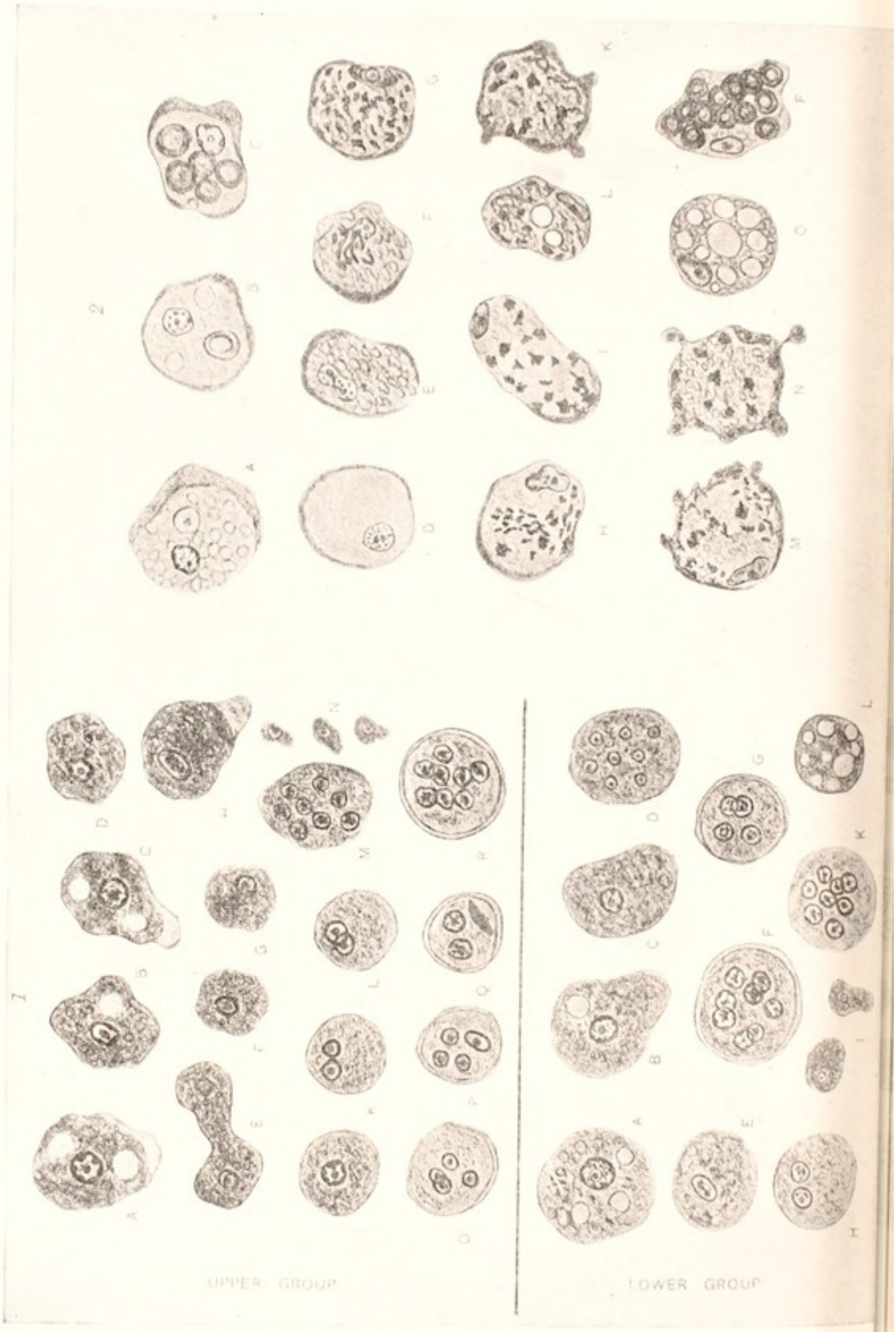


FIG. 260.

EXPLANATION OF FIG. 260

(All figures drawn by Charles F. Craig, M. D.)

I. Upper Group.—*Entamœba coli* stained with Giemsa stain.

A, *B*, and *C*. Vegetative organisms showing nuclear membrane, karyosome, and collections of chromatin upon the nuclear membrane and within the hyaloplasm. Vacuoles are also present.

D. An organism containing a protozoan parasite which might be mistaken for spores.

H. Division of nucleus (primitive mitosis).

E. Partially divided ameba containing two nuclei.

F, *G*. Ameba resulting from simple division.

M. Schizogony of Entamœba coli. Eight daughter nuclei in vegetative form.

N. Ameba resulting from schizogony.

I. Earliest stage in cyst formation. Cytoplasm clear of foreign bodies and nucleus showing collection of chromidial masses upon the inner side of the nuclear membrane.

K, *L*, *O*, *P*. Two- and four-nucleated stage of reproduction within the cyst.

Q. Encysted form containing two large nuclei and a mass of chromatin.

R. Fully developed cyst of Entamœba coli containing eight nuclei.

Lower Group.—*Entamœba coli*, fixed in sublimate alcohol and stained with Delafield's hematoxylin. Note the more delicate staining of the nucleus and the greater detail obtained with this method of staining.

A, *B*, *C*. Vegetative amebæ showing variations in the structure of the nucleus.

D. An organism during schizogony, containing eight nuclei.

E. Mitotic division of the nucleus as observed in this species.

F. A fully developed cyst of Entamœba coli containing eight daughter nuclei.

G. The four-nucleated cystic stage of Entamœba coli sometimes mistaken for the cyst of Entamœba tetragena.

H. Two-nucleated cyst of Entamœba coli.

I. Young amebæ originating from the cysts of Entamœba coli.

K. Fully developed cyst in which the cystic membrane is apparently absent.

L. Degenerated cyst of Entamœba coli, filled with vacuoles, and containing masses of chromatin. No nucleus is visible.

II. *Entamœba histolytica* stained with Giemsa stain.

A. Organism showing distinction between the ectoplasm and endoplasm, nucleus and vacuole.

B. Organism showing vacuole and red blood corpuscle and nucleus containing minute karyosome and chromatin dots in the hyaloplasm.

C. Organism showing nucleus and numerous red blood corpuscles.

D. Organism in first stage of nuclear division, showing division of the karyosome and minute dots of chromatin in hyaloplasm.

E. Organism showing later stage of nuclear division, the polar bodies being connected by a filament of chromatic substance.

F. First stage of formation of spore cysts; the nucleus distributing chromatin to the cytoplasm.

G to *I*. Stages in the process of formation of spore cysts, the chromatin being distributed to the cytoplasm and collected in threads or masses, while the nucleus is observed as a flattened body crowded against the periphery of the parasite.

L. Degenerated parasite containing vacuoles and free chromatin.

K, *M*, *N*. Entamœba histolytica in the final stage of the formation of spore cysts. The free chromatin has collected at the periphery, and surrounded by a small amount of cytoplasm, is being budded off from the parent organism.

O. Degenerated organism filled with vacuoles and free from chromatin. The nucleus stains abnormally and there is no distinction between the ectoplasm and endoplasm.

P. Entamœba histolytica filled with erthyrocytes, the nucleus being crowded to the periphery and staining abnormally (Charles F. Craig, M. D., in Journal of Medical Research, vol. XXVI, No. 1, April, 1912).

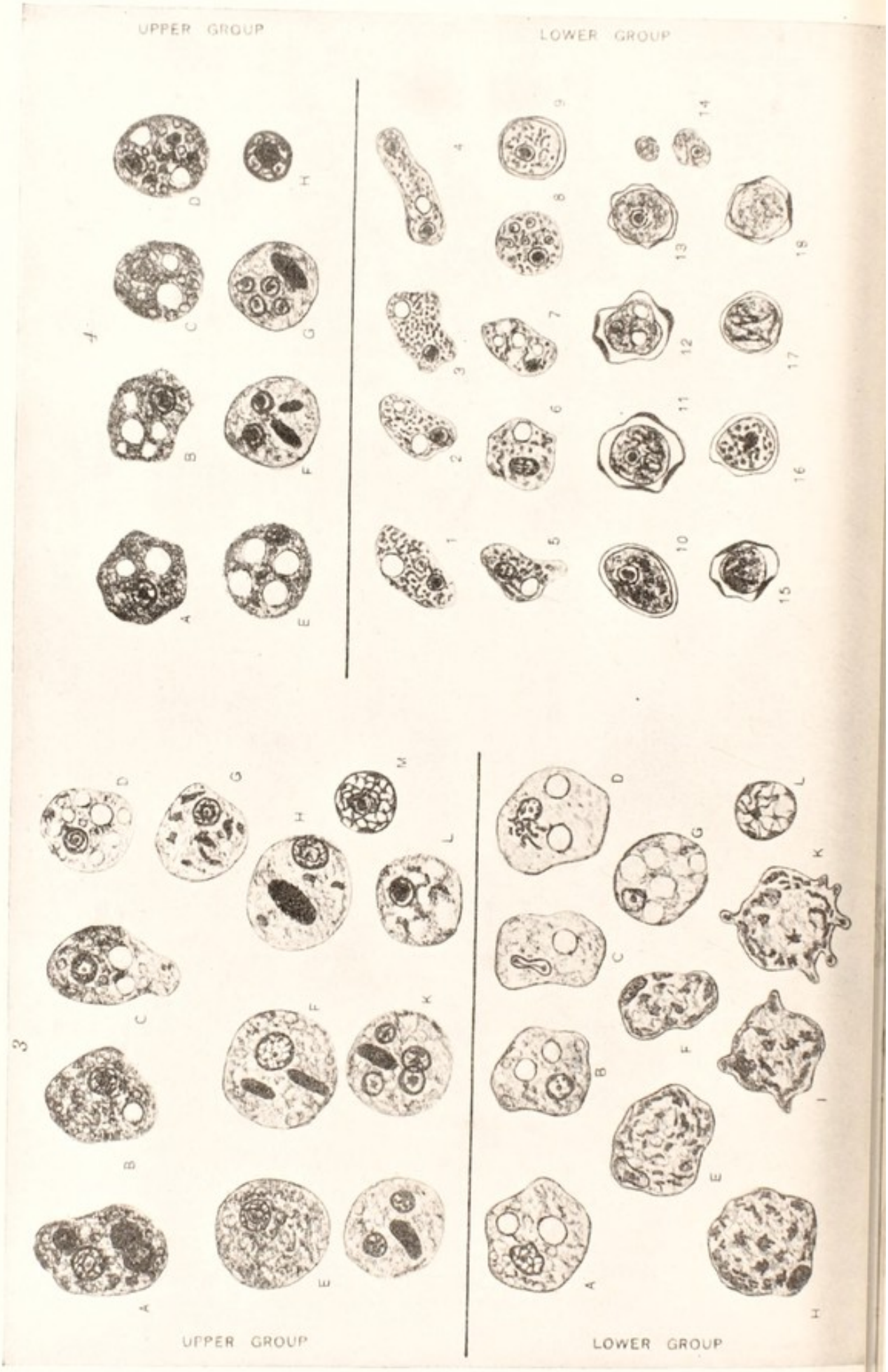


FIG. 261.

EXPLANATION OF FIG. 261

(All figures drawn by Charles F. Craig, M. D.)

III. Upper Group.—*Entamœba tetragena* fixed in sublimate alcohol and stained with Delafield's hematoxylin. Note the great delicacy of the staining when compared with the staining with the Giemsa method.

A. A vegetative parasite showing three erythrocytes in the cytoplasm and a nucleus in which the nuclear membrane, and the karyosome with its centriole are shown.

B. A vegetative organism showing thick nuclear membrane and karyosome containing a centriole.

C. A vegetative parasite containing vacuoles and nucleus showing karyosome containing a centriole surrounded by an unstained area.

D. A degenerative form filled with vacuoles and showing abnormal appearance of the nucleus.

E. Precystic form of Entamœba tetragena.

G. Another precystic form which is more typical in the free chromatin in the cytoplasm is visible. The form *E* would probably degenerate before the cyst wall was fully formed.

F. A cystic form of Entamœba tetragena showing two chromatin spindles in the cytoplasm and a nucleus having a centriole surrounded by an unstained area and a definite network upon which are arranged dots of chromatin.

H. An encysted form showing a very large mass of chromatin and a nucleus containing a karyosome and centriole.

I. Two-nucleated cyst of Entamœba tetragena showing mass of free chromatin and the morphology of the nuclei after division.

K. Fully developed cyst of Entamœba tetragena containing four daughter nuclei and a mass of chromatin.

L. Degenerated form of Entamœba tetragena containing some free chromatin and a nucleus in which the karyosome stains deeply and nearly fills the nucleus. This form might be mistaken for a free living ameba.

M. Illustrating the typical nuclear structure of Entamœba tetragena. Note the large karyosome containing a centriole surrounded by an unstained area.

Lower Group.—*Entamœba histolytica* fixed in sublimate alcohol and stained with Delafield's hematoxylin.

A and *B*. Vegetative organisms showing vacuoles and typical morphology of the nucleus. No distinction between the endoplasm and ectoplasm.

C. Vegetative form of Entamœba histolytica showing the type of mitosis during simple division.

D. First step in the formation of spore cysts. The distribution of the chromatin by the nucleus to the cytoplasm.

E, *F* and *H*. Organisms showing chromidia in the cytoplasm arranged in rods, threads, and masses, the nucleus being flattened out against the periphery and staining poorly.

G. A degenerative form of Entamœba histolytica filled with vacuoles and with an atypical nucleus.

I and *K*. Budding of the spore cysts from the periphery of Entamœba histolytica.

L. Illustrating the typical nuclear structure of Entamœba histolytica.

IV. Upper Group.—*Entamœba tetragena* stained with Giemsa stain.

A, *B*, *C*. Vegetative organisms. Note that the nuclear membrane and karyosome stain very heavily and are not as well differentiated as in specimens stained with hematoxylin.

D. Precystic form containing masses of chromatin in the cytoplasm.

E. Degenerative form containing vacuoles, masses of chromatin, and an atypically stained nucleus.

F. Two-nucleated stage of the cyst of Entamœba tetragena, showing heavy staining of the nuclear membrane and karyosome. Two masses of chromatin are present.

Lösch was the first to observe that quinin was destructive to intestinal amebas, and his observations have been reviewed by many others. Musgrave and Clegg found that active cultures of one ameba were killed in ten minutes by a 1:2500 solution of quinin hydrochlorate. The exposed organisms quickly encysted themselves and in from five to eight minutes many of them had broken up and disappeared. After ten minutes all were dead. Cultures of another ameba similarly treated gave a scanty growth after ten minutes.

Vedder found that emetin would kill ameba in dilutions up to 1:100,000, and Rogers has shown that this drug is the most destructive agent we possess as an amebicide. Unfortunately it does not kill the encysted forms.

Exposure to 1:1000 solution of formalin did not kill encysted amebas in twenty-four hours. Acetozone did not kill amebas in 1:1000 dilutions. If, however, the acetozone was made 1 per cent. acid to phenolphthalein the amebas were all killed by 1:5000 solutions in ten minutes.

Metabolic Products.—It seems as though Entamœba histolytica must produce some metabolic product that exerts an enzymic action upon the human tissues and thus accounts for the destructive nature of the lesions. This has not, however, been demonstrated as yet.

G. Fully developed cyst of Entamœba tetragena containing four nuclei and one mass of chromatin.

H. Illustrating the type of nucleus as observed in Entamœba tetragena in specimens stained with Giemsa stain.

Lower Group.—*Amœba lobospinosa* stained with Delafield's hematoxylin after fixation with sublimate alcohol.

1, 2, and 3. Vegetative organisms showing the large contractile vacuole and the typical nucleus containing a deeply stained karyosome almost filling the nucleus.

4. A vegetative ameba in which the nucleus has divided.

5, 6. Vegetative amebæ in which the nucleus is dividing. Polar bodies are present connected by filaments and a well-marked equatorial plate is apparent.

7. Degenerated vegetative ameba filled with vacuoles and with atypically staining nucleus.

8. Amœba lobospinosa containing a protozoan organism. These forms have been mistaken for sporulating amebæ.

9 and 10. Encysted forms of Amœba lobospinosa during the first few days in cultures.

11 to 18 (except 14). Various cystic forms of Amœba lobospinosa showing the character of the cyst wall in the older cysts. At 12 the cyst contains two vacuoles and the cyst membrane is folded in, an appearance frequently observed in cultures which have become dry; 15 and 17 represent cysts in which the cyst wall is cracked and a nucleus cannot be distinguished; 16 represents a cyst filled with deeply staining granules of chromatin derived from the degenerated nucleus; 18 is a cyst in which only the cystic membrane is visible, the ameba having escaped from the cyst.

14. A fragmenting ameba frequently mistaken for a budding organism before the separation of the fragments (Charles F. Craig, M. D., in Journal of Medical Research, vol. XXVI, No. 1, April, 1912).

DIFFERENTIAL FEATURES OF ENTAMŒBA COLI, ENTAMŒBA HISTOLYTICA, AND ENTAMŒBA TETRAGENA.*

Name	Size	Pseudopodia	Motility	Protoplasm	Nucleus	Cyst formation	Cultures	Methods of reproduction	Pathogenesis	Staining
Entamœba coli, Schaudinn, 1903.	Ten to 30 microns, generally smaller than Entamœba histolytica or Entamœba tetragena.	Small, blunt, and not clearly differentiated from rest of parasite.	Sluggish.	Ectoplasm not distinct, except when moving, and then only because it is free from granules. Is grayish in color and not very refractive. Endoplasm is gray, finely granular, few non-contractile vacuoles. Is not generally phagocytic for red blood-corpuscles.	Distinct, having a well-defined nuclear membrane and much chromatin. Large karyosome.	Present. Eight young amebas developed within cyst.	Doubtful.	By simple division; autogenous sexual reproduction in cyst; and by schizogony with the production of eight daughter amebas. Eight amebas are produced within the cyst.	Is not pathogenic, occurring in a large percentage of healthy individuals.	With Wright's stain, ectoplasm, light blue; endoplasm, dark blue; and nucleus red.
Entamœba histolytica, Schaudinn, 1903.	Ten to 70 microns, generally from 15 to 40 microns.	Blunt or slender and finger-shaped. Very refractive and clearly differentiated from rest of the parasite.	Active.	Ectoplasm is very distinct and refractive, in some instances even when motionless. Glassy appearing. Endoplasm is granular, contains numerous non-contractile vacuoles and red blood-corpuscles, when latter are present in feces.	Indistinct. No well-defined nuclear membrane and but little chromatin. Minute karyosome.	Minute spores developed by budding measure 3 to 5 microns. Possess a resistant membrane like a cystic covering. Development of the spores has not been studied.	Doubtful.	By simple division; gemmation; and by the budding of chromidial masses surrounded by protoplasm from the periphery of the mother parasite, forming minute spores.	Is the cause of a form of amebic dysentery.	With Wright's stain, ectoplasm, dark blue; endoplasm, light blue; and nucleus, pale red or pink.
Entamœba tetragena, Viereck, 1907.	Ten to 50 microns, about the size of Entamœba histolytica.	Lobose or finger-shaped. Very refractive and well differentiated from rest of parasite	Active.	Ectoplasm and endoplasm well differentiated. Ectoplasm hyaline in appearance. Endoplasm granular, containing numerous non-contractile vacuoles and red blood-corpuscles, when latter are present in feces.	Distinct, having definite nuclear membrane formed by chromatin. Large karyosome.	Present. Four amebas develop within cyst.	Negative.	By simple division and by autogamous sexual reproduction within cyst, four amebas being produced.	Is the cause of a form of amebic dysentery.	Does not stain well with Wright's stain

* Charles F. Craig, M. D., "Entamœba Tetragena as a Cause of Dysentery in the Philippine Islands," The Arch. of Inter. Med., vol. VII, No. 3, Mar. 15, 1911.

Pathogenesis.—Schaudinn was the first to prove the pathogenic action of the organism. He inspissated the evacuations of a case suffering from dysentery, so that it contained considerable numbers of encysted amebas. When this was fed to kittens they died in two weeks with the typical lesions of dysentery. Musgrave and Clegg had less satisfactory results with cats, dogs, and other laboratory animals, but were quite satisfied with the results secured with monkeys, which took the disease and sometimes died. The lesions resembled, but were less severe than those in man. Musgrave and Clegg would not admit that there were non-pathogenic intestinal amebas, but this was not in accord with the work of any other investigators, and was strongly opposed by Craig,* who found both

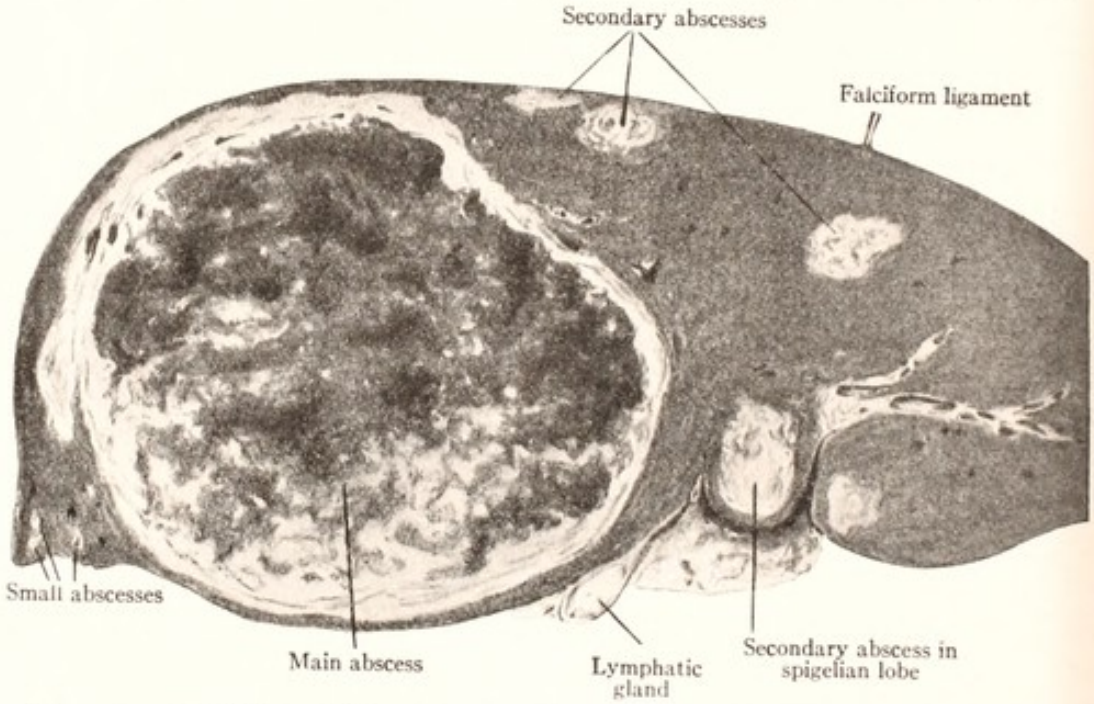


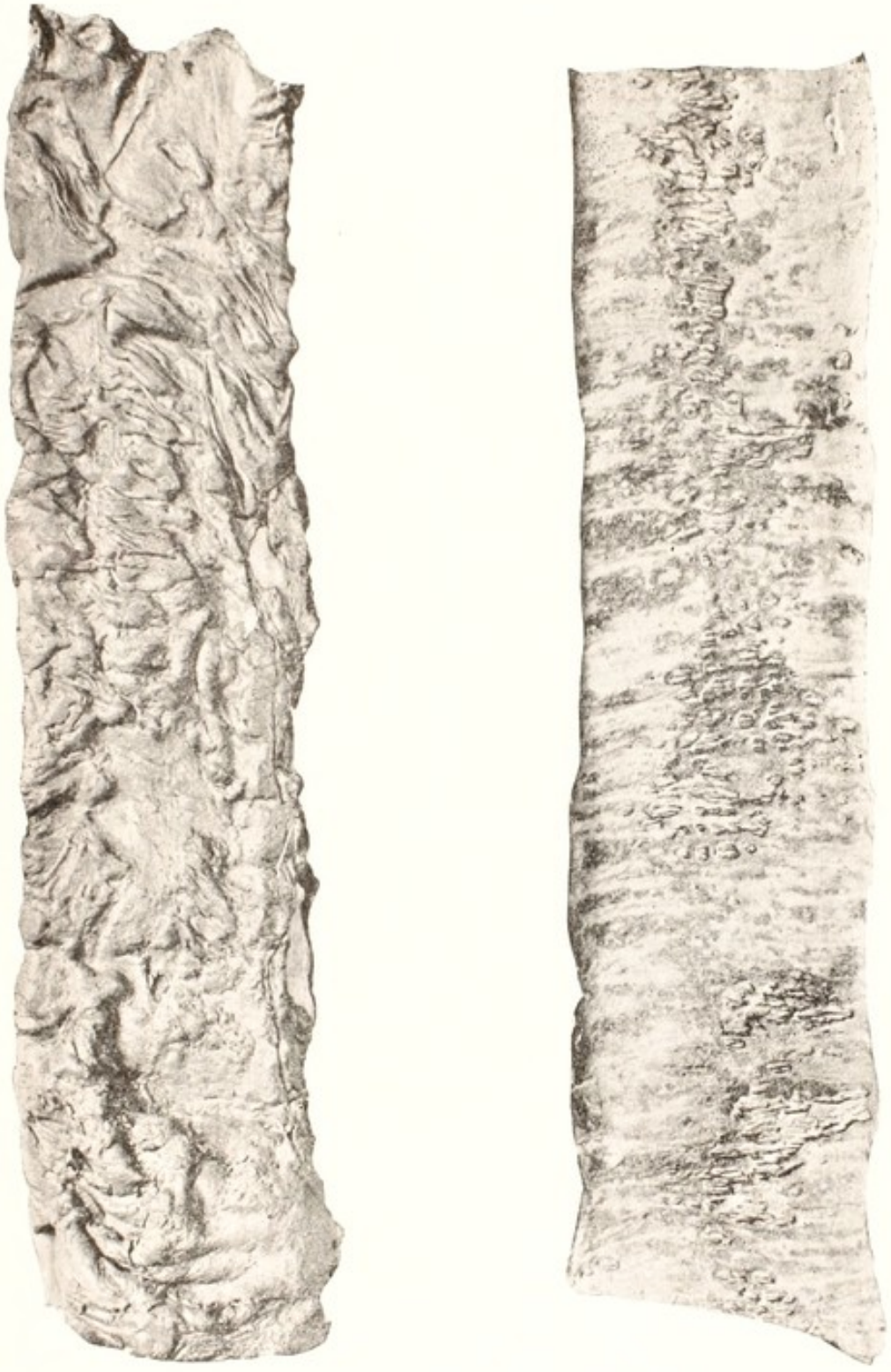
Fig. 262.—Multiple amebic abscesses of the liver (J. E. Thompson, in International Clinics, vol. II, 14th Series, J. B. Lippincott Co., Publishers).

varieties, and though he was never able to infect animals with Entamœba coli, was successful with the pathogenic varieties, and succeeded in infecting 50 per cent. of the kittens he experimented upon, by injecting the amebas into the rectum.

Lesions.—The gross morbid appearances of the intestinal lesions in both forms of dysentery are sufficiently distinct in typical cases to enable an experienced pathologist to differentiate them, yet not sufficiently distinct to make them easy of description. The one great characteristic feature of the amebic dysentery is abscess of the liver which occurs in nearly 25 per cent. of the cases, but which almost never occurs in bacillary dysentery.

The distinct and somewhat rigid ectoplasm of the Entamœba histolytica is supposed to make it easy for the organisms, which it

* "Journal of Infectious Diseases," 1908, v, p. 324.



Figs. 263, 264.—Colon. Tropical or amebic dysentery.

will be remembered are actively motile, to penetrate between the epithelial cells of the intestinal mucosa to the lymph-spaces of the submucosa below. Here the amebas multiply in large numbers, and by the enzymic action of their metabolic products produce necrosis of the suprajacent tissues with resulting exfoliation and the production of round, oval, or ragged ulcerations with markedly infiltrated and undermined edges. As the amebas continue to increase and fill up the lymphatics, and as bacteria add their effects to those occasioned by the amebas, the ulcers increase in extent and depth until the mucosa and submucosa may be almost entirely

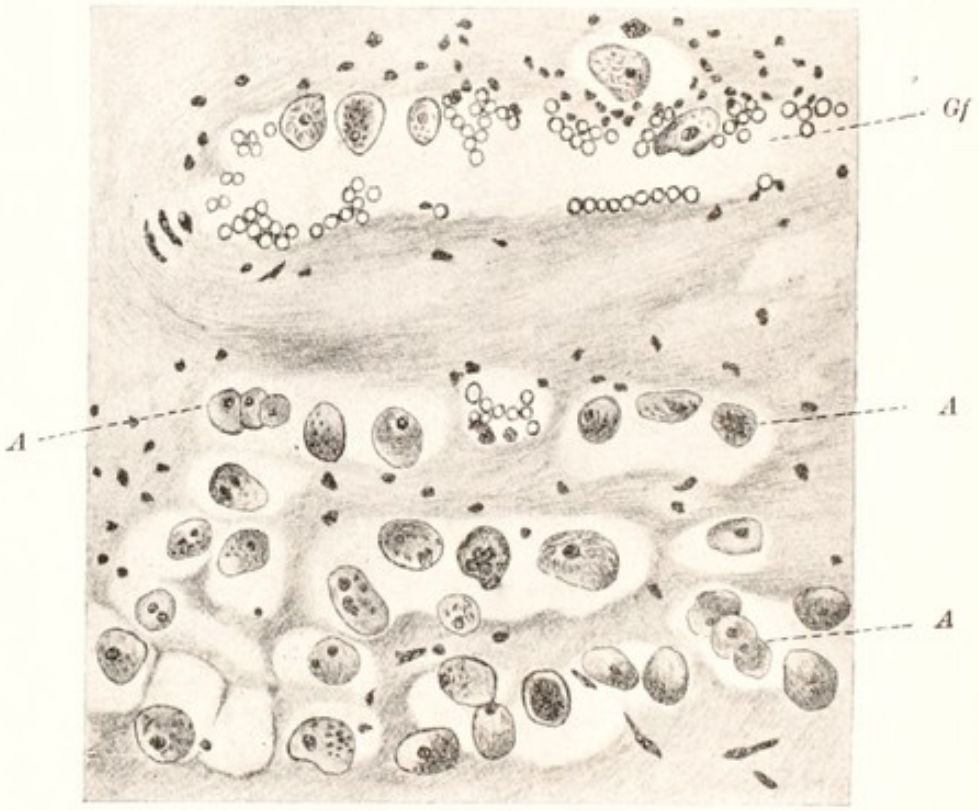


Fig. 265.—Entamœba histolytica. Section of the human intestinal wall showing the amebas at the base of a dysenteric ulcer: *A*, *A*, *A*, Amebas, some of which are in blood-vessels, *Gf* (Harris).

destroyed, leaving the entire large intestine denuded, except for occasional islands of much congested, inflamed, and partly necrotic mucous membrane. The diseased intestinal wall is the seat of much congestion and is much thickened. The amebas not only occur in great numbers in the interstices of the tissues about the base of the ulcers and in the lymphatics, but also enter the capillaries, through which they are carried to the larger vessels, and eventually to the liver, where their activities continue and give rise to the amebic abscess. The first expression of their injury to the liver parenchyma is shown by focal necroses. In each of these the organisms multiply and the lesion extends until neighboring necroses are brought into union, and eventuate in great collections of colliquated necrotic

material which may be so extensive as to involve the entire thickness of the organ. There is usually one large abscess, but there may be several small ones, or the liver may be riddled with minute abscesses. The content of the abscesses is pinkish necrotic material in which amebas are few. The walls are of semi-necrotic material, in which great numbers of amebas abound. The liver sometimes becomes adherent to the diaphragm, may perforate it, and after adhesion of the lung to the diaphragm may evacuate through the lung, the pinkish abscess contents with amebas being expectorated.

Sections of the intestinal wall and of the liver near the border of the abscess show the amebas well when stained with iron-hematoxylon, or perhaps still better by Mallory's differential method.*

1. Harden the tissue in alcohol.
2. Stain sections in a saturated aqueous solution of thionin three to five minutes.
3. Differentiate in a 2 per cent. aqueous solution of oxalic acid for one-half to one minute.
4. Wash in water.
5. Dehydrate in absolute alcohol.
6. Clear in alcohol.
7. Xylol-balsam.

The nuclei of the amebas and the granules of the mast-cells are stained brownish red; the nuclei of the mast-cells and of all other cells are stained blue.

II. BACILLARY DYSENTERY

Bacillus Dysenteriæ (Shiga)

General Characteristics.—A non-motile, non-flagellated, non-sporogenous, non-liquefying, aërobic and optionally anaërobic, non-chromogenic, non-aërogenic, pathogenic bacillus of the intestine, staining by ordinary methods, but not by Gram's method. It does not produce indol. It first acidifies, then alkalinizes milk, but does not coagulate it.

After considerable investigation of the epidemic dysentery prevalent in Japan, Shiga† came to the conclusion that a bacillus which he called Bacillus dysenteriæ was its specific cause.

It is not improbable that the bacillus of Shiga is identical with *Bacterium coli*, variety *dysenteriæ*, of Celli, Fiocca, and Scala,‡ a view that has been further confirmed by Flexner.‖ It may also be identical with an organism described in 1888 by Chantemasse and Widal.§

In 1899 Flexner,** while visiting the Philippine Islands, isolated a bacillus from the epidemic dysentery prevailing there, which he regarded as identical with Shiga's organism. In 1890 Strong and

* "Pathological Technic," 1911, p. 434.
† "Centralbl. f. Bakt. u. Parasitenk.," 1898, XXIV, Nos. 22–24.
‡ "Hygien. Institut. Rom. Univ.," 1895, and "Centralbl. f. Bakt. u. Parasitenk.," 1899.
‖ "Univ. of Penna. Med. Bulletin," Aug., 1901.
§ Deutsche med. Wochenschrift," 1903, No. 12.
** "Bulletin of the Johns Hopkins Hospital," 1900, IX.

Musgrave* isolated what appeared to be the same organism, also from cases of dysentery in the Philippines. Almost at the same time Kruse† was investigating an epidemic of dysentery in Germany, and succeeded in isolating a bacillus that also bore fair correspondence to that of Shiga. In 1901 Spronck‡ found a bacillus in cases of dysentery occurring in Utrecht, Holland, that corresponded with a slightly different organism first found and described by Kruse§ as a "pseudodysentery bacillus."

In 1902 Park and Dunham‖ investigated a small epidemic of dysentery in Maine, and there found a bacillus similar to those already described. In 1903 Hiss and Russell described a bacillus "Y" from a case of fatal diarrhea in a child.

Bacillus dysenteriæ was also found by Vedder and Duval** in the epidemic and sporadic dysentery of the United States. Duval and Bassett†† and Martha Wollstein‡‡ found Bacillus dysenteriæ in cases of the summer diarrheas of infants, especially when such diarrheas were epidemic.

Lentz§§ has shown that dysentery and pseudodysentery bacilli present differences in their behavior toward sugars. Various observers found differences in the behavior of the various bacilli to the agglutinating effects of artificially prepared immune serum. The outcome of these investigations is the discovery that Bacillus dysenteriæ is a species in which there are a number of different varieties well characterized, but by differences too slight to permit them to be regarded as separate species. This thought—that we are dealing with a group of varieties and not a single well-defined organism—is essential to an intelligent understanding of the bacteriology of dysentery.

Varieties of the Dysentery Bacillus.—Three varieties of the dysentery bacillus may now be described:

1. The Shiga-Kruse variety.
2. The Flexner variety.
3. The Hiss-Russell variety.

The differences by which they are separated are to be found in their varying agglutinability by artificially prepared immune serums, each of which exerts a far more pronounced effect upon its own variety than upon the others, and in the behavior toward sugars with reference to acid formation and gas production. It seems not improbable that the future will have much to say about the dys-

* "Report Surg. Gen. U. S. Army," Washington, 1900.
† "Deutsche med. Wochenschrift," 1900, XXVI.
‡ "Ref. Baumgarten's Jahresberichte," 1901.
§ "Deutsche med. Wochenschrift," 1901, Nos. 23 and 24.
‖ "New York Bull. of Med. Sciences," 1902.
** "Journal of Experimental Medicine," 1902; vol. VI, No. 2, "American Medicine," 1902.
†† "American Medicine," Sept. 13, 1902, vol. iv, No. 11, p. 417.
‡‡ "Jour. Med. Research," 1904, X, p. 11.
§§ "Zeitschrift f. Hygiene," etc., 1902, XLI.

entery bacillus, and that the validity of much that is accepted at present may have to be amended. This seems to be particularly true with regard to the matter of fermentation, the details of which are displayed in the table taken from Muir and Ritchie's "Manual of Bacteriology" (p. 650).

Morphology.—The organism is a short rod with rounded ends, generally similar to the typhoid bacilli. It usually occurs singly, but may occur in pairs. It is frequently subject to involutional changes. It is doubtfully motile and is probably without flagella.

Staining.—When stained with methylene-blue the ends color more deeply than the middle; and organisms from old cultures show numerous involution forms and irregularities. It stains with ordinary solutions, but not by Gram's method. It has no spores.

Cultivation.—The organism grows well in slightly alkaline media under aërobic conditions.

Colonies.—The colonies upon gelatin plates are small and dew-drop-like in appearance. Upon microscopic examination they are seen to be regular and of spheric form. By transmitted light they appear granular and of a yellowish color. They do not spread out in a thin pellicle like those of the colon bacillus, and there are no essential differences between superficial and deep colonies.

Gelatin Punctures.—The growth in the puncture culture consists of crowded, rounded colonies along the puncture. A grayish-white growth forms upon the surface. There is no liquefaction of the medium.

Agar-agar.—Upon the surface of agar-agar, cultures kept in the incubating oven show large solitary colonies at the end of twenty-four hours. They are bluish-white in color and rounded in form. The surface appears moist. In the course of forty-eight hours a transparent border is observed about each colony, and the bacilli of which it is composed cease to stain evenly, presenting involution forms.

Glycerin agar-agar seems less well adapted to their growth than plain agar-agar. Blood-serum is not a suitable medium.

Litmus Milk.—Milk is not coagulated. As the growth progresses there is slight primary acidity, which later gives place to an increasing alkalinity.

Potato.—Upon boiled potato the young growth resembles that of the typhoid bacillus, but after twenty-four hours it becomes yellowish brown, and at the end of a week forms a thick, brownish-pink pellicle.

Bouillon.—In bouillon the bacillus grows well, clouding the liquid. No pellicle forms on the surface.

Metabolic Products.—The organism does not form indol, does not ferment dextrose, lactose, saccharose, or other carbohydrates.

TABLE SHOWING CHARACTERS OF THE GRAM-NEGATIVE BACILLI OF THE COLI-TYPHOID GROUP

Bacterium	Motility	Gelatin	Glucose	Lactose	Saccharose	Mannite	Dulcite	Adonite	Sorbite	Inosite	Inulin	Litmus Milk			Indol	Voges' and Proskauer's Reaction
												1 day	3 days	15 days		
Bacillus coli communis	+	–	A. G.	A. G.	–	A. G.	A. G.	–	A. G.	–	–	A. C.	A. C.	A. C.	+	–
B. typhosus	+	–	A.	–	–	A.	–	–	A.		–	A.	A.	Alk. or A.	–	–
B. paratyphosus	+	–	A. G.	–	–	A. G.	A. G.		A. G.		–	A.	Alk.	Alk.	–	–
B. enteritidis (Gaertner)	+	–	A. G.	–	–	A. G.	A. G.		A. G.		–	A.	Alk.	Alk.	–	–
B. dysenteriæ (Shiga)	–	–	A.	–	–	–	–	–	–		–	A.	Alk.	Alk.	–	–
B. dysenteriæ (Flexner)	–	–	A.	–	–	A.	–		–		–	A.	Alk.	Alk.	+	–
B. "Morgan's No. 1"	+	–	A. G.	–	–	–	–		–		–	°	°	Alk.	+ +	–
B. lactis aërogenes	–	–	A. G.	A. G.	A. G.	A. G.	–	A. G.	A. G.	A. G.	–	A. C.	A. C.	A. C.	–	+
B. acidi lactici (Hüppe)	–	–	A. G.	A. G.	–	A. G.	–	A. G.	A. G.	–	–		A. C.		+	–
B. cloacæ	+	+	A. G.	A. G.	A. G.	A. G.	–	–	A. G.	A.	–	–	A. C.	Alk.	+	+
B. fæcalis alcaligenes	+	–	–	–	–	–						Alk.	Alk.			
B. coli anaërogenes	–	–	A.	A.		A.		–			–	A.	A.	A.	+	
B. oxytocus perniciosus*	–	+	A. G.	A. G.	A. G.	A. G.	A. G.	A. G.	A. G.	A. G.	A. G.	A. C.	A. C.	A. C.	+	+
B. vesiculosus*	–	–	A. G.	A. G.	–		–	–		–	–	A. C.	A. C.	A. C.	+	–
B. "McConkey's No. 71"*	+	–	A. G.	A. G.	A. G.		A. G.	–		–	–	A. C.	A. C.	A. C.	+	–
B. Friedländer*	–	–	A. G.	A. G. or A.	A. G.	A. G.	A. G.	A. G.	A. G.		–	A. C.	or	A.	+	–

+ in Motility column = presence of motility; in Gelatin = liquefaction; in Indol = presence of indol; in Voges and Proskauer = presence of reaction.

– in Motility column = absence of motility; in Gelatin = no liquefaction; in Indol = absence of indol; in Voges and Proskauer = absence of reaction; in other columns = absence of change.

"A" = acid production; "G" = gas; "C" = clot; "Alk." = development of alkalinity.

* McConkey, "Journal of Hygiene," v, 333; ix, 86. (From Muir and Ritchie's "Manual of Bacteriology," courtesy of Oxford University Press, Publishers.)

Acids are produced in moderate quantities after twenty-four hours. Milk is not coagulated. Gelatin is not liquefied.

Toxins, chiefly endotoxins, are produced. They may best be prepared by making massive agar-agar cultures in Kitasato flasks or flat-sided bottles, and after growth is complete washing off the bacillary mass with a very small quantity of sterile salt solution, and after killing the bacilli by exposure to 60°C. for fifteen to thirty minutes, permitting the rich suspension to autolyze for three days. The toxins may be precipitated from the sodium chlorid solution by ammonium sulphate.

Vital Resistance.—The thermal death-point is 68°C. maintained for twenty minutes. It grows slowly at ordinary temperatures, rapidly at the temperature of the body.

Pathogenesis.—Shiga and Flexner found that infection of young cats and dogs could be effected by bacilli introduced into the stomach, and that lesions suggestive of human dysentery were present in the intestines. Kazarinow* found that when guinea-pigs and young rabbits were narcotized with opium, the gastric contents alkalinized with 10 cc. of a 10 per cent. NaOH solution, and a quantity of Shiga bacilli introduced into the stomach with an esophageal bougie, it was possible to bring about diarrhea and death with lesions similar to those described by Vaillard and Dopter.

In these experiments it was found that rapid passage through animals greatly increased the virulence of the bacilli, and it was also observed that though 0.0005 cc. of a virulent culture introduced into the peritoneal cavity would cause fatal infection, to produce infection by the mouth as above stated required the entire mass of organisms grown in five whole culture-tubes.

The virulent organisms are infectious for guinea-pigs and other laboratory animals, and cause fatal generalized infection without intestinal lesions.

Lesions.—The lesions found in human dysentery are usually fairly destructive. They consist of a severe catarrhal and pseudomembranous colitis, which later passes into a stage of marked ulceration. There is great thickening of the submucosa and the whole of the intestinal lining is corrugated. For the most part the ulcerations are more superficial than those of the amebic dysentery, and the edges of the ulcerations show less tumefaction and less undermining. Abscess of the liver does not occur in bacillary dysentery.

Diagnosis.—The blood-serum of those suffering from epidemic dysentery or from those recently recovered from it causes a well-marked agglutinative reaction. This agglutination was first carefully studied by Flexner, and is peculiar in that the serums prepared from the different varieties of the bacillus, while they exert

* "Archiv. f. Hyg.," Bd. L, Heft 1, p. 66; see also "Bull. de l'Inst. Past.," 15 Aout, 1904, p. 634.

some action upon all varieties of the organism, exert a much more powerful influence upon the particular variety used in their preparation. The same is true of the patient's serum, hence, in making use of the agglutination reaction for the diagnosis of the disease, the blood of the patient should be tested by contact with all of the different cultures.

Serum Therapy.—By the progressive immunization of horses to an immunizing fluid, the basis of which is a twenty-four-hour-old agar-agar culture dried *in vacuo*, Shiga prepared an antitoxic serum with which, in 1898, in the Laboratory Hospital 65 cases were treated, with a death-rate of 9 per cent.; in 1899, in the Laboratory Hospital, 91 cases, with a death-rate of 8 per cent.; in 1899, in the Hirowo Hospital, 110 cases, with a death-rate of 12 per cent. These results are very significant, as the death-rate in 2736 cases simultaneously treated without the serum averaged 34.7 per cent., and in consideration of the frequency and high death-rate of the disease, Japan alone, between the years 1878 and 1899, furnishing a total of 1,136,096 cases, with 275,308 deaths (a total mortality for the entire period of 24.23 per cent.).*

BALANTIDIUM DIARRHEA

Balantidium Coli (Malmsten)

In certain rare cases a severe form of diarrhea, or a mild form of dysentery appears to depend neither upon Entamœba histolytica nor Bacillus dysenteriæ, but upon an infusorian parasite known as Balantidium coli. This organism was first observed by Malmsten† in 1857 in the intestines of a man who had suffered from cholera two years before and had ever since suffered from diarrhea. Upon investigation, an ulceration was found in the rectum just above the internal sphincter. In the bloody pus from this ulcer numerous balantidia were seen swimming about. Although the ulcer healed, the diarrhea did not cease. Since this original observation and up to 1908, Braun‡ had been able to collect 142 cases of human infection. In all of these cases the presence of the balantidium was accompanied by obstinate diarrhea with bloody discharges (dysentery) in some, and many of the cases ended in death.

Morphology.—The Balantidium coli is a ciliate protozoan micro-organism of ovoid or ellipsoidal form, measuring from 30 to 200 μ in length and from 20 to 70 μ in breadth. The body is surrounded by a distinct ectosarc completely covered by short fine cilia. The anterior end, which is usually a little sharper than the posterior, presents a deep indentation, the peristome, which continues, in an infundibuliform manner, deeply into the endosarc. The peristome is surrounded by a circle of longer cilia—adoral cilia—than those elsewhere upon the body. At the opposite pole there is a small opening in the ectosarc, the anus. The mouth is the simple termination of the infundibuliform extension of the peristome and opens directly into the endosarc, so that the small bodies upon which the organism feeds, and which are continually being caught in the vortex caused by the rapidly vibrating adoral cilia are driven down the short tubulature directly into the endosarc.

The endosarc is granular and contains fat and mucin granules, starch grains, bacteria, and occasionally red and white blood-corpuscles.

There are usually two contractile vacuoles, sometimes more, and as the quiet

* "Public Health Reports," Jan. 5, 1900, vol. XV, No. 1.
† "Archiv. f. pathologische Anatomie," etc., XII, 1857, p. 302.
‡ "Tierische Parasiten des Menschen," Würzburg, 1908.

organism is watched these large clear spaces can be seen alternately to contract and expand.

There are two nuclei. The larger, or macronucleus, is bean-shaped, kidney-shaped, or, more rarely, oval. The smaller, the micronucleus, is spherical. There is no digestive tube; the nutritious particles are directly in the endosarc, in which they are digested, any residuum being extruded from the anus.

Motility.—The organism is actively motile, swimming rapidly at a steady pace or darting here and there.

Staining.—The organism can be most easily and satisfactorily studied while alive. To stain it a drop of the fluid containing the balantidia is spread upon a slide and permitted to dry. Just before the moisture disappears from the film, methyl alcohol may be poured upon it to kill and fix the organisms. The staining may then be performed with Giemsa's polychrome methylene-blue or iron-hematoxylon. The cilia usually do not show.

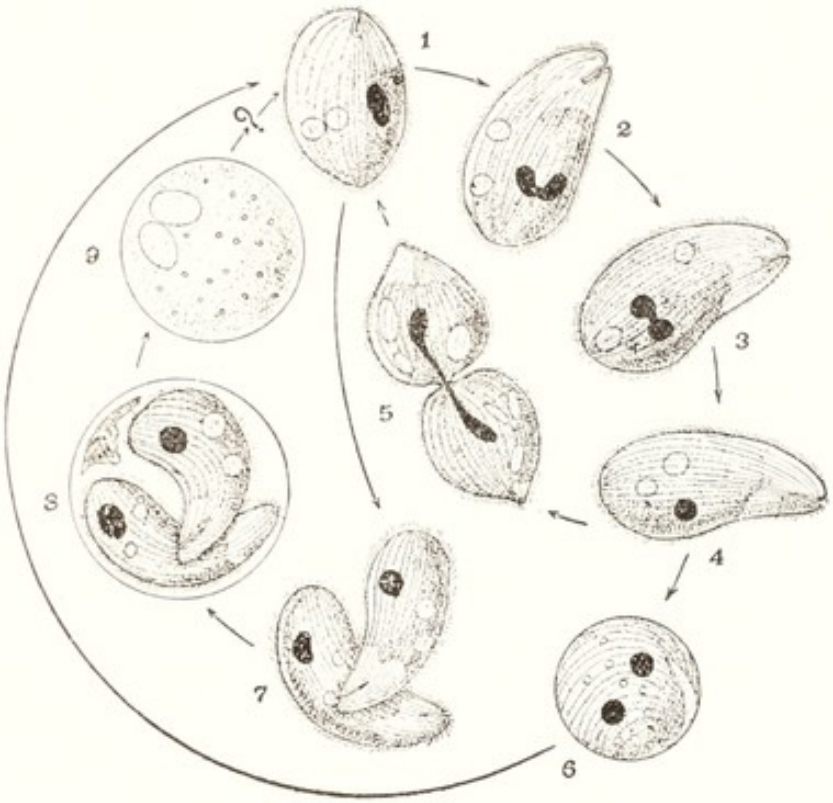


Fig. 266.—Reproduction of Balantidium coli: 1–5, Asexual reproduction by division; 6, encysted form of single individuals; 7, conjugation of two individuals; 8, reproductive cyst; 9, cyst with peculiar contents whose further development has not been followed (Brumpt).

Reproduction.—This commonly takes place by karyokinesis, followed by transverse division, and in cases of experimental infection so rapidly that the organisms have not time to grow to the full size before dividing again. The result is that many appear that are no more than 30 μ in length. In addition to multiplication by division, there is a sexual cycle of development with conjugation. This was first pointed out by Gourvitsch,* studied by Leger and Duboscq,† and further confirmed by Brumpt.‡ In the process of conjugation two individuals come together, become attached lengthwise, and fuse into a single large organism that forms a cyst several times as large as a balantidium, and with contents no longer recognizable as such. The contents of this cyst eventually divide into a number of spheres, but how these subsequently develop appears not to have been determined.

* "Russ. Archiv f. Path. klin. Med. u. Bact. St. Petersb.," 1896, quoted by Braun.

† "Archiv de Zoöl. Exper.," 1904, II, No. 4.

‡ "Compt.-rendu de la Soc. de Biol.," July 10, 1909.

Habitat.—The balantidium is unknown except as a parasite of the colon. It is very common in hogs and has been found in the orang-outang, in certain lower monkeys (Macacus cynomolgus), and in man.

Cultivation.—The organism quickly dies when transplanted to artificial media and has not yet been cultivated artificially.

Pathogenesis.—The presence of the organisms, in whatever kind of animal, gives rise to colitis, which is at first catarrhal, but soon becomes more or less ulcerative. Some doubt has been expressed as to the exact rôle of the balantidia in the causation of the inflammation, some believing them to be rather accidental factors than the true etiologic excitants. As the organisms descend into the ulcerated tissues and from the denuded surfaces invade the lymphatics, there seems to be little doubt of their pathogenic importance.

Animal Inoculation.—Experiments made by Casagrandi and Barbagallo,* Klimenko,† and others upon kittens and pups have failed to produce the disease

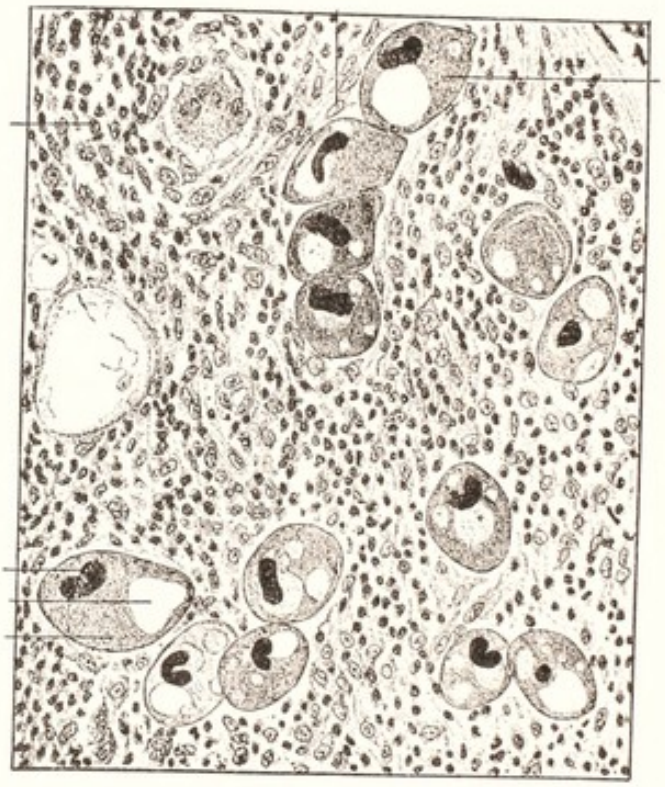


Fig. 267.—Balantidium coli deeply situated in the interglandular tissue of the intestinal mucosa (Brumpt).

even when the colon was already inflamed. Brumpt,‡ on the contrary, succeeded in reproducing it in monkeys and pigs by introducing the encysted organisms into the already inflamed intestine via the anus.

Lesions.—In the majority of fatal cases postmortem examination of the colon shows it to be in a state of catarrhal inflammation with numerous superficial ulcerations with considerable surrounding infiltration of the mucosa. Twenty-four hours from the time of the death of the patient the balantidia are all dead. Strong and Musgrave,‖ Solowiew,§ Klimenko,** and others have shown that in microscopic sections of the inflamed tissues the micro-organisms could be found deep down in the blood-vessels and lymphatic spaces about the ulcerated areas, sometimes penetrating as deeply as the serous coat of the bowel. Metastatic

* "Bal. coli," etc., Catania, 1896, quoted by Braun.
† "Beiträge zur. path. Anat. u. allg. Path.," 1903, XXXII, 281.
‡ "Précis de Parasitology," 1910, 152.
‖ "Bulletin of the Johns Hopkins Hospital," 1901, XII, 31.
§ "Centralbl. f. Bakt.," etc., 1 Abl., 1901, XXIX, 821, 849.
** Loc. cit.

abscess of the liver may be caused by balantidia, and has been reported by Manson,* and a case of abscess of the lung caused by the organism by Winogradow and Stokvis.†

Transmission.—The transmission of the disease can only come about through the encysted form of the parasites. Great numbers are passed in the feces of the infected animals, but except the encysted forms all die very quickly as the fecal matter dries. Unfortunately the further life-history of the encysted forms is unknown.

CRAIGIA HOMINIS (*Calkins*)‡

Craigia hominis is an ameboid and flagellated intestinal protozoan parasite of man, described in 1906 by Craig§ and recently carefully and elaborately studied by Barlow.‖ It is a minute organism and has an amebic stage during which it reproduces by simple division like a typical ameba for several generations or as long as conditions are favorable. It then encysts, and within the cysts numerous small bodies called "swarmers" develop and escape. Each of these has a long single protoplasmic flagellum and is actively motile. The swarmers

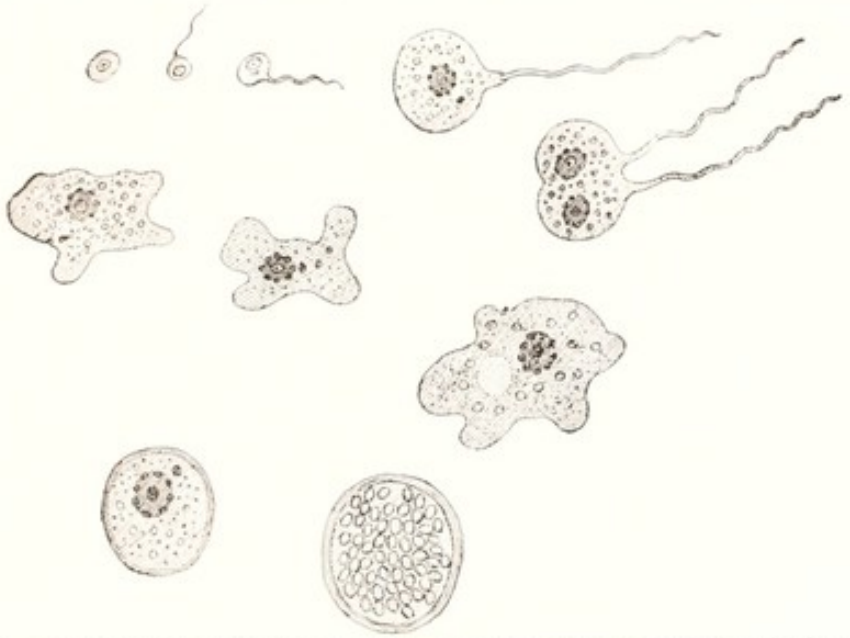


Fig. 268.—Craigia hominis (Barlow, in American Journal of Tropical Diseases).

multiply by longitudinal division for several generations after which the flagella disappear and the amebic stage begins again.

In 56 cases of infection by this parasite studied by Barlow, diarrhea was the most invariable symptom. Enterrhagia is less frequent and less severe in craigiosis than in amebiasis. Of the 56 cases, 11 developed abscess of the liver, one a pulmonary abscess, two appendicitis, one arthritis, two duodenal ulcer, while others had more vague complications and sequelæ. It seems, from Barlow's studies, that the parasite deserves considerable attention. The discovery of the parasite was made in the Philippine Islands, but Barlow's cases were in Honduras. One case has been reported in Texas, another in Tennessee.

Barlow recognizes two species, Craigia hominis and Craigia migrans.

HARMLESS FLAGELLATES OF THE HUMAN INTESTINES

In certain cases of diarrhea, flagellates—Trichomonas intestinalis, Cercomonas intestinalis, and Lamblia (Megastomum) intestinalis have been discovered. As, however, they seem to be frequent denizens of normal intestines, it is doubtful whether their presence is more than incidental.

* "Tropical Diseases," 1900, p. 394.
† "Niederl. Tijdschr. v. Geneeskde.," 1884, XX, No. 2, quoted by Braun.
‡ Trans, xvth Internat. Congress of Hygiene and Demography, 1912, II, 287.
§ Amer. Jour. Med. Sciences, 1906, CXXXII, 214.
‖ The American Journal of Tropical Diseases, etc., 1915, II, 680.

CHAPTER XXIX

TUBERCULOSIS

Bacillus Tuberculosis (Koch)

General Characteristics.—A non-motile, non-flagellate, non-sporogenous, non-liquefying, non-chromogenic, non-aërogenic, distinctly aërobic, acid-proof, purely parasitic, highly pathogenic organism, staining by special methods and by Gram's method. Commonly occurring in the form of slender, slightly curved rods with rounded ends, not infrequently showing branches, hence probably not a bacillus, but an organism belonging to the higher bacteria. It does not produce indol or acidulate or coagulate milk.

Tuberculosis is one of the most destructive and, unfortunately, one of the most common diseases. It is no respecter of persons, but affects alike the young and old, the rich and poor, the male and female, the enlightened and savage, the human being and the lower animals. It is the most common cause of death among human beings, and is common among animals, occurring with great frequency among cattle, less frequently among goats and hogs, and sometimes, though rarely, among sheep, horses, dogs, and cats.

Wild animals under natural conditions seem to escape the disease, but when caged and kept in zoölogic gardens, even the most resistant of them—lions, tigers, etc.—are said at times to succumb to it, while it is the most common cause of death among captive monkeys.

The disease is not limited to mammals, but occurs in a somewhat modified form in birds, and it is said even at times to affect reptiles, batrachians and fishes.

The disease has been recognized for centuries; and though, before the advent of the microscope, it was not always clearly differentiated from cancer, it has not only left unmistakable signs of its existence in the early literature of medicine, but has also imprinted itself upon the statute-books of some countries, as the kingdom of Naples, where its ravages were great and the means taken for its prevention radical.

Specific Organism.—Although the acute men of the early days of pathology clearly saw that the time must come when the parasitic nature of tuberculosis would be proved, and Klebs, Villemin, and Cohnheim were "within an ace" of its discovery, and Baumgarten* probably saw it in tissues cleared with lye, it remained for Robert Koch† to demonstrate and isolate the Bacillus tuberculosis, the specific cause of the disease, and to write so accurate a description

* "Virchow's Archives," Bd. LXXXII, p. 397.
† "Berliner klin. Wochenschrift," 1882, 15.

of the organism, and the lesions it produces, as to be almost without a parallel in medical literature.

Distribution.—So far as is known, the tubercle bacillus is a purely parasitic organism. It has never been found except in the bodies and discharges of animals affected with tuberculosis, and in dusts of which these are component parts. This purely parasitic nature interferes with the isolation of the organism, which cannot be grown upon the ordinary culture-media.

The widespread distribution of tuberculosis at one time suggested that tubercle bacilli were ubiquitous in the atmosphere, that we all inhaled them, and that it was only our *vital resistance* that prevented us all from becoming its victims. Cornet,* however,

Fig. 269.—Tubercle bacillus in sputum (Fränkel and Pfeiffer).

showed the bacilli to be present only in dusts with which pulverized sputum was mixed, and to be most common where the greatest uncleanliness prevailed.

Morphology.—The tubercle bacillus is a slender, rod-shaped organism with slightly rounded ends and a slight curve. It measures from 1.5 to 3.5 μ in length and from 0.2 to 0.5 μ in breadth. It commonly occurs in pairs, which may be associated end to end, but generally overlap somewhat and are not attached to each other. Organisms found in old pus and sputum show a peculiar beaded appearance caused by fragmentation of the protoplasm and the presence of metachromatic granules. The tubercle bacillus forms no endospores.

The fragments, originally thought by Koch to be spores, are irregular in shape, have ragged surfaces, and are without the high refraction peculiar to spores. Spores also resist heat strongly, but

* "Zeitschrift für Hygiene," 1888, v, pp. 191–321.

the fragmented bacilli are no more capable of resisting heat than others.

The bacilli not infrequently present projecting processes or branches, this observation having changed our views regarding the classification of the organism, which is probably erroneously placed among the bacilli, belonging more properly to the higher bacteria.

The organism is not motile, and does not possess flagella.

Staining.—The tubercle bacillus belongs to a group of organisms which, because of their peculiar behavior toward stains, are known as "säurefest" or *acid-proof*. It is difficult to stain after it has lived long enough to invest itself with a waxy capsule, requiring that the dye used shall contain a mordant (Koch). It is also tenacious of color once assumed, resisting the decolorizing power of strong mineral acids (Ehrlich).

Fig. 270.—Bacillus of tuberculosis, showing branched forms with involution (Migula).

Koch* first stained the bacillus with a solution consisting of 1 cc. of a concentrated solution of methylene blue mixed with 20 cc. of distilled water, well shaken, and then, before using, receiving an addition of 2 cc. of a 10 per cent. solution of caustic potash. Cover-glasses were allowed to remain in this for twenty-four hours and subsequently counterstained with vesuvin. Ehrlich subsequently modified Koch's method, showing that pure anilin was a better mordant than potassium hydrate, and that the use of a strong mineral acid would remove the color from everything but the tubercle bacillus. This modification of Koch's method, given us by Ehrlich, probably remains the best method of staining the bacillus.

* "Mittheilungen aus dem Kaiserlichen Gesundheitsamte," 1884, II.

Nearly all of the recent methods of staining are based upon the impenetrability of the bacillary substance by mineral acids which characterizes the *acid-fast* or *acid-proof* (säurefest) micro-organisms. But it is not improbable that we have been led into error by the assumption, upon inadequate grounds, that this is a constant and uniform quality of the tubercle bacillus and similar micro-organisms. The interesting observations of Much* have shown that many of the paradoxes of tuberculosis can be accounted for by the fact that during certain stages, or under certain conditions, the bacilli are not acid-proof at all. Thus, caseous masses from the lungs of cattle show complete absence of tubercle bacilli when examined by the usual method, yet cause typical tuberculosis when implanted into guinea-pigs, with typical bacilli, recoverable upon culture-media, in the lesions. This is certainly due to the inability of the bacilli in the bovine lesions mentioned to endure the acids, for when the same tissues are stained by Gram's method many organisms can be found. This shows that Gram's method is really a more useful method for demonstrating the bacillus than those in which acids are employed. Much has found two forms of the tubercle bacillus, one rod-like, the other granular, that are not acid-proof, and has succeeded in changing one into the other by experimental manipulation. He believes that the acid-proof condition has some bearing upon virulence, and speculates that the more acid-proof the organisms are, the less virulent they will be found.

In this connection the work of Maher,† who claims to be able, by appropriate methods of cultivation, to make many of the ordinary saprophytic bacteria (Bacillus coli, B. subtilis, etc.) thoroughly acid-proof, must be mentioned.

In all cases where the detection of tubercle bacilli in pus or secretions is a matter of clinical importance, it must be remembered that the quantity of material examined by the staining method is extremely small, so that a few bacilli in a relatively large quantity of matter can easily escape discovery.

As the purpose for which the staining is most frequently performed is the differential diagnosis of the disease through the demonstration of the bacilli in sputum, the method by which this can be accomplished will be first described.

Staining the Bacillus in Sputum.—When the sputum is mucopurulent and nummular, any portion of it may suffice for examination, but if the patient be in the early stages of tuberculosis, and the sputum is chiefly thin, seromucus, and flocculent, care must be exercised to see that such portion of it as is most likely to contain the micro-organisms be examined.

If one desires to make a very careful examination, it is well to

* "Berliner klin. Wochenschrift," April 6, 1908, p. 691.

† "International Conference on Tuberculosis," Philadelphia, 1907.

have the patient cleanse the mouth thoroughly upon waking in the morning, and after the first fit of coughing expectorate into a clean, wide-mouthed bottle.

The best result will be secured if the examination be made on the same day, for if the bacilli are few they occur most plentifully in small flakes of caseous matter, which are easily found at first, but which break up and become part of a granular sediment that forms in decomposed sputum.

The sputum should be poured into a watch-glass and held over a black surface. A number of grayish-yellow, irregular, translucent fragments somewhat smaller than the head of a pin can usually be found. These consist principally of caseous material from the tuberculous tissue, and are the most valuable part of the sputum for examination. One of the fragments is picked up with a pointed match-stick and spread over the surface of a perfectly clean cover-glass or slide. If no such fragment can be found, the purulent part is next best for examination.

The material spread upon the glass should not be too small in amount. Of course, a massive, thick layer will become opaque in staining, but should the layer spread be, as is often advised, "as thin as possible," there may be so few bacilli upon the glass that they are found with difficulty.

The film is allowed to dry thoroughly, is passed three times through the flame for fixation, and is then stained and examined.

Where examination by these means fails to reveal the presence of bacilli because of the small number in which they occur, recourse may be had to the use of caustic potash or, what is better, antiformin (*q.v.*) for digesting the sputum. A considerable quantity of sputum is collected, receives the addition of an equal volume of the antiformin, is permitted to stand until the formed elements and pus-corpuscles have been dissolved, is then shaken and poured into centrifuge tubes and whirled for fifteen to thirty minutes. The sediment at the bottom of the tubes is then spread upon the glasses and stained and will often reveal the bacilli which, having been freed from the viscid materials in the sputum, are thrown down in masses by the centrifuge.

The purpose of the staining being the discovery of the tubercle bacillus, success is only possible when the method employed enables that particular micro-organism to be recognized, as such, so soon as it is seen. This can be accomplished by taking advantage of the "acid-proof" quality of the micro-organism, which permits it to take up the penetrating stains employed, but does not permit it to let them go again in the bleaching agents, and assume the counter stain. It is owing to this peculiarity that the tubercle bacillus alone is colored blue by the Koch-Ehrlich method, and the tubercle bacillus alone red by the Ziehl method, and it is because no advantage is taken of the acid-proof peculiarity in using Gram's

method, that the latter, which colors all micro-organisms stained, the same blue-black color, and hence is not differential, is never used for diagnostic purposes.

Ehrlich's Method, or the Koch-Ehrlich Method.—Cover-glasses thus prepared are floated, smeared side down, or immersed, smeared side up, in a small dish of Ehrlich's anilin-water gentian violet solution:

Anilin	4
Saturated alcoholic solution of gentian violet	11
Water	100

and kept in an incubator or paraffin oven for about twenty-four hours at about the temperature of the body. Slides upon which smears have been made can be placed in Coplin jars containing the stain and stood away in the same manner. When removed from the stain, they are washed momentarily in water, and then alternately in 25 to 33 per cent. nitric acid and 60 per cent. alcohol, until the blue color of the gentian violet is entirely lost. A total immersion of thirty seconds is enough in most cases. After final thorough washing in 60 per cent. alcohol, the specimen is counterstained in a dilute aqueous solution of Bismarck brown or vesuvin, the excess of stain washed off in water, and the specimen dried and mounted in balsam. The tubercle bacilli are colored a fine dark blue, while the pus-corpuscles, epithelial cells, and other bacteria, having been decolorized by the acid, will appear brown.

This method, requiring twenty-four hours for its completion, is no longer used.

Ziehl's Method.—Among clinicians, Ziehl's method of staining with carbol-fuchsin has met with just favor. It is as follows: After having been spread, dried, and fixed, the cover-glass is held in the bite of an appropriate forceps (cover-glass forceps), or the slide spread at one end is held by the other end as a handle, and the stain (fuchsin, 1; alcohol, 10; 5 per cent. phenol in water, 100) dropped upon it from a pipet. As soon as the entire smear is covered with stain, it is held over the flame of a spirit lamp or Bunsen burner until the stain begins to volatilize a little. When vapor is observed the heating is sufficient, and the temperature can be maintained by intermittent heating.

If evaporation take place, a ring of encrusted stain at the edge prevents the prompt action of the acid. To prevent this, more stain should now and then be added. The staining is complete in from three to five minutes, after which the specimen is washed off with water, and then with a 3 per cent. solution of hydrochloric acid in 70 per cent. alcohol, 25 per cent. aqueous sulphuric, or 33 per cent. aqueous nitric acid solution dropped upon it for thirty seconds, or until the red color is extinguished. The acid is carefully washed off with water, the specimen dried and mounted in Canada balsam. Nothing will be colored except the tubercle bacilli, which appear red.

Gabbet's Method.—Gabbet modified the method by adding a little methylene blue to the acid solution, which he makes according to this formula:

Methylene blue	2
Sulphuric acid	25
Water	75

In Gabbet's method, after staining with carbol-fuchsin, the specimen is washed with water, acted upon by the methylene-blue solution for thirty seconds, washed again with water until only a very faint blue remains, dried, and finally mounted in Canada balsam. The tubercle bacilli are colored red; the pus-corpuscles, epithelial cells, and unimportant bacteria, blue.

Pappenheim,* having found bacilli stained red by Ziehls' method in the sputum of a case which subsequent postmortem examination showed to be one of gangrene of the lung without tuberculosis, condemns that method as not being sufficiently differential, and recommends the following as superior to methods in which the mineral acids are employed:

1. Spread the film as usual.
2. Stain with carbol-fuchsin, heating to the point of steaming for a few minutes.
3. Pour off the carbol-fuchsin and without washing—

* "Berl. klin. Wochenschrift," 1898, No. 37, p. 809.

4. Dip the spread from three to five times in the following solution, allowing it to run off slowly after each immersion:

Corallin	1 frm.
Absolute alcohol	100 cc.
Methylene-blue	ad sat.
Glycerin	20 cc.

5. Wash quickly in water.
6. Dry.
7. Mount.

The entire process takes about three minutes. The tubercle bacilli alone remain red.

Any possible relation that the number of bacilli in the expectoration of consumptives might bear to the progress of the disease was investigated by Nuttall.*

Fig. 271.—Bacillus tuberculosis in sputum, stained with carbolic fuchsin and aqueous methylene-blue. × 1000 (Ohlmacher).

But a glance down the columns of figures in the original article is sufficient to show that the number of bacilli is devoid of any practical interest, as is only to be expected when one considers the pathology of the disease and remembers that accident may cause wide variations in the quality, if not in the quantity of the sputum.

Staining the Bacillus in Urine.—The detection of tubercle bacilli in the urine is sometimes easy, sometimes difficult. The centrifuge should be used and the collected sediment spread upon the glass. If there be no pus or albumin in the urine, it is necessary to add a little white of egg to secure good fixation of the urinary sediment to the glass. The method of staining is the same as that for sputum

* "Bull. of the Johns Hopkins Hospital," May and June, 1891, II, 13.

but as the *smegma bacillus* (*q.v.*) is apt to be present in the urine, the precaution should be taken to use Pappenheim's solution for differentiation or to wash the stained film with absolute alcohol, that it may be decolorized and confusion avoided.

Staining the Bacillus in Feces.—It is difficult to find tubercle bacilli in the feces because of the relatively small number of bacilli and large bulk of feces.

Staining the Bacillus in Sections of Tissue.—*Ehrlich's Method for Sections.*—Ehrlich's method must be recommended as the most certain and best. The sections of tissue, embedded in paraffin, should be cemented to the slide and then freed from the embedding material.

They are then placed in the stain for from twelve to twenty-four hours and kept at a temperature of 37°C. Upon removal they are allowed to lie in water for about ten minutes. The washing in nitric acid (20 per cent.) which follows may have to be continued for as long as two minutes. Thorough washing in 60 per cent. alcohol follows, after which the sections can be counterstained, washed, dehydrated in 96 per cent. and absolute alcohol, cleared in zylol, and mounted in Canada balsam.

Unna's Method for Sections.—Unna's method is as follows: The sections are placed in a dish of twenty-four-hour-old, newly filtered Ehrlich's solution, and allowed to remain twelve to twenty-four hours at the room temperature or one to two hours in the incubator. From the stain they are placed in water, where they remain for about ten minutes to wash. They are then immersed in acid (20 per cent. nitric acid) for about two minutes, and become greenish black. From the acid they are placed in absolute alcohol and gently moved to and fro until the pale-blue color returns. They are then washed in three or four changes of clean water until they become almost colorless, and then removed to the slide by means of a section-lifter. The water is absorbed with filter-paper, and then the slide is heated over a Bunsen burner until the section becomes shining, when it receives a drop of xylol balsam and a cover-glass.

It is said that sections stained in this manner do not fade so quickly as those stained by Ehrlich's method.

Gram's Method.—The tubercle bacillus stains well by Gram's method and by Weigert's modification of it, but these methods are not adapted for differentiation. They should not be neglected when no tubercle bacilli are demonstrable by the other methods, as they are particularly well adapted to the demonstration of such of the organisms as may not be acid-proof.

Isolation.—Piatkowski* has suggested that the cultivation of the tubercle bacillus and other "acid-proof" organisms may be achieved by taking advantage of their ability to resist the action of formaldehyd. The material containing the acid-proof organism is mixed thoroughly with 10 cc. of water or bouillon, which receives an addition of 2 or 3 drops of 40 per cent. formaldehyd or "formalin." After standing from fifteen to thirty minutes transfers are made to appropriate culture-media, when the acid-proof organisms may develop, the others having been destroyed by the formaldehyd.

Still further improvement in the means by which the tubercle bacilli can be secured free from contamination with other organisms and from surrounding unnecessary and undesirable materials, has accrued from the use of *antiformin*. This commercial product, patented in 1909 by Axel Sjöo and Törnell, consists of Javelle water

* "Deutsche med. Wochenschrift," June 9, 1904, No. 23, p. 878.

to which sodium hydrate is added. To make it in the laboratory one first makes the Javelle water as follows:

K_2CO_3	58
$CaO(OCl)_2$	80
Water	q. s. 1000

and after dissolving the salts add an equal volume of 15 per cent. aqueous solution of caustic soda.

Uhlenhuth and Xylander* investigated its usefulness and recommend it highly for assisting in manipulating the tubercle bacillus. The sputum or tissue supposed to contain these organisms receives an addition of antiformin, by which the tissue elements, the pus cells, the mucous and other objectionable substances, and bacteria are quickly dissolved, leaving the tubercle bacilli uninjured. It is then centrifugalized, the fluid poured off and replaced by sterile water or salt solution, and the bacilli washed, after which they are again centrifugalized and caught at the bottom of the tube. This sediment, rich in bacilli, may be immediately transferred to appropriate culture-media, where the organisms frequently grow quite well, or can be used for the inoculation of guinea-pigs.

The most certain method of obtaining a culture of the tubercle bacillus from sputum, pus, etc., is to first inoculate a guinea-pig, allow artificial tuberculosis to develop, and then make cultures from one of the tuberculous lesions.

To make such an inoculation with material such as sputum, in which there are many associated micro-organisms that may destroy the guinea-pig from septicemia, Koch advised the following method, with which he never experienced an unfavorable result.

With a sharp-pointed pair of scissors a snip about ½ cm. long is made in the skin of the belly-wall. Into this the points of the scissors are thrust, between the skin and the muscles for at least 1 cm., and the scissors opened and closed so as to make a broad subcutaneous pocket. Into this pocket the needle of the hypodermic syringe containing the injection, or the slender glass point of a pipette containing it, is introduced, a drop of fluid expressed and gently rubbed about beneath the skin. When the inoculating instrument is withdrawn, the mouth of the pocket is left open. A slight suppuration usually occurs and carries out the organisms of wound infection, while the tubercle bacilli are detained and carried to the inguinal nodes, which usually enlarge during the first ten days. The guinea-pigs usually die about the twenty-first day after infection.

The guinea-pig is permitted to live until examination shows the inguinal glands are well enlarged, and toward the middle of the third week is chloroformed to death. The exterior of the body is then wet with 1:1000 solution of bichlorid of mercury and the animal stretched out, belly up, and tacked to a board or tied to an autopsy

* "Arbeiten a. d. Kaiserlichen Gesundheilsamte," 1909, XXXI, 158; "Centralbl. f. Bakt. u. Parasitenk.," Referata, 1910, XLV, 686.

tray. The skin is ripped up and turned back. The exposed abdominal muscles are now washed with bichlorid solution and a piece of gauze wrung out of the solution temporarily laid on to absorb the excess. With fresh sterile forceps and scissors the abdominal wall is next laid open and fastened back. With fresh sterile instruments the spleen, which should be large and full of tubercles, is drawn forward and, one after another, bits the size of a pea cut or torn off and immediately dropped upon the surface of appropriate culture-media in appropriate tubes. The fragments of tissue from the spleen of the tuberculous guinea-pig are not crushed or comminuted, but are simply laid upon the undisturbed surface of the culture medium and then incubated for several weeks. If no growth is apparent after this period, the bit of tissue is stirred about a little and the tube returned to the incubator, where growth almost immediately begins from bacilli scattered over the surface as the bit of tissue was moved. As the appropriate medium, blood-serum was recommended by Koch; glycerin agar-agar, by Roux and Nocard; glycerinized potato, by Nocard; coagulated dogs' blood-serum, by Smith, or coagulated egg, by Dorset, may be mentioned. The most certain results seem to follow the employment of the dogs' serum and egg media.

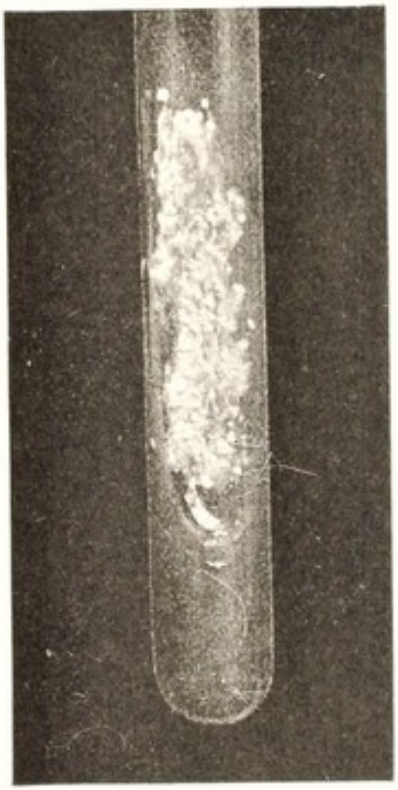


Fig. 272.—Bacillus tuberculosis on "glycerin agar-agar."

Cultivation.—*Blood-serum.*—Koch first achieved artificial cultivation of the tubercle bacillus upon blood-serum, upon which the bacilli are first apparent to the naked eye in about two weeks, in the form of small, dry, whitish flakes, not unlike fragments of chalk. These slowly increase in size at the edges, and gradually form small scale-like masses, which under the microscope are found to consist of tangled masses of bacilli, many of which are in a condition of involution. The medium is so ill adapted to the requirements of the tubercle bacillus and gives such uncertain results that it is no longer used.

Glycerin Agar-agar.—In 1887 Nocard and Roux* gave a great impetus to investigations upon tuberculosis by the discovery that the addition of from 4 to 8 per cent. of glycerin to bouillon and agar-agar made them suitable for the development of the bacillus, and that a much more luxuriant development could be obtained upon

* "Ann. de l'Inst. Pasteur," 1887, No. 1.

such media than upon blood-serum. The growth upon "glycerin agar-agar" resembles that upon blood-serum. A critical study of the relationship of massive development and glycerin was made by Kimla, Poupé, and Vesley,* who found that the most luxuriant growth occurred when the culture-media contained from 5 to 7 per cent. of glycerin.

Fig. 273.—Glass-capped culture-tube used by Theobald Smith for the isolation of the tubercle bacillus.

Dogs' Blood-serum.—A very successful method of isolating the tubercle bacillus has been published by Smith.†

A dog is bled from the femoral artery, the blood being caught in a sterile flask, where it is allowed to coagulate. The serum is removed with a sterile pipette, placed in sterile tubes, and coagulated at 75° to 76°C. Reichel has found it advantageous to add to each 100 cc. of the dogs' serum 25 cc. of a mixture of glycerin 1 part, and distilled water 4 parts. The whole is then carefully shaken without making a froth, and dispensed in tubes, 10 cc. to a tube. The coagulation and sterilization he effects by once heating to 90°C. for three to five hours. At the Henry Phipps Institute in Philadelphia this medium was employed with thorough satisfaction for the isolation of many different tubercle bacilli. Smith prefers to use a test-tube with a ground cap, having a small tubular aperture at the end, instead of the ordinary test-tube with the cotton-plug. The purpose of the ground-glass cap is to prevent the contents of the tube from drying during the necessarily long period of incubation; that of the tubulature, to permit the air in the tubes to enter and exit during the contraction and expansion resulting from the heating incidental to sterilization.

To the same end the ventilators of the incubator are closed, and a large evaporating dish filled with water is stood inside, so that the atmosphere may be constantly saturated with moisture.

Egg Media.—Dorset‡ recommends an egg medium, which has the advantage of being cheap and easily prepared. Eggs are always at hand, and can be made into an appropriate medium in an hour or two. He also claims that the chemic composition of the eggs makes them particularly adapted for the purpose.

The medium is prepared by carefully opening the egg and dropping its contents into a wide-mouth sterile receptacle.

The yolk is broken with a sterile wire and thoroughly mixed with the white by gentle shaking. The mixture is then poured into sterile tubes, about 10 cc. in each, inclined in a blood-serum sterilizer, and sterilized and coagulated at 70°C. on two days, the temperature being maintained for four or five hours each day. The medium appears yellowish and is usually dry, so that before using it is well to add a few drops of water.

Potato.—Pawlowski§ was able to isolate the bacillus upon potato. Sander found that it could be readily grown upon various vegetable

* "Revue de la Tuberculose," 1898, VI, p. 25.

† "Transactions of the Association of American Physicians," 1898, vol. XIII p. 417.

‡ "American Medicine," 1902, vol. III, p. 555.

§ "Ann. de l'Inst. Pasteur," 1888, t. VI.

compounds, especially upon acid potato mixed with glycerin Rosenau* has shown that it can grow upon almost any cooked and glycerinized vegetable tissue.

Animal Tissues.—Frugoni† recommends that the tubercle bacillus be isolated and cultivated upon animal tissue and organs used as culture-media. He especially recommends rabbit's lung and dog's lung for the purpose. The tissues are first cooked in a steam sterilizer, then cut into prisms, placed in a Roux tube, an addition of 6 to 8 per cent. glycerin-water added, so as to bathe the lower part of the tissue and keep it moist, and the whole then sterilized in the autoclave.

The organisms are planted upon the tissue, the top of the tube closed with a rubber cap, and the culture placed in the thermostat. The tubercle bacilli grow quickly and luxuriantly.

Bouillon.—Upon bouillon to which 6 per cent. of glycerin has been added the bacillus grows well, provided the transplanted material be in a condition to float. The organism being purely aërobic grows only at the surface, where a much wrinkled, creamy white, brittle pellicle forms.

Non-albuminous Media.—Instead of requiring the most concentrated albuminous media, as was once supposed, Proskauer and Beck‡ have shown that the organism can be made to grow in non-albuminous media containing asparagin, and that it can even be induced to grow upon a mixture of commercial ammonium carbonate, 0.35 per cent.; primary potassium phosphate, 0.15 per cent.; magnesium sulphate, 0.25 per cent.; glycerin, 1.5 per cent. Tuberculin was produced in this mixture.

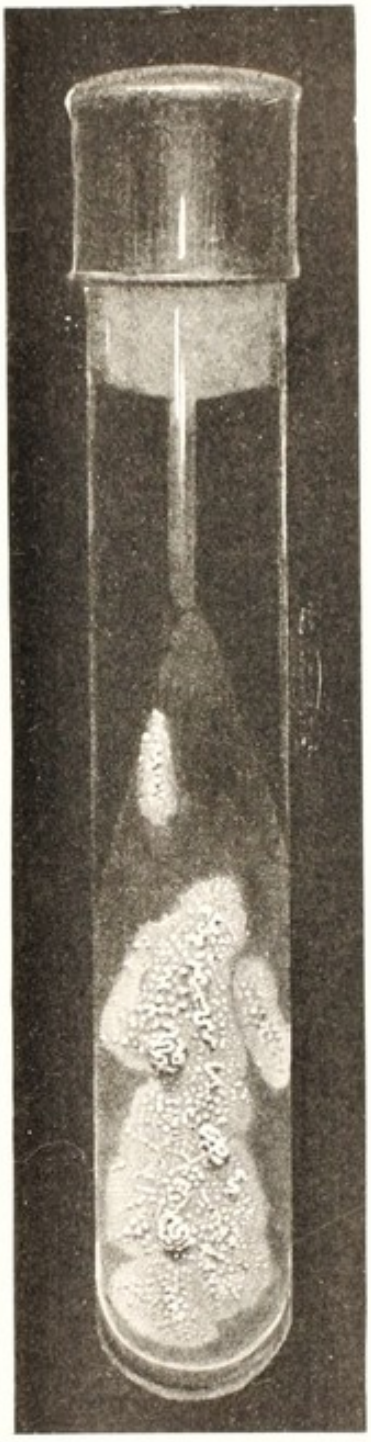


Fig. 274.—Bacillus tuberculosis; glycerin agar-agar culture, several months old (Curtis).

Gelatin.—The tubercle bacillus can be grown in gelatin to which glycerin has been added, but as its development takes place only at 37° to 38°C., a temperature at which gelatin is always liquid, its use for the purpose has no advantages.

* "Jour. Amer. Med. Assoc.," 1902.
† "Centralbl. f. Bakt. u. Parasitenk.," I. Abl. Orig., 1910, LIII, 553.
‡ "Zeitschrift für Hygiene," Aug. 10, 1894, XVIII, No. 1.

Appearance of the Cultures.—Irrespective of the media upon which they are grown, cultures of the tubercle bacillus present certain characteristics which serve to separate them from the majority of other organisms, though insufficient to enable one to identify them with certainty.

The bacterial masses make their appearance very slowly. As a rule very little growth can be observed at the end of a week, and sometimes a month must elapse before the growth is distinct.

They usually develop more rapidly upon fluid than upon solid media. The organism is purely aërobic, and the surface growth formed upon liquids closely resembles that upon solids.

Fig. 275.—Bacillus tuberculosis; adhesion cover-glass preparation from a fourteen-day-old blood-serum culture. × 100 (Fränkel and Pfeiffer.)

It is dry and lusterless, coarsely granular, wrinkled, slightly yellowish, and does not penetrate into the substance of the culture-medium. It sometimes extends over the surface of the medium and spreads out upon the contiguous surface of moist glass.

When the medium is moist, the bacterial mass may in rare instances be shining in spots. When the medium is dry, it is apt to be scaly and almost chalky in appearance.

The organism grows well when once successfully isolated, and, when once accustomed to artificial media, not only lives long (six to nine months) without transplantation, but may be transplanted indefinitely.

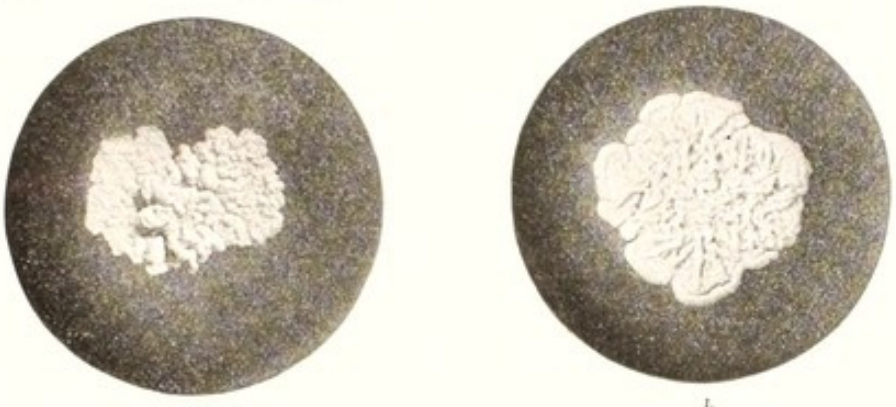
Reaction.—The tubercle bacillus will grow upon otherwise appropriate media whether the reaction be feebly acid or feebly alkaline.

Relation to Oxygen.—The tubercle bacillus requires oxygen, and grows only upon the surface of the culture-media.

Temperature Sensitivity.—The bacillus is sensitive to temperature variations, not growing below 29°C. or above 42°C. Rosenau* found that an exposure to 60°C. for twenty minutes destroys the infectiousness of the tubercle bacillus for guinea-pigs.

Effect of Light.—It does not develop well in the light, and when its virulence is to be maintained should always be kept in the dark. Sunlight kills it in from a few minutes to several hours, according to the thickness of the mass of bacilli exposed to its influence.

Pathogenesis.—Channels of Infection.—The channels by which the tubercle bacillus enters the body are numerous. A few cases are on record where the micro-organisms have passed through the *placenta*, a tuberculous mother infecting her unborn child. It is not impossible that the passage of bacilli through the placenta in this



a *b*

Fig. 276.—Bacillus tuberculosis: *a*, Source, human; *b*, source, bovine. Mature colonies on glycerin-agar. Actual size (Swithinbank and Newman.)

manner causes the rapid development of tuberculosis after birth, the disease having remained latent during fetal life, for Birch-Hirschfeld has shown that fragments of a fetus, itself showing no tuberculous lesions, but coming from a tuberculous woman, caused fatal tuberculosis in guinea-pigs into which they were inoculated.

The most frequent channel of infection is the *respiratory tract*, into which the finely pulverized pulmonary discharges of consumptives and the dusts of infected rooms and streets enter. Flügge, Laschtschenko, Heyman-Sticher, and Beninde† found that the greatest danger of infection was from the atomized secretions, discharged during cough, from the tuberculous respiratory apparatus. Nearly every one discharges finely pulverized secretions during coughing and sneezing, as can easily be determined by holding a mirror before the face at the time. Even though discharged by consumptives, these atoms of moisture are not infectious except when there are open lesions in the lungs, etc. Experiment showed that they usually do not pass farther than 0.5 meter from the patient, though occasionally they may be driven 1.5 meters. A knowledge

* "Hygienic Laboratory," Bulletin No. 24, Jan., 1908.

† "Zeitschrift für Hygiene," etc., Bd. XXX, pp. 107, 125, 139, 163, 193.

of these facts teaches us that visits to consumptives should not be prolonged; that no one should remain continually in their presence, nor habitually sit within 2 meters of them; also that patients should always hold a handkerchief before the face while coughing. The rooms occupied by consumptives should also be frequently washed with a disinfecting solution.

Probably all of us at some time in our lives inhale living virulent tubercle bacilli, yet not all suffer from tuberculosis. Personal variations in predisposition seem to account in part for this, as it has been shown that without the formation of tubercles virulent bacilli may sometimes be present for considerable lengths of time in the bronchial lymphatic glands—the dumping-ground of the pulmonary phagocytes.

In order that infection shall occur, it does not seem necessary that the least abrasion or laceration shall exist in the mucous lining of the respiratory tract.

Infection also commonly takes place through the *gastro-intestinal tract* from infected food. Present evidence points to danger from tubercle bacilli in the milk of cattle affected with tuberculosis.

The ingested bacilli may enter the tonsils and be carried to the cervical lymph-glands, but seem more commonly to reach the intestine, from which they enter the lymphatics, sometimes to produce lesions immediately beneath the mucous membrane, sometimes to invade the more distant mesenteric lymphatic glands, but more frequently to enter the thoracic duct and then through the venous system find their way to the lungs. Passing this barrier they may distribute through the arterial systemic circulation. The entrance of tubercle bacilli into the systemic circulation with subsequent deposition in the brain, bones, joints, etc., explains primary lesions of these tissues.

Koch* believed that human beings are infected only by bacilli from other human beings, and his paper upon this subject has stimulated extensive experimentation on the problem. Most authorities believe both human and bovine bacilli to be equally infectious for man. Behring† believes that nearly all children become infected by ingesting tubercle bacilli in milk, though a certain predisposition is necessary before the disease can develop. Baumgarten believes that all children harbor bacilli taken in the food, but that the disease does not develop until a certain susceptibility occurs.

Infection also occasionally takes place through the *sexual apparatus*. In sexual intercourse tubercle bacilli from tuberculous testicles can enter the female organs, with resulting bacillary implantation. Sexual infections are usually from the male to the female, primary

* "International Congress on Tuberculosis," London, 1901, and Washington, 1908.

† "Deutsche med. Wochenschrift," 1903, No. 39.

tuberculosis of the testicle being more common than of the uterus or ovaries.

Wounds are also occasional avenues of entrance for tubercle bacilli. Anatomic tubercles are not uncommon upon the hands of anatomists and pathologists, most of these growths being tuberculous in nature. Such dermal lesions usually contain few bacilli.

Lesions.—The macroscopic lesions of tuberculosis are too familiar to require a description of any considerable length. They consist of nodules, or collections of nodules, called tubercles, irregularly scattered through the tissues, which are more or less disorganized by their presence and retrogressive changes.

When tubercle bacilli are introduced beneath the skin of a guinea-pig, the animal shows no sign of disease for a week or two, then begins to lose appetite, and gradually diminishes in flesh and weight. Examination usually shows a nodule at the point of inoculation and enlargement of the neighboring lymphatic glands. The atrophy increases, the animal shows a febrile reaction, and dies at the end of a period of time varying from three to six weeks. Post-mortem examination usually shows a cluster of tubercles at the point of inoculation, tuberculous enlargement of lymphatic glands both near and remote from the primary lesion, and a widespread tuberculous invasion of the lungs, liver, spleen, peritoneum, and other organs. Tubercle bacilli are demonstrable in immense numbers in all the invaded tissues. The disease in the guinea-pig is usually more widespread than in other animals because of its greater susceptibility, and the death of the animal occurs more rapidly for the same reason. Intraperitoneal injection of tubercle bacilli in guinea-pigs causes a still more rapid disease, accompanied by widespread lesions of the abdominal organs. The animals die in from three to four weeks. In rabbits the disease runs a longer course with similar lesions. In cattle and sheep the infection is commonly first seen in the alimentary apparatus and associated organs, and may be limited to them though primary pulmonary disease also occurs. In man the disease is chiefly pulmonary, though gastro-intestinal and general miliary tuberculosis are common. The development of the lesions in whatever tissue or animal always depends upon the distribution of the bacilli by the lymph or the blood.

The experiments of Koch, Prudden, and Hodenpyl,* and others have shown that when dead tubercle bacilli are injected into the subcutaneous tissues of rabbits, small local abscesses develop in the course of a couple of weeks, showing that the tubercle bacilli possess chemotactic properties. These chemotactic properties seem to depend upon some other irritant than that by which the chief lesions of tuberculosis are caused. When the dead tubercle bacilli, instead of being injected *en masse* into the areolar tissue, are introduced by intravenous injection and disseminate themselves singly

* "New York Med. Jour.," June 6–20, 1891.

or in small groups, the result is quite different, and the lesions closely resemble those caused by the living organisms.

Baumgarten, whose researches were made upon the iris, found that the first irritation caused by the bacillus is followed by multiplication of the fixed connective-tissue cells of the part. The cells increase in number by karyokinesis, and form a minute cellular collection or primitive tubercle.

The group of epithelioid cells and lymphocytes constituting the primitive tubercle scarcely reaches visible proportions before

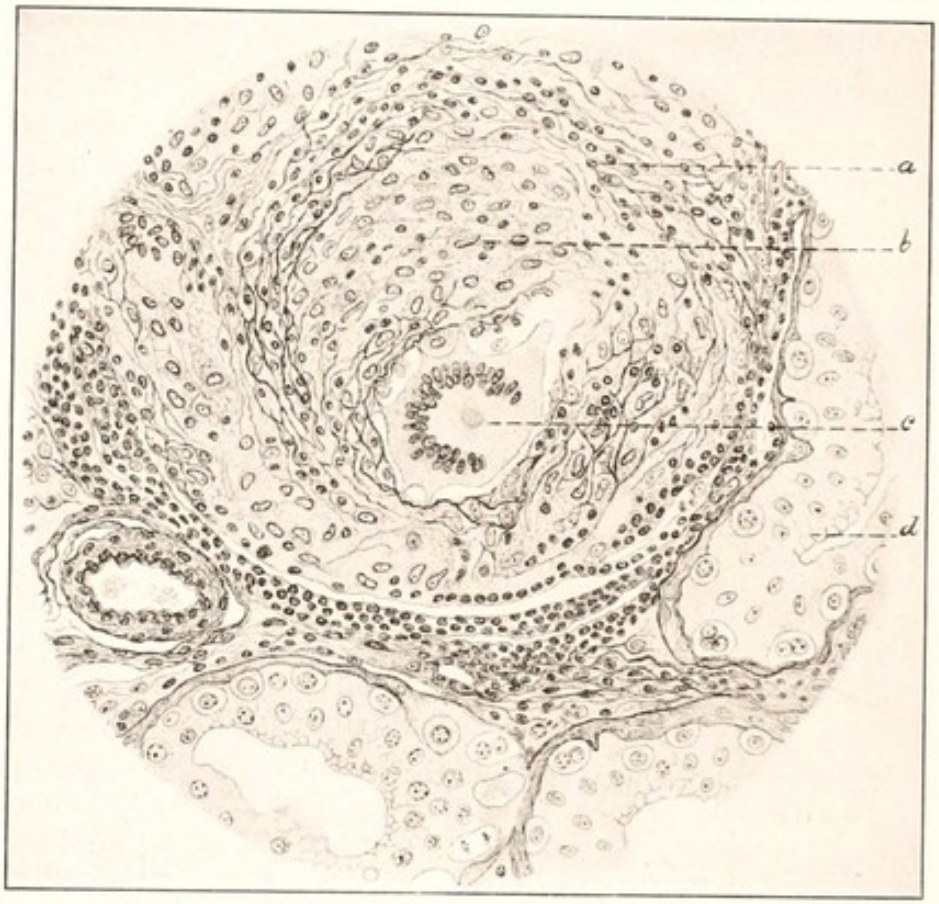


Fig. 277.—Miliary tubercle of the testicle: *a*, Zone of epithelioid cells and leukocytes; *b*, area of coagulation-necrosis; *c*, giant cell with its processes; peripherally arranged nuclei and necrotic center; *d*, seminiferous tubule (Cameron, in "International Text-book of Surgery").

central coagulation-necrosis begins. The cytoplasm of the cells takes on a hyaline character; the chromatin of the nuclei becomes dissolved in the nuclear juice and gives a pale but homogeneous appearance to the stained nuclei. As the tubercle grows, large protoplasmic masses—*giant cells*—which contain many nuclei are formed. They sometimes occur near the center, more frequently near the periphery of the lesion.

Giant cells are not always formed in tubercles, as the necrotic changes are sometimes too rapid and widespread.

Tubercles are constantly avascular—*i.e.*, in them no new capillary blood-vessels form—and the coagulation-necrosis soon destroys pre-

existing capillaries. Avascularity may be a factor in the necrosis of the larger tuberculous masses, though probably playing no important part in the degeneration of the small tubercles, which is purely toxic.

Fig. 278.—Tuberculosis of the lung: the upper lobe shows advanced cheesy consolidation with cavity-formation, bronchiectasis, and fibroid changes; the lower lobe retains its spongy texture, but is occupied by numerous miliary tubercles.

The minute primitive tubercle was first called a *miliary tubercle*, and small aggregations of these, "crude tubercles," by Laennec. As almost all tissues contain a supporting connective-tissue

framework whose fibers are more resistant to necrosis than the cells, after the cells of a tubercle have been destroyed, fibers may still be visible among the granules, and give the tubercle a reticulated appearance.

As a rule, tubercles progressively increase in size by the invasion of fresh tissue. The tubercle bacilli are usually observed in greatest number at the edges, among the healthy cells, where the nutrition is good. From this position they are swept along by currents of lymph or occasionally are picked up by leukocytes and transported through the lymph-spaces, until the phagocyte falls a prey to its prisoner, dies, and sows the seed of a new tubercle. It is by such continuous invasion of new tissue, the formation of necrotic areas in the lungs, and evacuation through the air-tubes that cavities are formed. In pulmonary tuberculosis the process of destruction is greatly accelerated by inspired saprophytic bacteria that live in the necrotic tissue. The patient also suffers from secondary infections, especially by the streptococcus and pneumococcus.

If the vital condition of the individual becomes so changed that the invasive activity of the bacilli is checked or their death brought about, the tubercle begins to cicatrize, and becomes surrounded by a zone of newly formed contracting fibrillar tissue, by which it is circumscribed and isolated. This constitutes recovery from tuberculosis. Sometimes the process of repair is accomplished without the destruction of the bacilli, which are incarcerated and retained. Such a condition is called *latent tuberculosis*, and may at a future time be the starting-point of a new infection.

Virulence.—The virulence of tubercle bacilli varies considerably according to the sources from which they are obtained. Bacilli from different cases are of different degrees of virulence, and bacilli from different animals vary still more. Lartigau,* in an instructive paper upon "Variation in Virulence of the Bacillus Tuberculosis in Man," found much variation among bacilli secured from the lesions of human tuberculosis. The virulence was tested by employing cultures only for inoculation, and taking of each bacillary mass exactly 5 mg. by weight, suspending it in 5 cc. of an indifferent fluid until the density was uniform and the microscope showed no clumps, and injecting into rabbits and guinea-pigs, pairs of animals being injected in the same manner, with the same material, at the same time, and being subsequently kept under similar conditions. The occurrence of tuberculosis in the inoculated animals was decided by both macroscopic and microscopic tests.

Lartigau found that human tubercle bacilli from different sources induced varying degrees of tuberculosis in animals; that the injection of the same culture in different amounts produces different results; that the extent and rapidity of development usually cor-

* "Journal of Medical Research," July, 1901, vol. VI, No. 1; N. S., vol. 1, No. 1, p. 156.

respond to the virulence of the culture; that doses of 1 mg. of a very virulent culture may induce general tuberculosis in rabbits in a very short time; that 20 mg. of a bacillus of low virulence may fail to produce any lesion in rabbits or guinea-pigs; that no morphologic relationship could be observed between the bacilli and their virulence; that highly virulent bacilli grew scantily on culture-media and were short lived; that bacilli of widely different virulence may be present in any one of the various human tuberculous lesions; that in scrofulous lymphadenitis the bacilli are usually of low virulence; the bacilli in pulmonary tuberculosis with ulceration are of feeble virulence, those of miliary tuberculosis of very great virulence; that the so-called "healed tubercles" of the lung may contain virulent or attenuated bacilli; that individuals suffering from infection with a bacillus of a low grade of virulence may be again infected with extremely virulent tubercle bacilli; that chronic tuberculosis of the bones may contain bacilli of high or low virulence, and that variations in virulence among human tubercle bacilli may possibly sometimes depend, like many other qualities among tubercle bacilli, on peculiarities inherited through serial transmissions in other than human hosts.

Chemistry of the Tubercle Bacillus.—Klebs* found that the tubercle bacillus contains two fatty bodies, one of which, having a reddish color and melting at 42°C., can be extracted with ether. It forms about 20 per cent. by weight of the bacillary substance. The other is insoluble in ether, but soluble in benzole, with which it can be extracted. It melts at about 50°C. and constitutes 1.14 per cent. of the bacillary substance. After removing these fatty bodies the bacilli fail to resist the decolorant action of acids when stained by ordinary methods, so that it seems probable that their acid-resisting power depends upon them.

De Schweinitz† showed that it was possible to extract from the tubercle bacillus an acid closely resembling, if not identical with, teraconic acid. It melts at 161° to 164°C. and is soluble in ether, water, and alcohol. He thinks the necrotic changes caused by the organism depend upon it.

Ruppel‡ believes that three different fatty substances are present in the tubercle bacillus, making up from 8 to 26 per cent. by weight. The first can be extracted with cold alcohol, the second with hot alcohol, the third with ether. In addition to the fatty substance Ruppel also found what he believes to be a protamin, and calls *tuberculosamin*. It seems to be combined with nucleinic acid, and, indeed, from it he isolated an acid for which he proposes the name *tuberculinic acid*.

* "Centralbl. f. Bakt.," 1896, XX, p. 488.

† "Trans. Assoc. of Amer. Phys.," 1897; "Centralbl. f. Bakt.," etc., Sept. 15, 1897, Bd. XXII, p. 200.

‡ "Zeitschrift für physiol. Chemie," 1899, XXVI.

Behring* found that this acid contained a histon-like body whose removal left chemically pure tuberculinic acid. One gram of this acid is capable of killing a 600-gram guinea-pig when administered beneath the skin. One gram is fatal to 90,000 grams of guinea-pig when introduced into the brain. If injected into tuberculous guinea-pigs it is much more fatal, 1 gram destroying 60,000 when injected subcutaneously and 40,000,000 when injected into the brain.

Levene† also found free and combined nucleinic acid varying in phosphorus content from 6.58 to 13.19 per cent. He also found a glycogen-like substance that reduced Fehling's solution when heated with a mineral acid.

Toxic Products.—In 1890 Koch‡ announced some observations upon the toxic products of the tubercle bacillus and their relation to the diagnosis and treatment of tuberculosis, which at once aroused an enormous though transitory enthusiasm. The observations are, however, of great importance. Koch found that when guinea-pigs are inoculated with tubercle bacilli, the wound ordinarily heals readily, and soon all signs of local disturbance other than enlargement of the lymphatic glands of the neighborhood disappear. In about two weeks, however, there appears, at the point of inoculation a slight induration, which develops into a hard nodule, ulcerates, and remains until the death of the animal. If, however, in a short time the animals be reinoculated, the course of the local lesion is changed, and, instead of healing, the wound and the tissue surrounding it assume a dark color, become obviously necrotic, and ultimately slough away, leaving an ulcer which rapidly and permanently heals without enlargement of the lymph-glands.

This observation was made by injecting cultures of the living bacillus, but Koch observed that the same changes also occur when the secondary inoculation is made with killed cultures of the bacilli.

It was also observed that if the material used for the secondary injections was not too concentrated and the injections not too often repeated (only every six to forty-eight hours), the animals treated improved in condition, and continued to live, sometimes (Pfuhl) as long as nineteen weeks.

Tuberculin.—Koch also discovered that a 50 per cent. glycerin extract of cultures of the tubercle bacillus—*tuberculin*—produced the same effect as the dead cultures originally used, and announced the discovery of this substance to the scientific world, in the hope that the prolongation of life observed to follow its use in the guinea-pig might also be true of man.

The active substance of the "tuberculin" seems to be an albuminous derivative (bacterioprotein) insoluble in absolute alcohol.

* "Berliner klin. Wochenschrift," XXXVI.
† "Jour. of Med. Research," 1, 1901.
‡ "Deutsche med. Wochenschrift," 1891, No. 343.

It is a protein substance and gives all the characteristic reactions. It differs from the toxalbumins in being able to resist exposure to 120°C. for hours without change. Tuberculin is almost harmless for healthy animals, but extremely poisonous for tuberculous animals, its injection into them being followed either by a violent febrile reaction or by death, according to the extent of the disease and size of the dose administered.

Preparation of Tuberculin.—The preparation of tuberculin is simple. Flasks made broad at the bottom so as to expose a considerable surface of the contained liquid are filled to a depth of about 2 cm. with bouillon containing 4 to 6 per cent. of glycerin, and preferably made with veal instead of beef infusion. They are inoculated with pure cultures of the tubercle bacillus, care being taken that the bacillary mass floats upon the surface, and are kept in an incubator at 37°C. In the course of some days a slight surface growth becomes apparent about the edges of the floating bacillary mass, which in the course of time develops into a firm, coarsely granular, wrinkled pellicle. At the end of some weeks development ceases and the pellicle sinks, a new growth sometimes occurring from floating scraps of the original.

Some bacteriologists prefer to use small Erlenmeyer flasks for the purpose, but large flasks, which contain from 500 cc. to 1 liter, are more convenient. The contents of a number of flasks of well-grown cultures are poured into a large porcelain evaporating dish, concentrated over a water-bath to one-tenth their volume, and filtered through a Pasteur-Chamberland filter. This is *crude* tuberculin.

When doses of a fraction of a cubic centimeter of crude tuberculin are injected into tuberculous animals, an inflammatory and febrile reaction occurs. Superficial tuberculous lesions (lupus) sometimes ulcerate and slough away. The febrile reaction is sufficiently characteristic to be of diagnostic value, though tuberculin can only be used with perfect safety as a diagnostic agent upon the lower animals.

From the "crude" or original tuberculin Koch prepared a purified or "*refined*" tuberculin by adding one and one-half volumes of absolute alcohol, stirring thoroughly, and standing aside for twenty-four hours. At the end of this time a flocculent deposit will be seen at the bottom of the vessel. The supernatant fluid is carefully decanted and an equal volume of 60 per cent. alcohol poured into the vessel for the purpose of washing the precipitate, which is again permitted to settle, the fluid decanted, and the washing thus repeated several times, after which it is finally washed in absolute alcohol and dried in a vacuum exsiccator. The white powder thus prepared is fatal to tuberculous guinea-pigs in doses of 2 to 10 mg. It is soluble in water and glycerin and gives the protein reactions. The tuberculin as Koch prepared it is now known as "concentrated" or "Koch's tuberculin," to differentiate it from the "diluted tuberculin" sometimes sold in the shops, which is the same thing so diluted with 1 per cent. aqueous carbolic acid solution that 1 cc. equals a dose. The dose of the concentrated tuberculin is 0.4 to 0.5 cc.; that of the diluted tuberculin, 1 cc.

Tuberculin does not exert the slightest influence upon the tubercle bacillus, but acts upon the tuberculous tissue, augmenting the poisonous influence upon the cells surrounding the bacilli, destroying their vitality, and removing the conditions favorable to bacillary growth, which for a time is checked. This action is accompanied by marked hyperemia of the perituberculous tissue, with transudation of serum, softening of the tuberculous mass, and absorption into the blood, a marked febrile reaction resulting from the intoxication.

Virchow, who well understood the action of the tuberculin, soon showed that as a diagnostic and therapeutic agent in man its use was attended by grave dangers. The destroyed tissue was absorbed,

but with it some of the bacilli, which, being transported to new tissue areas, could occasion a widespread metastatic invasion of the disease. Old tuberculous lesions which had been encapsulated were sometimes softened and broken down, and became renewed sources of infection to the individual, so that, a short time after an enthusiastic reception, tuberculin was placed upon its proper footing as an agent valuable for diagnosis in veterinary practice, but dangerous in human medicine, except in cases of lupus and other external forms of tuber-

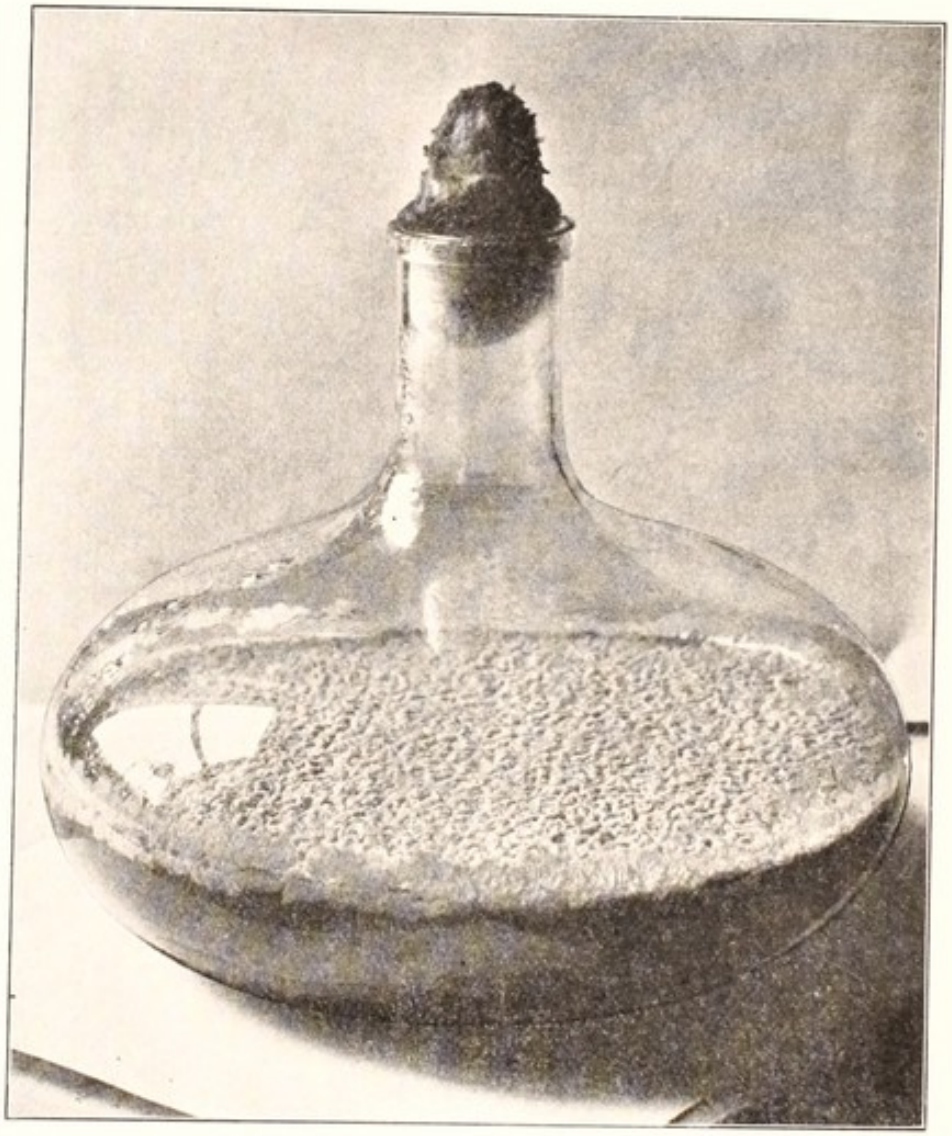


Fig. 279.—Massive culture of the tubercle bacillus upon the surface of glycerin-bouillon, used in the manufacture of tuberculin.

culosis where the destroyed tissue could be readily discharged from the surface of the body.

Many, however, continued to use it, and Petruschky* has reported, with careful details, 22 cases of tuberculosis which he claims have been cured by it.

* "Berliner klin. Wochenschrift," 1899, Dec. 18–25.

Recently there has been a return to the use of tuberculin for the diagnosis of tuberculosis, it being claimed that by the use of minute doses, several times repeated, the characteristic reaction and a positive diagnosis can be obtained without danger.

von Pirquet* found that if a drop or two of Koch's (old) tuberculin is placed upon the skin of a tuberculous child, and a small scarification made through the drop with a sterile lancet, a small papule develops at the point of inoculation that is not unlike a vaccine papule. It is at first bright, later on dark red, and remains for a week. Out of 500 tests made, the results were positive in nearly every case of clinical tuberculosis. The most characteristic reactions were obtained in tuberculosis of the bones and glands, and the method is recommended chiefly for the diagnosis of tuberculosis during the first year of life. This method of testing is called the "*dermotuberculin reaction.*"

A modification of this method by Ligniéres† is called by him the "*cutituberculin reaction.*" Ligniéres soaps and shaves the skin with a safety razor, avoiding scarification, but removing the superficial epidermal cells by scraping, and then applies 6 large drops of undiluted tuberculin, rubbing the reagent in with a pledget of cotton. The reaction obtained is purely local and without fever.

Moro ‡ has improved upon von Pirquet's method by using the tuberculin in the form of a 50 per cent. ointment made by mixing equal parts of "old tuberculin" and lanolin, which is rubbed into the skin without previous scarification.

Hiss§ says that "it is more simple and equally efficient to massage into the skin a drop of undiluted 'old tuberculin.'"

Calmette‖ suggested the '*ophthalmo-tuberculin reaction,*" which consists of instilling 1 drop of a solution of prepared tuberculin into the eye of the suspect. If no tuberculosis exists, no reaction follows, but if the patient be infected with tuberculosis, the eye becomes reddened in a few hours and soon shows all of the appearances of a more or less pronounced acute mucopurulent inflammation of the conjunctiva. This attains its maximum in six or seven hours, and entirely recovers in three days. It usually causes the patient very little discomfort, but a number of patients have been unfortunate enough to suffer from supervening corneal ulceration and other destructive lesions of the eye, so that the test is now rarely used, having been superseded by the dermal methods.

The method of preparing the solution employed by Calmette is to precipitate the tuberculin with alcohol, dry the precipitate and dissolve it in 100 parts of distilled water. One or two

* "Ibid., May 20, 1907.

† "Centralbl. f. Bakt. u. Parasitenk.," orig., XLVI, Hft. 4, March 10, 1908, p. 373.

‡ "Münch. med. Wochenschrift," 1906, p. 216.

§ "Text-book of Bacteriology," 1901, p. 489.

‖ "La Presse Médicale," June 19, 1907.

drops may be used. Ordinary tuberculin must be avoided, as the glycerin it contains causes too much irritation and masks the reaction.

Priority in regard to the theoretic aspects of these reactions seems to belong to Wolff-Eisner,* who was the first to point out that the injection of all albuminous substances resulted in hypersensitivity instead of immunity unless certain precautions were observed. Upon this ground Levy† gives him credit as the founder of the method. The reaction is undoubtedly an allergic phenomenon.

Klebs‡ made strong claims for his own modifications of tuberculin, known as *antiphthisin* and *tuberculocidin*, but according to the experimental studies of Trudeau and Baldwin, antiphthisin is only much diluted tuberculin, and exerts no demonstrable influence upon the tubercle bacillus *in vitro*, does not cure tuberculosis in guinea-pigs, and probably inhibits the growth of the tubercle bacillus upon culture-media to which it has been added only by its acid reaction.

The "bouillon-filtrate" (bouillon filtré), of Denys§ is a porcelain filtrate of bouillon culture of the tubercle bacillus and corresponds to Koch's original tuberculin before concentration, except in that it has not been subjected to heat.

Tuberculin-R.—TR or tuberculin-R appears to be an important addition to the immunology of tuberculosis, made by Koch.‖

TR signifies "tuberkel bacillen resten" or bacillary fragments.

Pursuing the idea of fragmenting the bacilli, or treating them chemically to increase their solubility, Koch found that a 10 per cent. sodium hydrate solution yielded an alkaline extract of the bacillus, which, when injected into animals, produced effects similar to those following the administration of tuberculin, except that they were more brief in duration and more constant in result; but the disadvantage of abscess formation following the injections remained. The fluid, when filtered, possessed the properties of tuberculin.

Mechanical fragmentation of bacilli had been employed by Klebs in his studies of *antiphthisin* and *tuberculocidin*, and Koch now used it with advantage. He pulverized living, virulent, but perfectly dry bacilli in an agate mortar, in order to liberate the toxic substance from its protecting envelope of fatty acid, triturating only very small quantities of the bacteria at a time.

Having thus reduced the bacilli to fragments, he removed them from the mortar, placed them in distilled water, washed them, and collected them by centrifugation, as a muddy residuum at the bottom of an opalescent, clear fluid. For convenience he named the clear fluid TO; the sediment, TR. TO was found to contain tuberculin. In order to separate the essential poison of the bacteria as perfectly as possible from the irritating tuberculin, the TR fragments were again dried perfectly, triturated once more, re-collected in fresh distilled water, and recentrifugated. After the second centrifugation microscopic examination showed that the bacillary fragments had not yet been resolved into a uniform

* "Centralbl. f. Bakt. u. Parasitenk.," 1904, Orig., XXXVII.
† "Verein für innere Medizin zu Berlin," Dec. 16, 1907.
‡ "Die Behandlung der Tuberculose mit Tuberculocidin," 1892.
§ "Acad. royale de med. de Belgique," Feb. 22, 1902; abst. "Centralbl. f. Bakt. u. Parasitenk.," Ref., 1902, XXXI, p. 563.
‖ "Deutsche med. Wochenschrift," 1897, No. 14.

mass, for when TO was subjected to staining with carbol-fuchsin and methylene blue it was found to exhibit a blue reaction, while in TR a cloudy violet reaction was obtained.

The addition of 50 per cent. of glycerin had no effect upon TO, but caused a cloudy white deposit to be thrown down from TR. This last reaction showed that TR contained fragments of the bacilli insoluble in glycerin.

In making the TR preparation Koch advises the use of a fresh, highly virulent culture not too old. It must be perfectly dried in a vacuum exsiccator, and the trituration, in order to be thorough, should not be done upon more than 100 mg. of the bacilli at a time. A satisfactory separation of the TR from TO is said to occur only when the perfectly clear TO takes up at least 50 per cent. of the solid substance, as otherwise the quantity of TO in the final preparation is so great as to produce undesirable reactions.

The fluid is best preserved by the addition of 20 per cent. of glycerin, which does not injure the TR and prevents its decomposition.

The finished fluid contains 10 mg. of solid constituents to the cubic centimeter, and before administration should be diluted with physiologic salt solution (not solutions of carbolic acid). When administering the remedy to man the injections are made with a hypodermic syringe into the tissues of the back. The beginning dose is $\frac{1}{500}$ mg., rapidly increased to 20 mg., the injections being made daily.

Experiment showed that TR had decided immunizing powers. Injected into tuberculous animals in too large a dose it produces a reaction, but its immunizing effects were entirely independent of the reaction. Koch's aim in using this preparation in the therapeutic treatment of tuberculosis was to produce immunity against the tubercle bacillus without reactions by gradual but rapid increase of the dose. In so large a number of cases did Koch produce immunity to tuberculosis by the administration of TR, that he believes it proved beyond a doubt that his observations are correct.

By proper administration of the TR he was able to render guinea-pigs so completely immune that they were able to withstand inoculation with virulent bacilli. The point of inoculation presents no change when the remedy is administered; and the neighboring lymph-glands are generally normal, or when slightly swollen contain no bacilli.

In speaking of his experiments upon guinea-pigs, Koch says:

"I have, in general, got the impression in these experiments that full immunization sets in two or three weeks after the use of large doses. A cure in tuberculous guinea-pigs, animals in which the disease runs, as is well known, a very rapid course, may, therefore, take place only when the treatment is introduced early—as early as one or two weeks after the infection with tuberculosis.

"This rule avails also for tuberculous human beings, whose treatment must not be begun too late. . . . A patient who has but a few months to live cannot expect any value from the use of the remedy, and it will be of little use to treat patients who suffer chiefly from secondary infection, especially with the streptococcus, and in whom the septic process has put the tuberculosis entirely in the background."

One very serious objection, first urged against commercially prepared TR by Trudeau and Baldwin,* is that it is possible for it to contain unpulverized, and hence still living, virulent tubercle bacilli.

* "Medical News," Aug. 28, 1897.

Thelling* could not observe any good effect to result from the use of Koch's TR-tuberculin, and, like Trudeau, found living, virulent bacilli in the preparation secured from Höchst. Many others have since discovered the same danger. In the preparation of the remedy it will be remembered that no antiseptic or germicide was added to the solutions by which the effects of accidental failure to crush every bacillus could be overcome, Koch having specially deprecated such additions as producing destructive changes in the TR. Until this possibility of danger can be removed, and our confidence that attempts to cure patients may not result in their infection be restored, it becomes a question whether TR can find a place in human medicine, or must remain an interesting laboratory product.

Baumgarten and Walz† find that the administration of tuberculin-R to guinea-pigs is without curative effect. They insist that the results obtained are like those of the old tuberculin; that "small doses are of no advantage, while the larger the doses one employs, the greater are the disadvantages that result from their employment."

During his experiments upon the agglutination of tubercle bacilli, to be descrived below, Koch‡ found that animals injected with an emulsion of tubercle bacilli showed great increase in the agglutinative power of the blood. This led him to suggest that a new preparation, "*bacillary emulsion*" Bazillenemulsion, be investigated for its immunizing and curative properties. Many are still using it and some claim good results.

It is almost impossible to make an accurate estimation of the usefulness or uselessness of therapeutic preparations of tubercle bacilli at the present time, not only because of their diversity of composition and the enthusiasm with which many have been exploited, but also because of our inability to compare the results attained with any definite standard. The advantages or disadvantages of any preparation, therefore, depend upon the personal opinions of those employing them rather than upon any demonstration regarding them—a very unscientific state of knowledge.

The suggestion of A. E. Wright that the administration of all such products should be controlled by an examination of the opsonic power of the blood, the remedy being withheld if this was high and applied if low, the utmost care being taken not to prolong the "negative phase," seemed to be an excellent one, affording the beginning of a scientific method of studying the disease, but unfortunately it seems not to have been successful in practice, and the tedium and expense of the examinations makes them impracticable.

* "Centralbl. f. Bakt.," etc., July 5, 1902, XXXII, No. 1, p. 28.
† "Centralbl. f. Bakt. und Parasitenk.," April 12, 1898, XXIII, No. 14, p. 593.
‡ "Deutsche med. Wochenschrift," 1901, No. 48, p. 829.

Agglutination.—Arloing* and Courmont† found it possible to prepare homogenized cultures of the tubercle bacillus, and saw them agglutinated by the serum of immunized animals and by the serum of tuberculous patients. The subject was investigated by Koch,‡ who carefully reviewed the details of technic and investigated the method, which, he concluded, was valueless for the diagnosis of human infection, though a good guide to the extent of immunization achieved by the therapeutic administration of tuberculin-R. Thelling§ has also shown the reaction to be too irregular to be of practical diagnostic importance.

The technic of the agglutination test as given by Koch‖ is as follows:

> Any culture of the tubercle bacillus can be made useful by the following treatment: Collect the bacillary masses upon a filter-paper and press between layers of filter-paper to remove the fluid. Weigh out, say, 0.2 gm. of the solid mass and rub it in an agate mortar, adding, drop by drop, a $\frac{1}{50}$ normal sodium hydroxid solution until the proportion of 1 part of the culture to 100 parts of the solution is reached.
>
> It is necessary that the rubbing be thorough in order that the firm connection between the bacilli shall be broken up and the organisms distributed throughout the fluid. The operation usually lasts fifteen minutes. The fluid is then placed in a hand centrifuge and whirled for six minutes, then pipetted off, and rendered feebly alkaline by adding diluted hydrochloric acid solution. The fluid thus obtained is too concentrated to be used in this form, so must be diluted with 0.5 per cent. carbolic acid in 0.85 per cent. sodium chlorid solution. This solution should be repeatedly filtered before receiving the bacillary suspension. The quantity of bacillary suspension to be added should make the final product a 3000 dilution of the original. It should look like water by transmitted light, but slightly opalescent by reflected light.
>
> The serum to be tested is added in proportions of 1:10, 1:25, 1:50, 1:75, 1:100, 1:200, 1:300, etc., and is to stand for twenty-four hours. By inclining the tube and looking through a thin stratum of the fluid the agglutinations can be at once detected.

Antitubercle Serums.—Tizzoni and Centanni,** Bernheim,†† Paquin,‡‡ Viquerat§§ and others have experimented in various ways, hoping that the principles of serum therapy might apply to tuberculosis. Nothing has, however, been achieved. Maragliano's‖‖ antitubercle serum has been used in a very large number of cases in human medicine, but the glittering results reported by its author have not been confirmed. Behring*** comments upon it by saying that "Maragliano's tubercle antitoxin contains no antitoxin."

* "Congress de méd. int. Montpellier," 1898; "Compt. rendu Acad. de Sciences de Paris," 1898, T. CXXVI, pp. 1319–1321.

† "Compt. rend. Soc. de Biol. de Paris," 1898, No. 28, V; "Congr. pour l'etude de la Tuberculose," Paris, 1898.

‡ "Deutsche med. Wochenschrift," 1901, No. 48, p. 829.

§ Loc. cit.

‖ "Deutsche med. Wochenschrift," 1901, No. 48, p. 829.

** "Centralbl. f. Bakt.," etc., 1892, Bd. XI, p. 82.

†† "Ibid., 1894, Bd. XV, p. 654.

‡‡ "New York Med. Record," 1895.

§§ "Zur Gewinnung von Antituberkulin, Centralbl. f. Bakt.," etc., Nov. 5, 1896, XX, Nos. 18, 19, p. 674.

‖‖ "Berliner klin. Wochenschrift," 1895, No. 32.

*** "Fortschritte der Med.," 1897.

Babes and Proca,* Maffucci and di Vestea,† McFarland,‡ De Schweinitz,§ Fisch,‖ and Patterson** have all endeavored to obtain serums of therapeutic value by immunizing animals against living or dead tubercle bacilli or their products, but without success.

From these discordant observations, the more favorable of which are probably the hasty records of inadequate or incomplete experiments, the conclusion that little is to be hoped from immune serums in the treatment of tuberculosis is inevitable.

Prophylaxis.—It is the duty of every physician to use every means in his power to prevent the spread of tuberculous infection in the households under his care. To this end patients should cease to kiss the members of their families and friends; should have individual knives, forks, spoons, cups, napkins, etc., carefully kept apart—secretly if the patient be sensitive upon the subject—from those of the family, and scalded after each meal; should have their napkins and handkerchiefs, as well as whatever clothing or bed-clothing is soiled by them, kept apart from the common wash, and boiled; and should carefully collect the expectoration in a suitable receptacle, that is sterilized or disinfected, without being permitted to dry, as it has been shown that the tubercle bacillus can remain alive in dried sputum as long as nine months. The physician should also give directions for disinfecting the bed-room occupied by a consumptive before it becomes the chamber of a healthy person, though this should be as much the function of the municipality as the disinfection practised after scarlatina, diphtheria, and smallpox.

Boards of health are now becoming more and more interested in tuberculosis, and, though exceedingly slow and conservative in their movements, are disseminating literature with the hope of achieving by volition that which might otherwise be regarded as cruel compulsion.

So long as tuberculosis exists among men or cattle, it shows that existing hygienic precautions are insufficient. While condemning any unreasonable isolation of patients, we should favor the registration of tuberculous cases as a means of collecting accurate data concerning their origin; insist upon the careful domestic sterilization and disinfection of all articles used by the patients; recommend public disinfection of the houses they cease to occupy; and approve of special hospitals for as many (especially of the poorer classes, among whom hygienic measures are almost always opposed) as can be persuaded to occupy them.

* "La Med. Moderne," 1896, p. 37.
† "Centralbl. f. Bakt.," etc., 1896, Bd. XIX, p. 208.
‡ "Jour. Amer. Med. Assoc.," Aug. 21, 1897.
§ "Centralbl. f. Bakt. und Parasitenk.," Sept. 15, 1897, Bd. XXII, Nos. 8 and 9.
‖ "Jour. Amer. Med. Assoc.," Oct. 30, 1897.
** "Amer. Medico-Surg. Bull.," Jan. 25, 1898.

BOVINE TUBERCULOSIS

Bacillus Tuberculosis Bovis

The tuberculous diseases of the lower animals and especially cattle have lesions closely resembling those of human tuberculosis, and containing bacilli similar both in morphology and in staining reaction to those found in human tuberculosis. The conclusion that they are identical seems inevitable, but in his monograph upon tuberculosis Koch called attention to certain morphologic and cultural differences that exist between bacilli obtained from human and from animal tuberculosis. Unfortunately, very little attention was paid to the subject until Theobald Smith* carefully compared a series of bacilli obtained from human sputum with another series obtained from cattle, horses, hogs, cats, dogs, and other animals.

His observations form the foundation of the following description of the bovine tubercle bacillus:

Morphology.—The size of the bovine bacillus is quite constant, the individuals being quite short (1–2 μ). They are straight, not very regular in outline, and sometimes of a spindle, sometimes a barrel, and sometimes an oval shape. The human bacilli, on the other hand, are prone to take an elongate form under artificial cultivation.

Staining.—The bovine bacillus usually stains homogeneously; the human bacillus commonly shows the so-called "beaded appearance."

Vegetation.—The human bacillus grows upon dogs' serum much more luxuriantly and rapidly than the bovine bacillus.

Metabolic Products.—Smith† observed that cultures of the two organisms in glycerin bouillon differ in the induced reaction of the media. The cultures of the bovine bacillus tend toward neutrality, those of the human bacillus toward acidity.

Pathogenesis.—(a) **Guinea-pigs.**—The bovine bacilli are more virulent than those of human tuberculosis, intraperitoneal inoculation of the former producing death in adult animals in from seven to sixteen days; of the latter, in from ten to thirty-eight days. Subcutaneous inoculation of the bovine bacillus causes death in less than fifty days; of the human bacillus, in from fifty to one hundred days.

(b) **Rabbits.**—Rabbits inoculated into the ear vein with the bovine bacillus die in from seventeen to twenty-one days. Those receiving human bacilli sometimes live several months.

(c) **Cattle.**—Cows and heifers receiving intrapleural and intra-abdominal injections of the human bacilli usually gain in weight and show no symptoms. When examined *postmortem*, circumscribed

* "Trans. Assoc. Amer. Phys.," 1896, XI, p. 75, and 1898, XIII, p. 417; "Jour. of Experimental Medicine," 1898, III, 495.

† "Trans. Assoc. Amer. Phys.," 1903, vol. XVIII, p. 109.

chronic lesions were found. Those inoculated with the bovine bacillus lose weight, suffer from constitutional symptoms, and show extensive lesions at the necropsy. Two-thirds of the cattle inoculated experimentally with the bovine bacillus die.

Lesions.—In general the lesions produced by the bovine bacillus are rapid, extensive, and necrotic. Many bacilli are present. Those produced by the human bacillus are more apt to be productive, chronic, and contain relatively few bacilli. The bacilli of human tuberculosis produce lesions with many giant cells; those of bovine tuberculosis, lesions with rapid coagulation necrosis. The lesions resulting from the intravenous injection of human bacilli into rabbits resembled those observed by Prudden and Hodenpyl* after the intravenous injection of boiled, washed tubercle bacilli.

From these data it is evident that the bovine bacillus is by far the more virulent and dangerous organism.

At the International Congress on Tuberculosis, held in London, 1901, Koch expressed the opinion that bovine tuberculosis was not communicable to man. The matter is of the utmost importance to the medical profession and of far-reaching influence upon many important sanitary measures that bear directly upon the public health.

Koch's opinion, being opposed to all that had been believed before, received almost universal disapproval. The papers by Arloing,† Ravenel,‡ and Salmon§ contain evidence showing that under certain conditions bovine tuberculosis can be communicated to man.

Ravenel‖ has reported 3 cases of accidental cutaneous inoculation of bovine tuberculosis in man. All were veterinary surgeons who became infected through wounds accidentally inflicted during the performance of necropsies upon tuberculous cattle. The tubercle bacilli were demonstrated in some of the excised cutaneous nodules.

Theobald Smith,** in studying 3 cases of supposed food infection, found what corresponded biologically with the human rather than the bovine bacillus.

In a later paper Koch†† analyzed the cases usually selected from the literature to prove the communicability of bovine tuberculosis to man, and showed that not one of the cases really proves what is claimed for it, and that the subject requires further careful investigation and demonstration before it will be possible to express any positive opinion in regard to it.

During the years that have elapsed since 1901 and the present time sentiment has been almost uniformly against Koch, and an enormous literature has accumulated that in reality means very

* "New York Med. Jour.," June 6–20, 1891.

† "Lyon Méd.," Dec. 1, 1901.

‡ "Univ. of Pa. Bulletin," XIV, p. 238, 1901; "Lancet," Aug. 17 and 19, 1901; "Medicine," July and Aug., 1902, vol. VIII.

§ "Bull. No. 33, Bureau of Animal Industry," U. S. Dept. of Agriculture, 1901.

‖ "Phila. Med. Jour.," July 21, 1900.

** "Amer. Jour. Med. Sciences," Aug., 1904, vol. CXXVIII, No. 389, p. 216.

†† Eleventh International Congress for Tuberculosis, Berlin, 1902.

little. The most important is that of the Royal Commission on Tuberculosis of Great Britain.* The general tenor of this report is contrary to Koch's views, and many believed it settled the question. At the International Congress on Tuberculosis in Washington, 1908, Koch reviewed the subject and stated his continued belief in the principle he had enunciated seven years before. Practically the same contentions were raised against him by much the same group of men, but the controversy was more bitter than before. Koch,† however, leaves us in no doubt upon the subject, summarizing his views in these words:

1. The tubercle bacilli of bovine tuberculosis are different from those of human tuberculosis.
2. Human beings may be infected by bovine tubercle bacilli, but serious diseases from this cause occur very rarely.
3. Preventive measures against tuberculosis should, therefore, be directed primarily against the propagation of human tubercle bacilli.

He weighed the contrary evidence that had been collected during seven years, showed how errors had crept into the investigations, and laid down certain rules to be observed before the experiments could be accepted. At the close of the congress the matter remained unsettled, Koch appearing to have the best of the argument.

The opponents of Koch based their opinions upon the supposed modifiability of the tubercle bacillus in different environments. When it lived in man, it was by virtue of the contact with the human juices and their chemical peculiarities compelled to assume the human form; in the cow, by virtue of the different chemical conditions, the bovine form, etc. Proofs of this were, however, wanting, and have not yet been published. On the other hand, Moriya‡ seems to have shown that such changes are either purely hypothetic or come about with great difficulty. He succeeded in keeping human and also bovine types of tubercle bacilli alive in tortoises for twelve months, at the end of which period each was found unmodified and possessed of its original characteristics.

It was Koch's hope to be able to finally settle the whole matter, and to this end he asked the coöperation of many laboratories throughout different parts of the world. Unfortunately he died before the results could be compiled, but much work had been done and much support thereby given his views. A most fertile research, the results of which form a valuable addition to our knowledge of the problem has been published by Park and Krumwiede,§ who, basing their opinions upon the following tabulation of 1224 cases, come to the following conclusions:

* See the "British Medical Journal," 1907 and 1908.
† "Jour. Amer. Med. Assoc.," Oct. 10, 1908, II, No. 15, p. 1256.
‡ "Centralbl. f. Bakt. u. Parasitenk.," 1909, I, Abt. Orig., LI, 460.
§ "Journal of Medical Research," 1910, XXIII, No. 2, p. 205; 1911, XXV, No. 2, p. 313.

COMBINED TABULATION CASES REPORTED AND OWN SERIES OF CASES

Diagnosis	Adults 16 years and over		Children 5 to 16 years		Children under 5 years	
	Human	Bovine	Human	Bovine	Human	Bovine
Pulmonary tuberculosis	644	(1?)	11	—	23	1
Tuberculous adenitis, axillary or inguinal	2	—	4	—	2	—
Tuberculous adenitis, cervical	27	1	36	21	15	21
Abdominal tuberculosis	14	4	8	7	9	13
Generalized tuberculosis, alimentary origin	6	1	2	3	13	12
Generalized tuberculosis	29	—	4	1	43	5
Generalized tuberculosis including meninges, alimentary origin	—	—	1	—	3	8
Generalized tuberculosis including meninges	5	—	7	—	52	1
Tubercular meningitis	1	—	3	—	27	4
Tuberculosis of bones and joints	27	1	38	3	26	—
Genito-urinary tuberculosis	17	1	2	—	—	—
Tuberculosis of skin	3	—	1	—	1	—
Miscellaneous cases:						
Tuberculosis of tonsils	—	—	—	1	—	—
Tuberculosis of mouth and cervical nodes	—	1	—	—	—	—
Tuberculous sinus or abscess	2	—	—	—	—	—
Sepsis, latent bacilli	—	—	—	—	1	—
Totals	777	10	117	36	215	65

Mixed or double infections, 4 cases.

Total cases, 1224.

Conclusions.—Bovine tuberculosis is practically a negligible factor in adults. It very rarely causes pulmonary tuberculosis or phthisis which causes the vast majority of deaths from tuberculosis in man, and is the type of disease responsible for the spread of the virus from man to man.

In children, however, the bovine type of tubercle bacillus causes a marked percentage of the cases of cervical adenitis, leading to operation, temporary disablement, discomfort, and disfigurement. It causes a large percentage of the rarer types of alimentary tuberculosis requiring operative interference or causing the death of the child directly or as a contributing cause in other diseases.

In young children it becomes a menace to life and causes from 6⅓ to 10 per cent. of the total fatalities from this disease.

Prophylaxis.—The prevention of tuberculosis in cattle is a matter of vast sanitary importance. Not only have we to consider the danger of infection from milk containing tubercle bacilli, but also the inferior quality and diminished usefulness of milk and flesh

coming from animals that are diseased. The extermination of bovine tuberculosis, therefore, becomes imperative, and the utmost efforts should be made to bring it about. Several separate measures must be considered:

1. Improvement in the methods of diagnosis, by which the recognition of the disease is made possible before its ravages are great. This is rapidly coming about with increasing information regarding the use and abuse of tuberculin, etc.

2. Means by which infected animals shall be destroyed. Here the municipal and state governments furnish inadequate funds to make possible the destruction of diseased cattle without adequate compensation—an injustice to the unfortunate owner.

3. Means of preventing the infection of healthy animals. In many places this is being achieved with brilliant success by separation of the herd, healthy and newly born animals constituting one part, suspicious animals the other. By these means valuable breeding animals can be kept for a time, at least, in usefulness. A second and less successful means of preventing infection is by means of prophylactic vaccination of the healthy animals with dead cultures, modified living cultures, or by bacteriotoxins made by comminuting them.

Experiments of this kind have been conducted by McFadyen,* on a large scale by von Behring,† by Pearson and Gilliland,‡ Calmette and Guérin,§ and by Theobald Smith,‖ all of whom think distinct resisting power against infection by the tubercle bacillus can thus be brought about.

Tuberculin Test for Tuberculosis of Cattle.—The febrile reaction caused by the injection of tuberculin into tuberculous animals is an important adjunct to our means of diagnosticating the disease. For the recognition of tuberculosis in cattle it is easily carried out.

To make a satisfactory diagnostic test the temperature of the animal should be taken every few hours for a day or two before the tuberculin is administered, in order that the normal diurnal and nocturnal variations of temperature shall be known. The tuberculin is then administered by hypodermic injection into the shoulder or flank, and the temperature subsequently taken every two hours for the next twenty-four hours. *A reaction of two degrees beyond that normal to the individual animal is positive of tuberculosis.* After one reaction of this kind the animal will not again react to an equal dose of tuberculin for a number of weeks.

* "Jour. Comp. Path. and Therap.," June, 1901.

† "Beiträge zur experimentellen Therapie," 1902, Hft. 5.

‡ "Jour. of Comp. Med. Vet. Archiv," Nov., 1902, "Univ. of Penna. Med. Bull.," April, 1905.

§ "Ann. de l'Inst. Pasteur.," Oct., 1905, May, 1906, and July, 1907; and "International Congress on Tuberculosis," Washington, 1908.

‖ "Journal of Medical Research," June, 1908, XVIII, No. 3, p. 451.

FOWL TUBERCULOSIS

Bacillus Tuberculosis Avium

The occasional spontaneous occurrence of tuberculosis in chickens, parrots, ducks, and other birds, observed as early as 1868 by Roloff* and Paulicki,† was originally attributed to Bacillus tuberculosis hominis, but the work of Rivolta,‡ Mafucci,§ Cadio, Gilbert and Roger,‖ and others has shown that, while similar to it in many respects, the organism found in the avian diseases has distinct peculiarities which make it a different variety, if not a separate species. Cadio, Gilbert, and Roger succeeded in infecting fowls by feeding them upon food containing tubercle bacilli, and keeping them in cages in which dust containing tubercle bacilli was placed. The

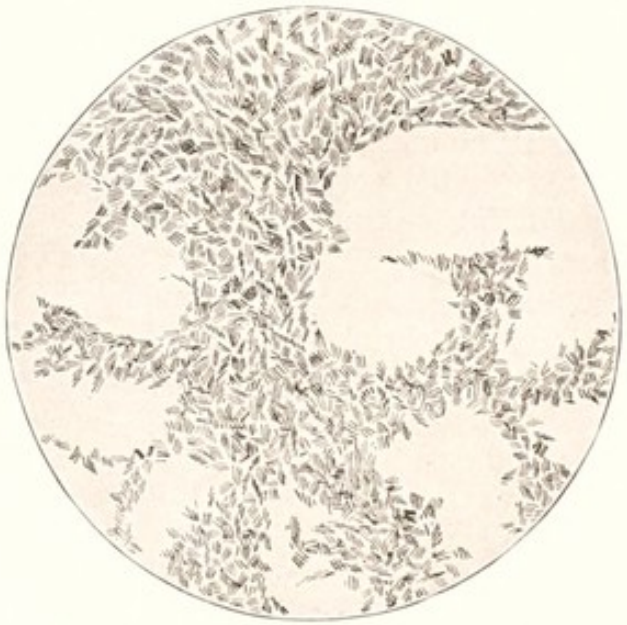


Fig. 280.—Bacillus tuberculosis avium.

infection was aided by lowering the temperature of the birds with antipyrin and lessening their vitality by starvation.

Morphologic Peculiarities.—Morphologically, the organism found in avian tuberculosis is similar to that found in the mammalian disease, but is a little longer and more slender, with more marked tendency to club and branched forms. Fragmented and beaded forms occur as in the human tubercle bacilli.

Staining.—The avian bacillus stains in about the same manner as the human and bovine bacilli and has an equal resistance to the decolorant effect of acids.

Cultivation.—Marked rapidity and luxuriance of growth are

* "Mag. f. d. ges Tierheilkunde," 1868.
† "Beitr. zur vergl. Anat.," Berlin, 1872.
‡ "Giorn. anat. fisiol. e. path.," Pisa, 1883.
§ "Zeitschrift für Hygiene," Bd. XI.
‖ "La Semaine medicale," 1890, p. 45.

characteristic of the avian bacillus, which grows upon ordinary agar-agar and bouillon prepared without glycerin.

The growth also lacks the dry quality characteristic of cultures of the human and bovine bacilli. Old cultures of the bacillus of fowl tuberculosis turn slightly yellow.

Thermic Sensitivity.—The bacillus also differs in its thermic sensitivity and will grow at 42° to 45°C. quite as well as at 37°C., while the growth of the human and mammalian bacilli ceases at 42°C. Moreover, growth at 43°C. does not attenuate its virulence. The thermal deathpoint is 70°C. Upon culture-media it is said to retain its virulence as long as two years.

Pathogenesis.—Birds are the most susceptible animals for experimental inoculation, the embryos and young being more susceptible than the adults. Artificial inoculation can be made in the subcutaneous tissue, in the trachea, and in the veins; never through the intestine. After inoculation the birds die in from one to seven months. The chief seat of the disease is the liver, where cellular (lymphocytic) nodes, lacking the central coagulation and the giant-cell formation of mammalian tuberculosis, and enormously rich in bacilli, are found. The disease never begins in the lungs, and the fowls that are diseased never show bacilli in the sputum or in the dung.

Guinea-pigs are quite immune, or after inoculation develop cheesy nodes, but do not die.

Rabbits are easily infected, an abscess forming at the seat of inoculation, nodules forming later in the lungs, so that the distribution is quite different from that seen in birds. It is possible that the avian bacillus occasionally infects man.

The possibility that this bacillus is derived from the same stock as the tubercle bacillus is strengthened by the experiments of Fermi and Salsano,* who succeeded in increasing its virulence until it became fatal to guinea-pigs, by adding glucose and lactic acid to the cultures inoculated.

FISH TUBERCULOSIS

Dubarre and Terre† isolated a bacillus having the tinctorial and morphologic characteristics of the tubercle bacillus from carp suffering from a tubercle-like affection. In respect to cultivation, however, it was unlike the tubercle bacillus, growing readily upon simple culture-media at 15° to 30°C., and not at 37°C.

Weber and Taube‡ found the same organism, or what seemed to be the same organism, in mud and in a healthy frog.

BACILLI RESEMBLING THE TUBERCLE BACILLUS

It is not improbable that the bacilli of human, bovine, and avian tuberculosis are closely related to one another, and, together with a few other micro-organisms of similar morphology and staining peculiarities, have a common ancestry

* "Centralbl. f. Bakt.," etc., XII, 750.

† "Compt. rendu de la Soc. de Biol. de Paris," 1897, 446.

‡ "Tuberkulose Arbeiten aus dem Kaiserlichen Gesundheitsamte," 1905.

and are descended from the same original stock. The most important of these similar organisms are *Bacillus lepræ* (*q.v.*), *B. smegmatis*, and *Moeller's grass bacillus*.

Bacillus Smegmatis

Alvarez and Tavel,* Matterstock,† Klemperer and Bittu,‡ Cowie,§ and others have described peculiar bacilli in smegma taken from the genitals of man and the lower animals, as well as from the moist skin in the folds of the groin, the axillæ, and the anus. They are also sometimes found in urine, and occasionally in the saliva and sputum.

Morphology and Staining.—The organisms are of somewhat variable morphology, but in general resemble the tubercle bacillus, stain with carbol-fuchsin, as does the tubercle bacillus, and resist the decolorant action of acids. They are, however, decolorized by absolute alcohol, though Moeller declares the smegma bacillus to be absolutely alcohol-proof as well as acid-proof, and admits no tinctorial difference between it and the tubercle bacillus. The bacillus, being about the size and shape of the tubercle bacillus, is very readily mistaken for it, and its presence in cases of suspected tuberculosis of the genito-urinary apparatus, and in urine and other secretions in which it is likely to be present, may lead to considerable confusion. The final differentiation may have to rest upon animal inoculation.

Cultivation.—The cultivation of the smegma bacillus is difficult and was first achieved by Czaplewski.‖ Doutrelepont and Matterstock cultivated it upon coagulated hydrocele fluid, but were unable to transplant the growth successfully.

Novy** recommends the cultivation of the smegma bacillus by inoculating a tube of melted agar-agar cooled to 50°C. with the appropriate material, and mixing with it about 2 cc. of blood withdrawn from a vein of the arm with a sterile hypodermic syringe. The blood-agar mixture is poured into a sterile Petri dish and set aside for a day or two at 37°C. The colonies that form are to be examined for bacilli that resist decolorization with acids.

Moeller†† found it comparatively easy to secure cultures of the smegma bacillus by a peculiar method. To secure small quantities of human serum for the purpose of investigating the phenomena of agglutination he applied small cantharidal blisters to the skins of various healthy and other men, and found large numbers of acid-proof bacilli in the serum saturated with epithelial substance, that remained after most of the serum had been withdrawn. He removed the skin covering from the blister, placed it in the remaining serum, and kept it in the incubator for three or four days, after which he found a dry, floating scum, which consisted of enormous numbers of the bacilli, upon the serum. From this growth he was subsequently able to start cultures of the smegma bacillus upon glycerin agar-agar. Human blood-serum is thus found to be the best medium upon which to start the culture.

Agar.—A culture thus isolated grew upon all the usual culture-media. Upon glycerin-agar, at 37°C., the colonies appeared as minute, dull, grayish-white, dry, rounded scales, which later became lobulated and velvety. At room temperature the dry appearance of the growth was retained. The water of condensation remained clear.

Potato.—On potato the growth was luxuriant, grayish, and dull.

Milk.—Milk is said to be an exceptionally good medium, growth taking place in it with rapidity and without coagulation.

Bouillon.—The growth forms a dry white scum upon the surface, the medium remaining clear.

Pathogenesis.—So far as is known, the smegma bacillus is a harmless saprophyte.

* "Archiv de Physiol. norm. et Path.," 1885, No. 7.
† "Mittheil. aus d. med. Klin. d. Univ. zu. Würzburg," 1885, Bd. VI.
‡ "Virchow's Archives," V, 103.
§ "Journal of Experimental Medicine," 1900–01, vol. V, p. 205.
‖ "Münchener med. Wochenschrift," 1897.
** "Laboratory Work in Bacteriology," 1899.
†† "Centralbl. f. Bakt. u. Parasitenk," March 12, 1902, (Originale), Bd. XXXI, No. 7, p. 278.

Moeller's Grass Bacillus

Bacilli found in milk, butter, timothy hay, cow-dung, etc., which stain like the tubercle bacillus and may be mistaken for it, have been described by Moeller.* The organisms so closely resemble the tubercle bacillus that guinea-pig inoculations must be resorted to in cases of doubt, but as some of these organisms sometimes kill the guinea-pigs after a month or two, and as small nodules or tubercles may be present in the mesentery, peritoneum, liver, lung, etc., of such animals, the diagnosis may have to be subjected to the further confirmation of a histologic examination of the lesions in order to exclude tuberculosis. In cases of this kind it should not be forgotten that the tubercle bacillus can be present in the substances mentioned, so that the exact differentiation becomes a very fine one. An instructive study of these organisms has been made by Abbott and Gildersleeve,† who, in an elaborate work upon the "Etiological Significance of the Acid-resisting Group of Bacteria, and the Evidence in Favor of Their Botanical Relation to Bacillus Tuberculosis," a work that gives complete references to the literature of the subject, come to the following conclusions:

1. That the majority of the acid-resisting bacteria may be distinguished from true tubercle bacilli by their inability to resist decolorization by a 30 per cent. solution of nitric acid in water.

2. That some of the acid-resisting bacteria are capable of causing in rabbits and guinea-pigs nodular lesions suggestive of tubercles; that these lesions, while often very much like tubercles in their histologic structure, may nevertheless usually be distinguished from them by the following peculiarities:

(*a*) When occurring as a result of intravenous inoculation, they are always seen in the kidneys, only occasionally in the lungs, and practically not at all in the other organs.

(*b*) They constitute a localized lesion, having no tendency to dissemination, metastasis, or progressive destruction of tissue by caseation.

(*c*) They tend to terminate in suppuration or organization rather than in progressive caseation, as is the case with true tubercles.

(*d*) They are more commonly and conspicuously marked by the actinomyces type of development of the organisms than is the case with true tubercles, and these actinomycetes are less resistant to decolorization by strong acid solutions than are those occasionally seen in tubercles.

3. That by subcutaneous, intravenous, and intrapulmonary inoculation of hogs (4) and calves (15) the typical members of the acid-resisting group are incapable of causing lesions in any way suggestive of those resulting from similar inoculations of the same animals with true tubercle bacilli.

4. That though occasionally present in dairy products, they are to be regarded as of no significance, etiologically speaking, but may be considered as accidental contaminations from the surroundings, and not as evidence of disease in the animals.

5. That the designation "bacillus" as applied to this group of bacteria and to the exciter of tuberculosis is a misnomer; they are more correctly classified as actinomyces.

The Butter Bacillus

Petri,‡ Rabinowitsch,§ and Korn‖ have described, as Bacillus butyricus, an acid-fast organism morphologically like the tubercle bacillus, which may at times be found in butter. Its chief importance lies in the confusion that may arise through mistaking it for the tubercle bacillus where attention is paid to the morphologic and tinctorial characters only, as tubercle bacilli may be found in butter made from cream from the milk of tuberculous cattle.

* "Deutsche med Zeitung," 1898, p. 135; "Deutsche med. Wochenschrift," 1898, p. 376, etc.

† "Univ. of Penna. Bulletin," June, 1902.

‡ "Arbeiten aus dem Kaiselichen Gesundheitsamte," 1897.

§ "Zeitschrift für Hygiene," etc., 1897.

‖ "Centralbl. f. Bakt.," etc., 1899.

Isolation and cultivation of these organisms is easy, and more than any other measure serves to differentiate them from the tubercle bacillus, as they grow upon nearly all the culture-media with rapidity and luxuriance.

PSEUDOTUBERCULOSIS

Bacillus Pseudotuberculosis

Pfeiffer,* Malassez and Vignal,† Eberth,‡ Chantemesse,§ Charrin, and Roger‖ have all reported cases of so-called pseudotuberculosis occurring in guinea-pigs, and characterized by the formation of cellular nodules in the liver and kidneys much resembling miliary tubercles. Cultures made from them showed the presence of a small *motile* bacillus which could easily be stained by ordinary methods. When introduced subcutaneously into guinea-pigs, the original disease was reproduced.

Morphology and Cultivation.—Bacillus pseudotuberculosis is characterized by Pfeiffer as follows: The organisms are rod-shaped, the rods varying in length (0.4 to 1.2 μ) and sometimes united in chains. They may be almost round, and then

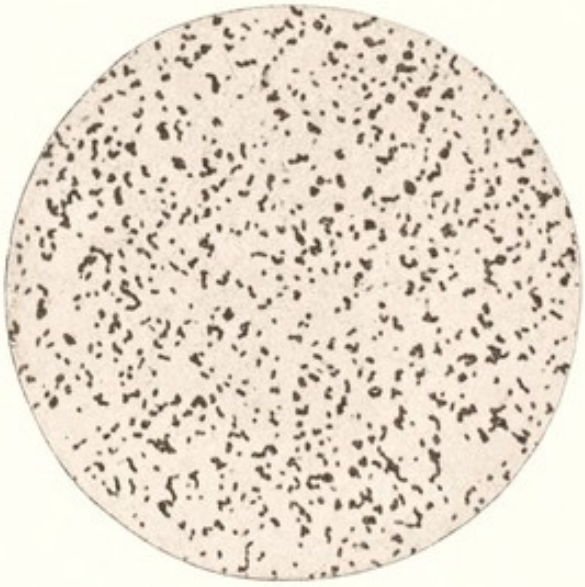


Fig. 281.—Bacillus pseudotuberculosis from agar-agar. × 1000 (Itzerott and Niemann.)

resemble diplococci. They stain by ordinary methods, but not by Gram's method. They are motile and have flagella like the typhoid and colon bacilli. They form no spores. Upon gelatin and agar-agar, circular colonies with a dark nucleus surrounded by a transparent zone are formed. In gelatin punctures the bacilli grow all along the line of puncture and form a surface growth with concentric markings. The gelatin is not liquefied. The bacilli grow readily upon agar and on potato, but without characteristic appearances. In bouillon a diffuse turbidity occurs, with floating and suspended flakes. Milk is not altered.

Pathogenesis.—The bacillus is fatal to mice, guinea-pigs, rabbits, hares, and other rodents in about twenty days after inoculation. At the seat of inoculation an abscess develops, the neighboring lymphatic glands enlarge and caseate, and nodules resembling tubercles form in the internal organs. Similar bacilli studied by Pfeiffer were isolated from a horse supposed to have glanders.

* "Bacilläre tuberculose, u. s. w.," Leipzig, 1889.
† "Archiv de Physiol. norm. et. Path.," 1883 and 1884.
‡ "Virchow's Archiv," Bd. CII.
§ "Ann. de l'Inst. Pasteur," 1887.
‖ "Compte-rendu de l'Acad. des Sci.," Paris, t. CVI.

CHAPTER XXX

LEPROSY

Bacillus Lepræ (Hansen)*

General Characteristics.—A non-motile, non-flagellate, non-sporogenous, chromogenic, non-liquefying, non-aërogenic, distinctly aërobic, parasitic and highly pathogenic, acid-resisting bacillus, staining by Gram's method, and cultivable upon specially prepared artificial media. It does not form indol, or acidulate or coagulate milk.

Leprosy very early received attention and study. Moses included in the laws to the people of Israel rules for its diagnosis, for the isolation of the sufferers, for the determination of recovery, and for the sacrificial observances to be fulfilled before the convalescent could once more mingle with his people. The Bible is replete with miracles wrought upon lepers, and during the times of biblical tradition it seems to have been an exceedingly common and malignant disease. Many of the diseases called leprosy in the Bible were, however, in all probability, less important parasitic skin affections.

Distribution.—At the present time, although we hear very little about it in the northern United States, leprosy is a widespread disease and exists much the same as it did several thousand years ago in Palestine, Syria, Egypt, and the adjacent countries, and is common in China, Japan, and India. South Africa has many cases, and Europe, especially Norway, Sweden, and parts of the Mediterranean coast, a considerable number. In certain islands, especially the Sandwich and Philippine Islands, it is endemic. In the United States the disease is uncommon, the Southern States and Gulf coast being chiefly affected.

A commission of the Marine-Hospital Service, formed for the purpose of investigating the prevalence of leprosy, in 1902 reported 278 existing cases in the United States. Of these, 155 occurred in the State of Louisiana. The other States with numerous cases were California, 24; Florida, 24; Minnesota, 20; and North Dakota, 16. No other State had more than 7 (New York). Of the cases, 145 were American born, 120 foreign born, the remainder uncertain.

Etiology.—The cause of leprosy is, without doubt, the lepra bacillus, discovered by Hansen in 1879.

Morphology.—The bacillus is about the same size as the tubercle bacillus. Its protoplasm commonly presents open spaces of frac-

* "Virchow's Archives," 1879.

tures, giving it a beaded appearance, like the tubercle bacillus. It occurs singly or in irregular groups. There is no characteristic grouping and filaments are unknown. It is not motile and has no flagella and no spores.

Duval found that the cultivated bacilli are longer, more curved, and show a greater irregularity in the distribution of the chromatin than those in the tissues where they are short, slender, and slightly curved. In artificial cultures there is a delicate filamentous arrangement of the bacilli, especially where they have become accustomed to a saprophytic existence. They often contain distinct metachromatic granules analogous to those met with in certain forms of the diphtheria bacillus. They are quite pleomorphous, and in the same culture all forms occur, from solidly staining coccoid

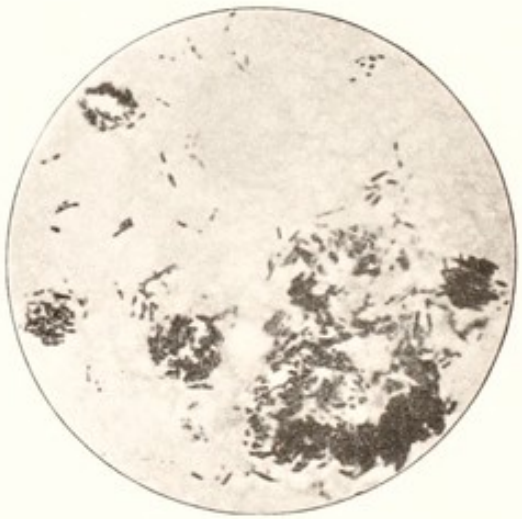


Fig. 282.—Lepra bacilli. Smear from a lepra node stained with carbol-fuchsin (Kolle and Wassermann).

shapes to slender slightly curved filaments, with numerous chromatic segments and occasional metachromatic granules. Sometimes the organisms are pointed at the ends.

Czaplewski found that the lepra bacilli in his cultures colored uniformly when young, but were invariably granular when old. The more rapidly the organism grew, the more slender it appeared.

Staining.—It stains in very much the same way as the tubercle bacillus, but permits of a more ready penetration of the stain, so that the ordinary aqueous solutions of the anilin dyes color it quite readily. The property of retaining the color in the presence of the mineral acids also characterizes the lepra bacillus, and the methods of Ehrlich, Gabbet, and Unna for staining the tubercle bacillus can be used for its detection. It stains well by Gram's method and by Weigert's modification of it, by which beautiful tissue specimens can be prepared.

Cultivation.—Many endeavors have been made to cultivate

this bacillus upon artificially prepared media, but in 1903 Hansen,* who discovered the organism, declared that no one had yet cultivated it.

Bordoni-Uffreduzzi† was able to cultivate a bacillus which partook of the staining peculiarities of the lepra bacillus as it appears in the tissues, but differed in morphology.

Czaplewski‡ confirmed the work of Bordoni-Uffredozzi, and

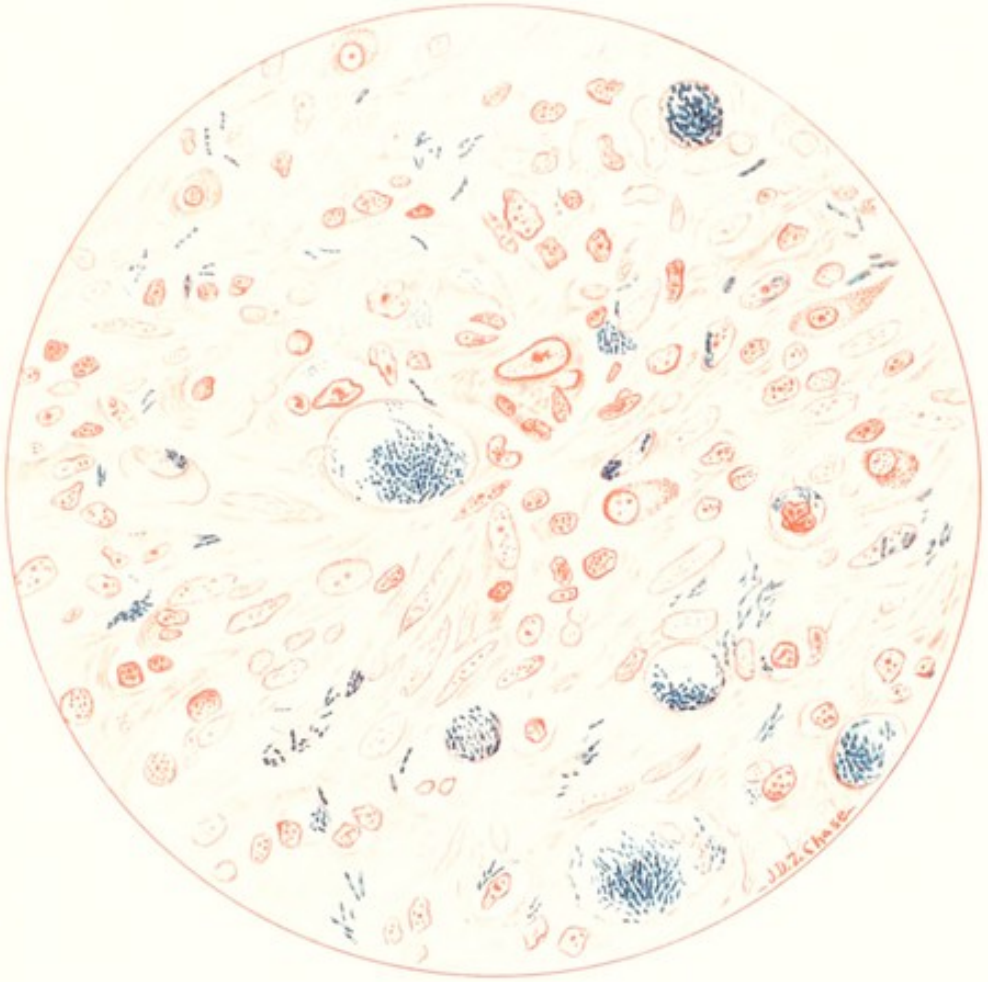


Fig. 283.—Section of one of the nodules from the patient shown in Fig. 285, stained by the Weigert-Gram method to show the lepra bacilli scattered through the tissue and inclosed in the large vacuolated "lepra-cells." Magnified 1000 diameters.

described a bacillus supposed to be the lepra bacillus, which he succeeded in cultivating from the nasal secretions of a leper.

The bacillus was isolated upon a culture-medium consisting of glycerinized serum without the addition of salt, peptone, or sugar. The mixture was poured into Petri dishes, coagulated by heat, and sterilized by the intermittent method.

* Kolle and Wassermann's "Handbuch der pathogenen Mikroörganismen," II, p. 184, 1903.

† "Zeitschrift f. Hygiene," etc., 1884, III.

‡ "Centralbl. f. Bakt. und Parasitenk.," Jan. 31, 1898, vol. XXIII, Nos. 3 and 4, p. 97.

The secretion, being rich in lepra bacilli, was taken up with a platinum wire and inoculated upon the culture-medium by a series of linear strokes. The dishes were then sealed with paraffin and kept in the incubating oven at 37°C.

Numerous colonies, chiefly of Staphylococcus aureus and the bacillus of Friedländer, developed, and in addition a number of colonies, composed of slender bacilli about the size and form of the lepra bacillus.

These colonies were grayish yellow, humped in the middle, 1 to 2 mm. in diameter, irregularly rounded, and uneven at the edges. They were firm and could be entirely inverted with the platinum wire, although the consistence was crumbly. They were excavated on the under side.

The colonies that formed upon agar-agar were much like those described by Bordoni-Uffreduzzi, and appeared as isolated, grayish, rounded flakes, thicker in the center than at the edges, and characterized by an irregular serrated border from which a fine irregular network extended upon the medium. These projections consisted of bundles of the bacilli.

When a transfer was made from one of these colonies to fresh media, the growth became apparent in a few days and assumed a band-like form, with a plateau-like elevation in the center.

The bacillus thus isolated grew with moderate rapidity upon all the ordinary culture-media except potato. Upon blood-serum the growth was more luxuriant and fluid than upon the solid media. Upon coagulated serum the growth was somewhat dry and elevated, and was frequently so loosely attached to the surface of the medium as to be readily lifted up by the platinum wire.

The growth was especially luxuriant upon sheep's blood-serum to which 5 per cent. of glycerin was added. The growth upon the Löffler mixture was also luxuriant.

Upon agar-agar the growth was more meager; it was more luxuriant upon glycerin agar-agar than upon plain agar-agar, the bacterial mass appearing grayish and flatter than upon blood-serum. The growth never extended to the water of condensation to form a floating layer.

The bacillus developed well upon gelatin after it had grown artificially for a number of generations and become accustomed to a saprophytic existence. Upon the surface of gelatin the growth was in general, similar to that upon agar-agar. In puncture cultures most of the growth occurred upon the surface to form a whitish, grayish, or yellowish wrinkled layer. Below the surface of the gelatin the growth occurred as a thick, granular column. The medium was not liquefied.

In bouillon, growth occurred only at the bottom of the tube in the form of a powdery sediment.

Spronck* believed that he had successfully cultivated the organism upon glycerinized, neutralized potatoes, first seeing the growth after the lapse of ten days. Cultures thus prepared were found to be agglutinated by the blood-serum of lepra cases, and he recommended the agglutination test for the diagnosis of obscure cases of the disease.

Ducrey claimed to have cultivated the lepra bacillus in grape-sugar, agar, and in bouillon *in vacuo*. His results need confirmation.

Rost† claimed to have isolated and cultivated the lepra bacillus upon media free from sodium chlorid. The technic of his method is thus described by Rudolph:‡

"Small lumps of pumice stone are washed and then dried in the sun, and then allowed to absorb a mixture of 1 ounce of meat extract and 2 ounces of water. This pumice stone is then placed in wide-mouthed bottles and placed in the autoclave. Each bottle is provided with a stopper through which pass two tubes, the one tube opening into the autoclave and reaching nearly to the bottom of the bottle, and the other leading from the top of the bottle into a condenser adjoining. When the cover of the autoclave is adjusted and the steam admitted, then in the case of each bottle, the steam passes by the one tube to the bottom of the bottle, and rising through the pieces of pumice stone, the steam, carrying with it the volatile constituents of the meat-extract, reaches the condenser by the second tube. The vapor in the condenser yields the salt-free nutrient medium in the proportion of 2 liters to each ounce of meat-extract originally used. The medium is collected from the condenser in sterilized Pasteur flasks which are kept plunged during the process in a freezing mixture in order to condense some of the volatile alkaloids from the beef that would otherwise escape. The nutrient fluid is now inoculated with the bacillus of leprosy and the flasks kept at 37°C. for from four to six weeks; at the end of this period when examined the flasks should present a turbid appearance with a stringy white deposit."

Clegg§ announced the cultivation of lepra bacilli from human leprous tissue in symbiosis with ameba and other bacteria. The organisms thus cultured he kept alive in subcultures. The method devised by Clegg was the starting-point of a more extended research by Duval,‖ who, after confirming the work of Clegg, found that the bacillus could be cultivated directly from human lesions upon culture-media containing tryptophan, without the symbiotic ameba or other bacteria. The initial culture was somewhat difficult to secure, but once the bacilli grew, transplantation was easily and successfully carried on for indefinite generations. He further found that the lepra bacillus could be successfully started to grow upon the ordinary laboratory media if bits of leprous tissue were placed upon them, and at the same time some symbiotic organism, such as the colon, typhoid, proteus, or other bacilli, added. Or if the tissue were already contaminated the lepra bacilli proceeded to multiply. Duval interprets this to mean that the lepra bacillus is unable to effect the destruction of the albumin molecule alone, and

* "Weekblad van het Nederlandsch Tijdschrift voor geneeskunde," Deel II, 1898, No. 14; abstract "Centralbl. f. Bakt.," etc., 1899, XXV, p. 257.

† "Brit. Med. Jour.," Feb. 22, 1905, and "Indian Med. Gazette," 1905.

‡ "Medicine," March, 1905, p. 175.

§ "Philippine Journal of Science," 1909, IV, 403.

‖ "Journal of Experimental Medicine," 1910, XII, 649; 1911, XIII, 365.

hence explains the advantage of adding tryptophan. The medium most successfully employed by Duval was as follows:

"Egg-albumen or human blood-serum is poured into sterile Petri dishes and inspissated for three hours at 70°C. The excised leprous nodule is then cut into thin slices, 2 to 4 mm. in breadth and 0.5 to 1 mm. in thickness, which are distributed over the surface of the coagulated albumin. By means of a pipette the medium thus seeded with bits of tissue is bathed in a 1 per cent. sterile solution of trypsin, care being taken not to submerge the pieces of leprous tissue. Sufficient fluid is added to moisten thoroughly the surface of the medium. The Petri dishes are now placed in a moist chamber at 37°C., and allowed to incubate for a week or ten days. They are removed from the plates from time to time, as evaporation necessitates, for the addition of more trypsin. It will be noted that after a week or ten days the tissue bits are partially sunken below the surface of the medium and are softened to a thick, creamy consistence, fragments of which are readily removed with a platinum needle. On microscopic examination of this material it is noted that the leprosy bacilli have increased to enormous numbers and scarcely a trace of the tissue remains. Separate lepra bacillus colonies are also discernible on and around the softened tissue masses. . . . The colonies are at first grayish white, but after several days they assume a distinct orange-yellow tint. . . . Subcultures may be obtained by transferring portions of the growth to a second series of plates or to slanted culture-tubes that contain the special albumin-trypsin medium. After the third or fourth generation the bacilli may be grown without difficulty upon glycerinated serum agar prepared in the following manner:

"Twenty grams of agar, 3 gm. of sodium chlorid, 30 cc. of glycerin, and 500 cc. of distilled water are thoroughly mixed, clarified, and sterilized in the usual way. To tubes containing 10 cc. of this material is added in proper proportion a solution of unheated turtle muscle infusion. Five hundred grams of turtle muscle are cut into fine pieces and placed in a flask with 500 cc. of distilled water. This is kept in the ice-chest for forty-eight hours and then filtered through gauze to remove the tissue. The filtrate is then passed through a Berkefeld filter for purposes of sterilization. By means of a sterile pipet, 5 cc. of the muscle filtrate is added to the agar mixture which has been melted and cooled to 42°C. The tubes are now thoroughly agitated and allowed to solidify in the slanted position.

"This medium is perfectly clear or of a light amber color, and admirably suited to the cultivation of the Bacillus lepra, once the initial culture has been started. Growth is luxuriant and reaches its maximum in forty-eight to sixty hours. On the surface of this medium the growth is moist and orange-yellow in color, while in the water of condensation, though growth apparently has not occurred, the detached bacilli collect in the dependent parts in the form of feathery masses without clouding the fluid.

"Ordinary nutrient agar may be used with trypsin as a plating medium instead of the inspissated serum where bits of tissue are employed. With the addition of 1 per cent. of tryptophan it answers every purpose, whether the bacilli are planted with tissue or alone. It also serves to start multiplication of lepra bacilli that are contaminated at the time of plating. In the latter case the medium is 'surface seeded' with an emulsion of the tissue juices in the same manner as in preparing 'streak' plates. The leprosy colonies in the thinner parts of the loop track are well separated and easily distinguished from those of other species by their color and by their appearance only after two to five days.

"In using an agar medium it is well to leave out the peptone and to titrate the reaction to 1.5 per cent. alkaline in order to prevent too profuse growth of the associated bacteria; besides, an alkaline medium seems best adapted for the multiplication of the lepra bacillus.

"Bacillus lepræ will also grow on the various blood-agar media once they are accustomed to artificial conditions. The Novy-McNeal agar for the cultivation of trypanosomes gives a luxuriant growth of the organism if 2 per cent. glycerin has been added; without the glycerin, growth is very scant. Fluid media are not suited for the artificial cultivation of leprosy bacilli unless they are kept upon the surface. Like the tubercle bacilli they require abundant oxygen. . . .

"Ordinarily the growth of Bacillus lepræ is very moist, and in this respect unlike that of Bacillus tuberculosis, except possibly the avian stain. Sometimes when the medium is devoid of water of condensation, the growth is dry and occasionally wrinkled, though it is easily removed from the surface of the medium

"The chromogenic property of lepra cultures is a constant and characteristic feature of the rapidly growing strains. The color varies in the degree of intensity depending upon the medium employed. If glycerinated agar (without peptone) is used, the colonies are faint lemon, while on inspissated blood-serum they are deep orange. It is noteworthy that the growth in the tissues and in the first dozen or so generations on artificial media is entirely without pigment."

Although each of the workers upon leprosy has begun by asserting that he had certainly cultivated the specific organism, a time comes when a more extended acquaintance with the bacteriology of the disease seems to cause him to doubt the results of his own work. This is particularly true of this work of Duval, which was prosecuted with enthusiasm, carried conviction with it, and then was partially repudiated by its author, for in the discussion before the 17th International Medical Congress in London in 1913, Duval* is reported as saying that "he knew less of the bacteriology of leprosy now than he did some four years ago. He had made several mistakes, had stated openly that he had cultivated the leprosy bacillus, but now admitted frankly that he was mistaken."

The interesting question that awaits settlement now seems to be, if these bacilli, and specially the bacillus of Duval, are not Bacillus lepræ, what are they? What relation do they bear to leprosy?

Pathogenesis.—Melcher and Ortmann* introduced fragments of lepra nodules into the anterior chambers of the eyes of rabbits, and observed the death of the animals after some months, with what they considered to be typical leprous lesions of all the viscera, especially the cecum; but the later careful experiments of Tashiro† show that most of the lower animals are entirely insusceptible to infection with the lepra bacillus, and that when they are inoculated the bacilli persistently diminish in numbers and finally disappear.

Nicolle‡ found it possible to infect monkeys with material rich in lepra bacilli taken from human beings. The lesions appeared only after an incubation period that was in some cases prolonged from twenty-two to ninety-four days. The lesions persisted but a short time and the monkeys recovered in from thirty to one hundred and fifty days.

Clegg§ and Sugai‖ found Japanese dancing mice susceptible to infection with leprous material, the micro-organisms not remaining localized at the seat of inoculation, but disseminating throughout the animal's body. Their observation has been confirmed by Duval,** who later†† was also able to infect monkeys—Macacus rhesus—with pure cultures of the organism and produce the typical disease.

* "Berliner klin. Wochenschrift," 1885–1886.

† "Centralbl. f. Bakt. u. Parasitenk," (Originale), March 12, 1902, XXXI, No. 7, p. 276.

‡ "Semaine medicale," 1905, No. 10, p. 110.

§ "Philippine Journal of Science," 1909, IV, 403.

‖ "Lepra," 1909, VIII, 203.

** "Journal of Experimental Medicine," 1910, XII, 649.

†† Ibid., 1911, XIII, 374.

Very few instances are recorded in which actual inoculation has produced leprosy in man. Arning* was able to experiment upon a condemned criminal, of a family entirely free from the disease, in the Sandwich Islands. Fragments of tissue freshly excised from a lepra nodule were introduced beneath his skin and the man was kept under observation. In the course of some months typical lesions began to develop at the points of inoculation and spread gradually, ending in general leprosy in about five years.

Sticker† is of the opinion that the primary infection in lepra takes place through the nose, supporting his opinion by observations upon 153 accurately studied cases, in which—

1. The nasal lesion is the only one constant in both the nodular and anesthetic forms of the disease.

2. The nasal lesion is peculiar—*i.e.*, characteristic—and entirely different from all other lepra lesions.

3. The clinical symptoms of lepra begin in the nose.

4. The relapses in the disease always begin with nasal symptoms, such as epistaxis, congestion of the nasal mucous membrane, a sensation of heat, etc.

5. In incipient cases the lepra bacilli are first found in the nose.

Lesions.—The lepra nodes in general resemble tuberculous lesions, but are superficial, affecting the skin and subcutaneous tissues. Rarely they may also occur in the organs. Virchow‡ has seen a case in which lepra bacilli could be found only in the spleen.

Once established in the body, the bacillus may grow in the connective tissues and produce chronic inflammatory nodes—the analogues of tubercles;—or in the nerves, causing anesthesia and trophic disturbances. On this account two forms of the disease, *lepra nodosa* (elephantiasis græcorum) and *lepra anæsthetica*, are described. These forms may occur independently of one another, or may be associated in the same case.

The nodes consist of lymphoid and epithelioid cells and fibers, and are vascular, so that much of the embryonal tissue completes its transformation to fibers without necrotic changes. This makes the disease productive rather than destructive, the lesions resembling new growths. The bacilli, which occur in enormous numbers, are often found in groups inclosed within the protoplasm of certain large vacuolated cells—the "lepra cells"—which seem to be partly degenerated endothelial cells. Sometimes they are anuclear; rarely they contain several nuclei (giant cells). Bacilli also occur in the lymph-spaces and in the nerve-sheath.

Lepra nodules do not degenerate like tubercles, and the ulceration, which constitutes a large part of the pathology of the disease,

* "Centralbl. f. Bakt.," etc., 1889, VI, p. 201.

† "Mittheilungen und Verhandlungen der internationalen wissenschaftlichen Lepra-Konferenz zu Berlin," Oct., 1897, 2, Theil.

‡ Ibid.

seems to be largely due to the injurious action of external agencies upon the feebly vital pathologic tissue.

According to the studies of Johnston and Jamieson,* the bacteriologic diagnosis of nodular leprosy can be made by spreading serum obtained by scraping a leprous nodule upon a cover-glass, drying, fixing, and staining with carbol-fuchsin and Gabbet's solution as for the tubercle bacillus. In such preparations the bacilli are present in enormous numbers, forming a marked contrast to tuberculous skin diseases, in which they are very few.

Fig. 284.—Lepra anæsthetica (McConnell).

In *anesthetic leprosy* nodules form upon the peripheral nerves, and by connective-tissue formation, as well as by the entrance of the bacilli into the nerve-sheaths, cause irritation, followed by degeneration of the nerves. The anesthesia following the peripheral nervous lesions predisposes to the formation of ulcers, etc., by allowing injuries to occur without detection and to progress without observation. The ulcerations of the hands and feet, with frequent loss of fingers and toes, follow these lesions, probably in the same manner as in syringomyelia.

The disease usually first manifests itself upon the face, extensor surfaces, elbows, and knees, and for a long time confines itself to

* "Montreal Med. Journal," Jan., 1897.

the skin. Ultimately it sometimes invades the lymphatics and extends to the internal viscera. Death ultimately occurs from exhaustion, if not from the frequent intercurrent affections, especially pneumonia and tuberculosis, to which the patients seem predisposed.

Specific Therapy.—Carrasquilla's* "leprosy serum" was prepared by injecting the serum separated from blood withdrawn from lepers, into horses, mules, and asses, and, after a number of injections, bleeding the animals and separating the serum. There is no reason for thinking that such a product could have therapeutic value. In practice it proved worthless.

Rost† prepared massive cultures of the lepra bacillus, filtered

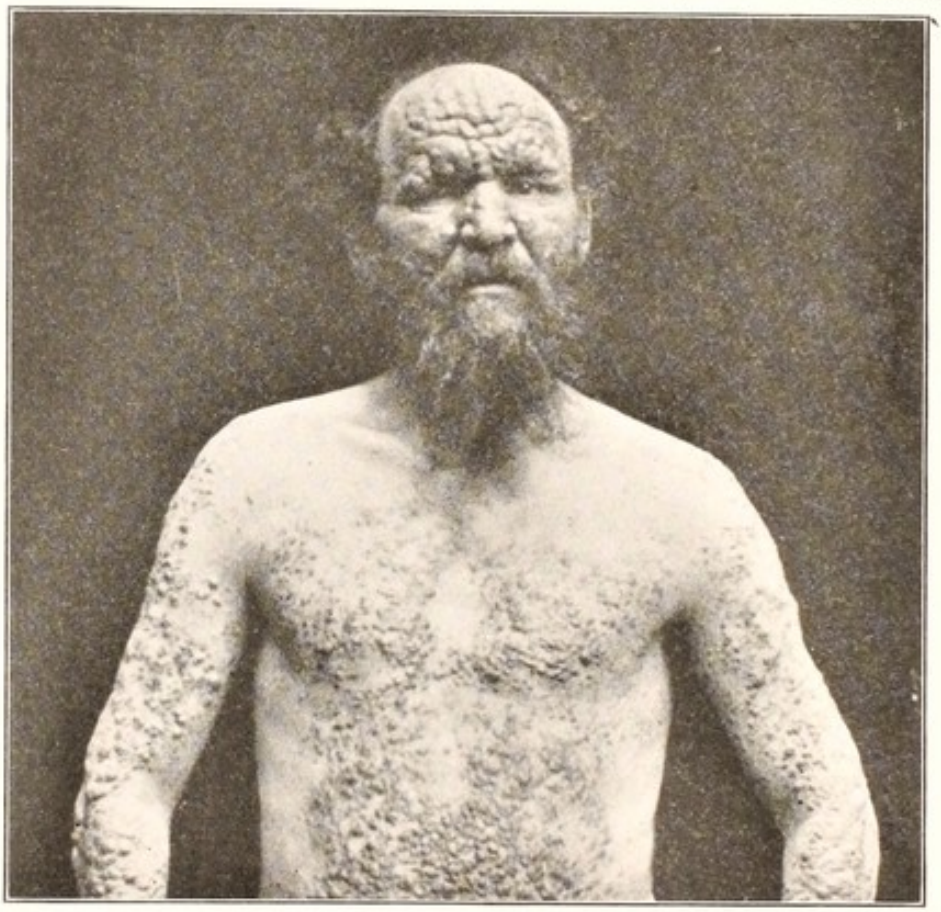


Fig. 285.—A case of lepra nodosa treated in the Medico-Chirurgical Hospital of Philadelphia.

them through porcelain, concentrated the filtrate to one-tenth of its volume, and mixed the filtrate with an equal volume of glycerin. The resulting preparation was called *leprolin* and was supposed to be analogous to tuberculin. With it he treated a number of lepers at the Leper Hospital at Rangoon, Burmah, many of whom greatly improved and some of whom seemed to be cured. Confirmation of the work by others is greatly desired.

Sanitation.—While not so contagious as tuberculosis, it has

* "Wiener med. Wochenschrift," No. 41, 1897.
† "Brit. Med. Jour.," Feb. 11, 1905.

been proved that leprosy is transmissible, and it may be regarded as an essential sanitary precaution that lepers should be segregated and mingle as little as possible with healthy persons. The disease is not hereditary, so that there is no reason why lepers should not marry among themselves. The children should, however, be taken from the parents lest they be subsequently infected.

CHAPTER XXXI

GLANDERS

Bacillus Mallei (Löffler and Schütz)*

General Characteristics.—A non-motile, non-flagellate, non-sporogenous, non-liquefying, non-chromogenic, non-aërogenic, aërobic and optionally anaërobic, acid-forming and milk coagulating bacillus, pathogenic for man and the lower animals, staining by ordinary methods, but not by Gram's method.

Glanders, "Rotz" (German) or "*morve*" (French), is an infectious mycotic disease which, fortunately, is almost entirely confined to the lower animals. Only occasionally does it secure a victim among hostlers, drovers, soldiers, and others whose vocations bring them in contact with diseased horses. Several bacteriologists have succumbed to accidental laboratory infection.

Glanders was first known to us as a disease of the horse and ass, characterized by the formation of discrete, cleanly cut ulcers upon the mucous membrane of the nose. The ulcers in the nose are formed by the breaking down of inflammatory nodules which can be detected in all stages upon the diseased membranes. Having once formed, they show no tendency to recover, but slowly spread and persistently discharge a virulent pus. The edges of the ulcers are indurated and elevated, their surfaces often smooth. The disease does not progress to any great extent before the submaxillary lymphatic glands begin to enlarge, soften, and ulcerate. The lungs may also become infected by inspiration of the infectious material from the nose and throat, and contain small foci of bronchopneumonia not unlike tubercles in their early appearance. The animals ultimately die of exhaustion.

Specific Organism.—In 1882, shortly after the discovery of the tubercle bacillus, Löffler and Schütz discovered in the discharges and tissues of the disease the specific micro-organism, the glanders bacillus (*Bacillus mallei*).

Distribution.—The glanders bacillus does not seem to find conditions outside the animal body suitable for its growth, and probably lives a purely parasitic existence.

Morphology.—The glanders bacillus is somewhat shorter and distinctly thicker than the tubercle bacillus, and has rounded ends. It measures about 0.25 to 0.4 × 1.5 to 3 μ, and is slightly bent. Coccoid and branched forms sometimes occur. It usually occurs singly, though upon blood-serum, and especially upon potato,

* "Deutsche med. Wochenschrift," 1882, 52.

conjoined individuals may occasionally be found. Long threads are never formed.

When stained with ordinary aqueous solutions of the aniline dyes, or with Löffler's alkaline methylene-blue, the bacillary substance does not usually appear homogeneous, but, like that of the diphtheria bacillus, shows marked inequalities, some areas being deeply, some faintly, stained.

The bacillus is non-motile, has no flagella, and does not form spores.

Staining.—The organism can be stained with the watery anilin-dye solutions, but not by Gram's method. The bacillus readily gives up the stain in the presence of decolorizing agents, so is dif-

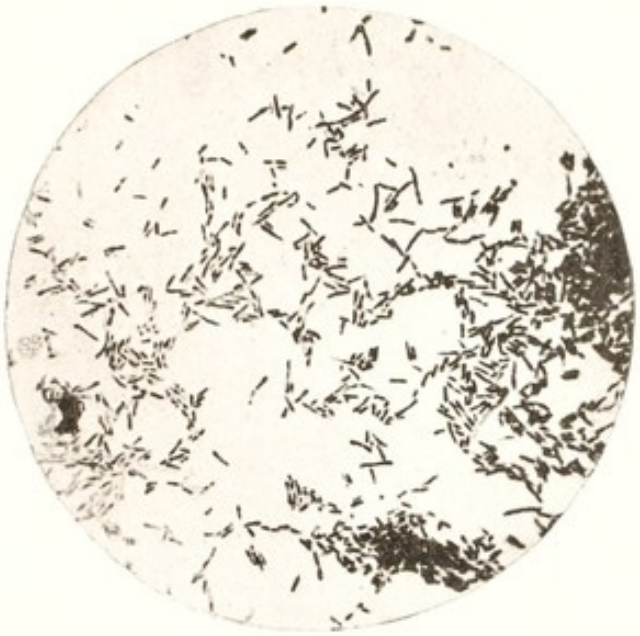


Fig. 286.—Bacillus mallei, from a culture upon glycerin agar-agar. × 1000 (Fränkel and Pfeiffer).

ficult to stain in tissues. Löffler accomplished the staining by allowing the sections to lie for some time (five minutes) in the alkaline methylene-blue solution, then transferring them to a solution of sulphuric and oxalic acids:

Concentrated sulphuric acid	2 drops
Five per cent. oxalic acid solution	1 drop
Distilled water	10 cc.

for five seconds, then to absolute alcohol, xylol, etc. The bacilli appear dark blue upon a paler ground. This method gives very good results, but has been largely superseded by the use of Kühne's carbolmethylene-blue.

Methylene-blue	1.5
Alcohol	10.0
Five per cent. aqueous phenol solution	100.0

Kühne stains the section for about half an hour, washes it in water,

decolorizes it carefully in hydrochloric acid (10 drops to 500 cc. of water), immerses it at once in a solution of lithium carbonate (8 drops of a saturated solution of lithium carbonate in 10 cc. of water), places it in a bath of distilled water for a few minutes, dips it into absolute alcohol colored with a little methylene-blue, dehydrates it in anilin oil containing a little methylene-blue in solution, washes it in pure anilin oil, not colored, then in a light ethereal oil, clears it in xylol, and finally mounts it in balsam.

Vital Resistance.—The organism grows only between 25° and 42°C. It is killed by exposure to 60°C. for two hours, or to 75°C. for one hour. Sunlight kills it after twenty-four hours' exposure. Thorough drying destroys it in a short time. When planted upon culture-media, sealed, and kept cool and in the dark, it may be kept alive for months and even years. Exposure to 1 per cent. carbolic acid destroys it in about half an hour; 1 : 1000 bichlorid of mercury solution, in about fifteen minutes. According to Hiss and Zinsser, it may remain alive in the water of horse-troughs for seventy days.

Isolation.—Attempts to isolate the glanders bacillus from infectious discharges, by the usual plate method, are apt to fail, on account of the presence of other more rapidly growing organisms.

A better method seems to be by infecting an animal and recovering the bacillus from its tissues. For this purpose the guinea-pig, being a highly susceptible as well as a readily procurable animal, is appropriate. When a subcutaneous inoculation of some of the infectious pus is made, a tumefaction can be observed in guinea-pigs in from four to five days. Somewhat later this tumefaction changes to a caseous nodule, which ruptures and leaves a chronic superficial ulcer with irregular margins. The lymph-glands speedily become invaded, and in four or five weeks signs of general infection appear. The lymph-glands, especially of the inguinal region, suppurate, and the testicles frequently undergo the same process. Later the joints are affected with a suppurative arthritis, the pus from which contains the bacilli. The animal eventually dies of exhaustion. No nasal ulcers form in guinea-pigs.

In field-mice the disease is much more rapid, no local lesions being visible. For two or three days the animal seems unwell, its breathing is hurried, it sits with closed eyes in a corner of the cage, and finally, without any other preliminaries, tumbles over dead.

From the tissues of the inoculated animals pure cultures are easily made. Perhaps the best places from which to secure a culture are the softened nodes which have not ruptured, or the joints.

Diagnosis of Glanders.—Straus* has given us a method which is of great use, both for isolating pure cultures of the glanders bacillus and for making a diagnosis of the disease.

* "Compt. rendu Acad. d. Sciences," Paris, CVIII, 530.

But a short time is required. The material suspected to contain the glanders bacillus is injected into the peritoneal cavity of a male guinea-pig. In three or four days the disease becomes established and the testicles enlarge; the skin over them becomes red and shining; the testicles themselves begin to suppurate, and often evacuate through the skin. The animal dies in about two weeks. If, however, it be killed and its testicles examined, the tunica vaginalis testis will be found to contain pus, and sometimes to be partially obliterated by inflammatory exudation. The bacilli are present in this pus, and can be secured from it in pure cultures.

The value of Straus' method has been somewhat lessened by the discovery by Kutcher,* that a new bacillus, which he has classed among the pseudo-tubercle bacilli, produces a similar testicular swelling when injected into the abdominal cavity; also by Levy and Steinmetz,† who found that Staphylococcus pyogenes aureus was also capable of provoking suppurative orchitis. However, the diagnosis is certain if a culture of the glanders bacillus be secured from the pus in the scrotum.

For the diagnosis of the disease in living animals, subcutaneous injections of mallein (*q.v.*) are also employed.

McFadyen‡ was the first to recommend agglutination of the glanders bacillus by the serum of supposedly infected animals as a test of the existence of glanders. The subject has been somewhat extensively tried and officially adopted by the Prussian government. Moore and Taylor,§ in a recent review and examination of the test, conclude that it is easier and quite as accurate as the mallein method and is applicable in cases where fever exists. The maximum dilution of normal horse-serum that will macroscopically agglutinate glanders bacilli is 1 : 500, but occurs in very few cases. The maximum agglutinative power of the serum of diseased horses not suffering from glanders is not higher than that of normal serum. The diagnosis is usually not difficult to make, but requires much care. Cultures of the glanders bacillus sometimes unexpectedly lose their ability to agglutinate.

The diagnosis of glanders by means of the complement-fixation method has been tried with glittering results by Mohler and Eichhorn.‖

Cultivation.—The bacillus is an aërobic and optionally anaërobic organism, and can be grown in bouillon, upon agar-agar, better upon glycerin agar-agar, very well upon blood-serum, and quite characteristically upon potato. The optimum temperature is 37.5°C.

Colonies.—Upon 4 per cent. glycerin agar-agar plates the colonies appear upon the second day as whitish or pale yellow, shining, round dots. Under the microscope they are brownish yellow, thick and granular, with sharp borders.

Bouillon.—In broth cultures the glanders bacillus causes turbidity, the surface of the culture being covered by a slimy scum. The medium becomes brown in color.

* "Zeitschrift für Hygiene," Bd. XXI, Heft 1, Dec. 6, 1895.
† "Berliner klin. Wochenschrift," March 18, 1895, No. 11.
‡ "Jour. Comp. Path. and Therap.," 1896, p. 322.
§ "Jour. Infectious Diseases," 1907, IV, p. 85, supplement.
‖ "Report of the Bureau of Animal Industry," 1910.

Gelatin is not liquefied. The growth upon the surface is grayish white and slimy, never abundant.

Agar-agar.—Upon agar-agar and glycerin agar-agar the growth occurs as a moist shining viscid layer.

Blood-serum.—Upon blood-serum the growth is rather characteristic, the colonies along the line of inoculation appearing as circumscribed, clear, transparent drops, which later become confluent and form a transparent layer unaccompanied by liquefaction.

Potato.—The most characteristic growth is upon potato. It first appears in about forty-eight hours as a transparent, honey-like, yellowish layer, developing only at incubation temperatures, and soon becoming reddish-brown in color. As this brown color of the colony develops, the potato for a considerable distance around it becomes greenish brown. Bacillus pyocyaneus sometimes produces somewhat the same appearance.

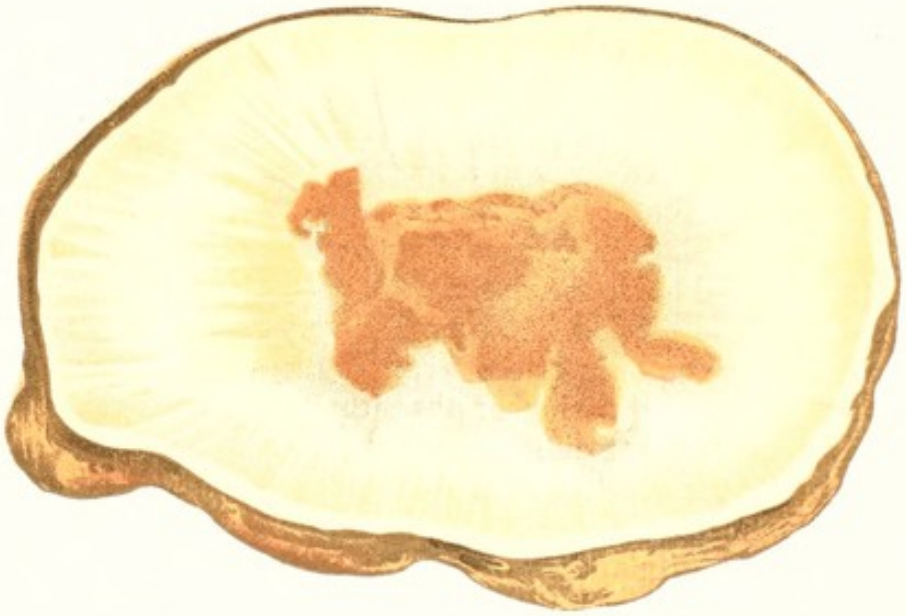


Fig. 287.—Culture of glanders upon cooked potato (Löffler).

Milk.—In litmus milk the glanders bacillus produces acid. A firm coagulum forms and subsequently separates from the clear reddish whey.

Metabolic Products.—The organism produces acids and curdling ferments. It forms no indol, no liquefying or proteolytic ferments. There is no exotoxin. All the poisonous substances seem to be endotoxins.

Mallein.—Babes,* Bonome,† Pearson,‡ and others have prepared a substance, *mallein*, from cultures of the glanders bacillus, and have employed it for diagnostic purposes. It seems to be useful in veteri-

* "Archiv de Med. exp. et d'Anat. patholog.," 1892, No. 4.
† "Deutsche med. Woch.," 1894, Nos. 36 and 38, pp. 703, 725, and 744.
‡ "Jour. of Comp. Med. and Vet. Archiv," Phila., 1891, XII, pp. 411–415.

nary medicine, the reaction following its injection into glandered animals being similar to that caused by the injection of tuberculin into tuberculous animals. The preparation of mallein is simple. Cultures of the glanders bacillus are grown in glycerin bouillon for several weeks and killed by heat. The culture is then filtered through porcelain, to remove the dead bacteria, and evaporated to one-tenth of its volume. Before use the mallein is diluted with nine times its volume of 0.5 per cent. aqueous carbolic acid solution. The dose for diagnostic purposes is 0.25 cc. for the horse. It has also been prepared from potato cultures, which are said to yield a stronger product. The agent is employed exactly like tuberculin, the temperature being taken before and after its hypodermic injection. A febrile reaction of more than 1.5°C. is said to be indicative of the disease.

Pathogenesis.—That the bacillus is the cause of glanders there is no room to doubt, as Löffler and Schütz have succeeded, by the inoculation of horses and asses, in producing the well-known disease.

The goat, cat, hog, field-mouse, wood-mouse, marmot, rabbit, guinea-pig, and hedgehog all appear to be susceptible. Cattle, house-mice, white mice, rats, and birds are immune.

Infection may take place through the mucous membranes of the nose, mouth, or alimentary tract, and apparently without preëxisting demonstrable lesions.

The disease assumes either an acute form, characterized by destructive necrosis and ulceration of the mucous membranes with fever and prostration, terminating in pneumonia, or, as is more frequent, a chronic form ("farcy"), in which the lesions of the mucous membranes are less destructive and in which there is a generalized distribution of the micro-organisms throughout the body, with resulting more or less widespread nodular formations (farcy-buds) in the skin. The acute form is quickly fatal, death sometimes coming on in from four to six weeks; the chronic form may last for several years and end in complete recovery.

Lesions.—When stained in sections of tissue the bacilli are found in small inflammatory areas. These nodules can be seen with the naked eye scattered through the liver, kidney, and spleen of animals dead of experimental glanders. They consist principally of leukocytes, but also contain numerous epithelioid cells. As is the case with tubercles, the centers of the nodules are prone to necrotic changes, but the cells show marked karyorrhexis, and the tendency is more toward colliquation than caseation. The typical ulcerations depend upon retrogressive changes occurring upon mucous surfaces, the breaking down of the nodules permitting the softened material to escape. At times the lesions heal with the formation of stellate scars.

Baumgarten* regarded the histologic lesions of glanders as much

* "Pathologische Mykologie," Braunschweig, 1890.

Fig. 288.—Pustular eruption of acute glanders as exhibited on the day of the patient's death, twenty-eight days after initial chill (Zeit).

Fig. 289.—Lesions of glanders in the skin of a horse. (Kitt).

like those of the tubercle. He first saw epithelioid cells accumulate, followed by the invasion of leukocytes. Tedeschi* was not able to confirm Baumgarten's work, but found the primary change to be necrosis of the affected tissue followed by invasion of leukocytes. The observations of Wright† are in accord with those of Tedeschi. He first saw a marked degeneration of the tissue, and then an inflammatory exudation, amounting in some cases to actual suppuration.

Glanders in Human Beings.—Human beings are but rarely infected. The disease has, however, occurred among those in frequent

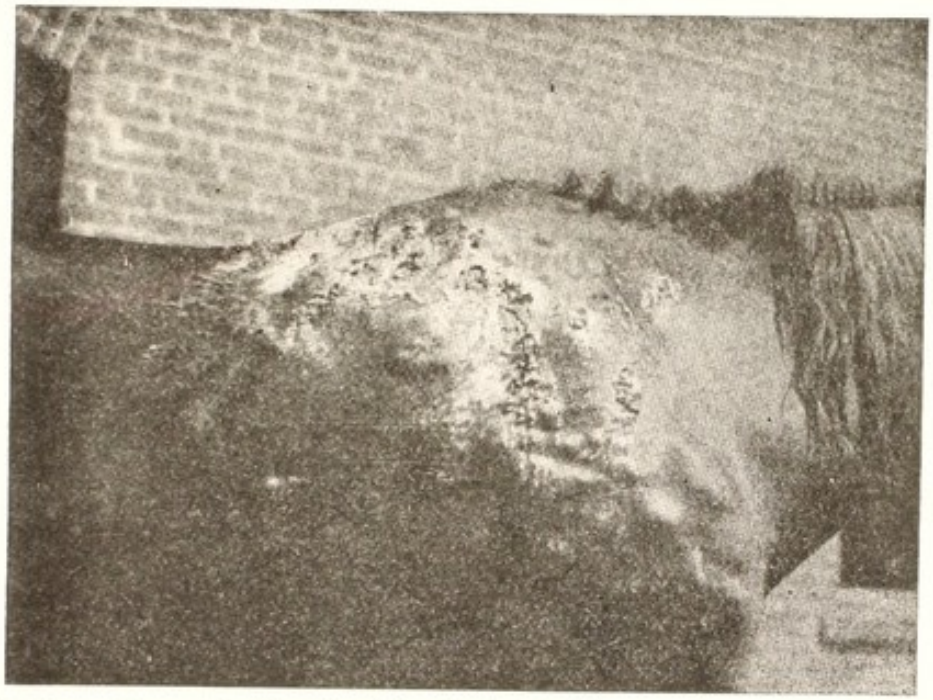


Fig. 290.—Farcy affecting the skin of the shoulder (Mohler and Eichhorn, in Twenty-seventh Annual Report of the Bureau of Animal Industry, U. S. Department of Agriculture, 1910).

contact with horses and among bacteriologists. It occurs either in an acute form in which, from whatever primary focus may have been its starting-point, the distribution of micro-organisms may be so rapid as to induce an affection with skin lesions resembling smallpox and terminating fatally in eight or ten days.

The chronic form in man is chiefly confined to the nasal and laryngeal mucosa. It is commonly mistaken for more simple infections, and though it sometimes shows its character by generalizing, it not infrequently recovers.

Virulence.—The organism is said to lose virulence if cultivated for many generations upon artificial media. While this is true, attempts to attenuate fresh cultures by heat, etc., have usually failed.

* "Zeigler's Beiträge z. path. Anat.," Bd. XIII, 1893.
† "Journal of Experimental Medicine," vol. 1, No. 4, p. 577.

Immunity.—Leo has pointed out that white rats, which are immune to the disease, may be made susceptible by feeding with phloridzin and causing glycosuria.

Babes has asserted that the injection of mallein into susceptible animals will immunize them against glanders. Some observers claim to have seen good therapeutic results follow the repeated in-

Fig. 291.—Lesions of glanders in the nasal septum of a horse (Mohler and Eichhorn, in Twenty-seventh Annual Report of the Bureau of Animal Industry, U. S. Department of Agriculture, 1910).

jection of mallein in small doses. Others, as Chenot and Picq,* find blood-serum from immune animals like the ox to be curative when injected into guinea-pigs infected with glanders.

Pseudo-glanders Bacillus.—Bacilli similar to the glanders bacillus in tinctorial and cultural peculiarities, but not pathogenic for mice, guinea-pigs, or rabbits, have been isolated by Babes,† and by Selter,‡ and called the pseudo-glanders bacillus.

* "Compte-rendu de la Soc. de Biol.," March 26, 1892.
† "Archiv de med. exp. et d'anat. path.," 1891.
‡ "Centralbl. f. Bakt.," etc., Feb. 18, 1902, XXXV, 5, p. 529.

CHAPTER XXXII

RHINOSCLEROMA

Bacillus Rhinoscleromatis (von Frisch*)

General Characteristics.—A non-motile, non-flagellate, non-sporogenous, non-chromogenic, non-aërogenic, aërobic and optionally anaërobic, capsulated bacillus, pathogenic for man and identical with Bacillus pneumoniæ of Friedländer, except that it stains by Gram's method.

A peculiar disease of the nares, characterized by the formation of circumscribed nodular tumors, and known as *rhinoscleroma*, is occasionally seen in Austria-Hungary, Italy, and some parts of

Fig. 292.—Rhinoscleroma (Courtesy of Mr. Owen Richards, Cairo, Egypt).

Germany. A few cases have been observed in Egypt and a few among the foreign-born residents of the United States. The nodular masses are flattened, may be discrete, isolated, or coalescent, grow with great slowness, and recur if excised. The disease commences in the mucous membrane and the adjoining skin of the nose, and spreads to the skin in the immediate neighborhood by a slow invasion, involving the upper lip, jaw, hard palate, and sometimes even the

* "Wiener med. Wochenschrift," 1882, 32.

pharynx. The growths are without evidences of acute inflammation, do not usually ulcerate, and upon microscopic examination consist of an infiltration of the papillary layer and corium of the skin, with round cells which in part change to fibrillar tissue. The tumors possess a well-developed lymph-vascular system. Sometimes the cells undergo hyaline degeneration.

In the nodes, von Frisch discovered bacilli closely resembling the pneumobacillus of Friedländer, both in morphology and vegetation, and, like it, surrounded by a capsule. The only differences between

Fig. 293.—Rhinoscleroma (Courtesy of Mr. Owen Richards, Cairo, Egypt).

the bacillus of rhinoscleroma and Bacillus pneumoniæ of Friedländer are that the former stains well by Gram's method, while the latter does not; that the former is rather more distinctly rod-shaped than the latter, and more often shows its capsule in culture-media.

The bacillus can be cultivated, and cultures in all media resemble those of the bacillus of Friedländer (*q.v.*) so closely as to be almost indistinguishable from it. The chief difference lies in its inability to endure acid media and to ferment carbohydrates. Even when inoculated into animals the bacillus behaves much like Friedländer's bacillus.

Inoculation has, so far, failed to reproduce the disease either in man or in the lower animals.

Pathogenesis.—The bacillus is said to be pathogenic for man only, producing granulomatous formations of the skin and mucous membranes of the anterior and posterior nares. These vary in

Fig. 294.—Bacillus rhinoscleromatis. Pure culture on glycerin agar-agar. Magnified 1000 diameters (Migula).

structure according to age. The young nodes consist of a loose fibrillar tissue composed of lymphocytes, fibroblasts, and fibers. Some of the cells are large and have a clear cytoplasm and are known as the cells of Mikulicz. In and between them the bacilli are found in considerable numbers. The older lesions consist of a firm sclerotic cicatricial tissue.

CHAPTER XXXIII

SYPHILIS

Treponema (Spirochæta) Pallidum (Schaudinn and Hoffmann)

General Characteristics.—A non-chromogenic, non-aërogenic, anaërobic, minute, slender, closely coiled, flexible, motile, flagellated, non-sporogenous, non-liquefying, spiral organism, cultivable upon specially prepared media, pathogenic for man and certain of the lower animals, staining by certain methods only and not by Gram's method.

Although syphilis has been well known for centuries, its specific cause has but recently been discovered. The supposition that the disease could not be successfully communicated to any of the lower animals was supposed to explain the delay, but has not proved to be the case, for in spite of the discovery of Metschnikoff and Roux* that chimpanzees could be successfully inoculated with virus from a human lesion, the confirmation of their work by Lassar† and others, and the additional discovery of Metschnikoff and Roux,‡ that it is also possible to infect macaques with syphilis, the specific organism was, after all, discovered for the first time in matter secured from human lesions.

It has long been known that preputial smegma and various ulcerative lesions of the generative organs contain certain spiral organisms. Bordet studied them with care, expecting to prove that they were concerned with the etiology of syphilis, but it remained for Schaudinn and Hoffmann§ to discover the specific microorganism. They point out that there are two separate species of spiral organisms commonly found in ulcerative lesions of the genitalia. One called by them Spirochæta refringens is of common occurrence, the other, called Spirochæta pallida, later, and more correctly, Treponema pallidum, is found only in syphilitic lesions—and is, therefore, their probable cause. The discovery of Treponema pallidum by Schaudinn and Hoffmann was quickly confirmed by Metschnikoff.‖ It is now universally accepted as the cause of syphilis.

Morphology.—The organism is a slender, flexible, closely coiled spiral, usually showing from eight to ten uniform undulations, but occasionally being so short as to show only two or three, or so long as to show as many as twenty.

* "Ann. de l'Inst. Pasteur," Dec., 1903, p. 809.
† "Berliner klin. Wochenschrift," 1903, p. 1189.
‡ "Annales de l'Inst. Pasteur," Jan., 1904.
§ "Deutsche med. Wochenschrift," May 4, 1905.
‖ "Bull. Acad. de med. de Paris," May 16, 1905.

It is very slender, measuring from 0.33 to 0.5 μ in breadth to 3.5 to 15.5 μ in length (Levaditi and McIntosh).

It forms no spores. Multiplication seems to take place by longitudinal division.

It is motile, and when observed alive with a dark field illuminator, can be seen to rotate slowly about its longitudinal axis at the same time that it slowly sways from side to side with a serpentine movement. The organisms are provided with flagella at one end, sometimes one at each end.

Noguchi* observed two types of treponema, one slender, one stouter. When carried through culture and used to inoculate rabbits their differences were found to be fairly constant. The lesions produced in rabbit's testicles varied with the variety of organism inoculated, one causing a diffuse, the other a nodular, orchitis. He conjectured that the distinction may be of value in explaining certain obscure points in human syphilis.

Staining.—*I. Films.*—The original discovery of the organism was achieved through the employment of Giemsa's stain—a modification of the Romanowsky method. But by this method the organisms appeared very pale and not very numerous. Goldhorn† improved it as follows:

In 200 cc. of water, 2 grams of lithium carbonate are dissolved and 2 grams of Merck's medicinal, Grübler's BX, or Koch's rectified methylene blue added. This mixture is heated moderately in a rice boiler until a rich polychrome has formed. To determine this a sample is examined in a test-tube every few minutes by holding it against an artificial light. As soon as a distinctly red color is obtained, the desired degree of heating has been reached. After cooling it is filtered through cotton in a funnel. To one-half of this polychrome solution 5 per cent. of acetic acid is gradually added until a strip of litmus-paper shows above the line of demarcation a distinct acid reaction, when the remaining half of the solution is added, so as to carry the reaction back to a low degree of alkalinity. A weak eosin solution is now prepared, approximately 0.5 per cent. French eosin, and this is added gradually while the mixture is being stirred until a filtered sample shows the filtrate to be of a pale bluish color with a slight fluorescence. The mixture is allowed to stand for one day and then filtered. The precipitate which has separated is collected on a double piece of filter-paper and dried at room temperature (heating spoils it). When completely dried it can easily be removed from the paper and may then be dissolved without further washing in commercial (not pure) wood alcohol. The solution should be allowed to stand a day, then filtered. The strength of this alcoholic solution is approximately 1 per cent. To use the stain, one drops upon an unfixed spread enough dye to cover it, permits it to act for three or four seconds, and then pours it off and introduces the glass slowly, spread side down, into clean water, where it is held for another four or five seconds, after which it is shaken to and fro in the water to wash it. It is next dried and examined at once or after mounting in balsam. The spirochætes appear violet in color.

Ghoreyeb‡ recommends the following rapid method of staining the organism in smears. A thin spread is to be preferred. No heat fixation is necessary:

* "Journal of Experimental Medicine," 1912, XV, No. 2, p. 201.
† Ibid., 1906, VIII, p. 451.
‡ "Jour. Amer. Med. Assoc.," May 7, 1910, LIV, No. 19, p. 1498.

1. Cover the smear with a 1 per cent. aqueous solution of osmic acid, and permit it to act for thirty seconds. This solution acts as a fixative and mordant.
2. Wash thoroughly in running water.
3. Cover the smear with a 1:100 dilution of Liquor plumbi subacetatis (freshly prepared). Permit it to act for ten seconds. The lead unites with the albumin to form lead albuminate which is insoluble in water.
4. Wash thoroughly in running water.
5. Cover the smear with a 10 per cent. aqueous solution of sodium sulphid. This is to act ten seconds, during which the salt transforms the lead albuminate into lead sulphid and causes the preparation to turn brown. The osmic acid when reapplied causes it to become black.
6. Wash thoroughly in running water.

The whole process is to be repeated in exactly the same manner three times, the washings all being very thorough. The preparation is then dried and mounted in Canada balsam. The micro-organisms and cellular detritus are stained black.

Fig. 295.—Treponema pallidum in the periosteum near an epiphysis (Bertarelli).

When serum from a primary sore or other syphilitic lesion is treated by these methods, a number of the spirochæta appear well stained and a number very palely stained, so that one is in doubt whether there may be many others unstained, and this seems to be the case, for when similar smears are treated by other methods many more can be found.

Stern* has applied the method of silver incrustation to the examination of films by the following simple procedure:

Spreads are made in the usual manner, dried in the air, and then for a few hours in an incubating oven at 37°C. They are next placed in a 10 per cent. solution of nitrate of silver in a colorless glass receptacle and allowed to rest in the diffused daylight of a comfortably lighted room for a few hours, until they become brownish metallic in appearance, when they are thoroughly washed in water. The spirochæta appear black, the background brownish.

* "Berliner klin. Wochenschrift," 1907, No. 14.

Burri* has recommended a simple and rapid method of demonstrating the treponema and other similar organisms by the use of India ink.

A drop of juice is squeezed from a chancre or mucous patch and mixed with a drop of India ink and then spread upon a glass slide as in making a spread of a drop of blood. As the ink dries it leaves a black or dark brown field upon which the spiral organisms stand out as shining, colorless, and hence conspicuous objects. Williams uses Higgins' water-proof ink, and Hiss recommends "chin-chin," Günther-Wagner liquid pearl ink, for the purpose.

The method is fairly satisfactory for diagnosis and can be applied in a few moments.

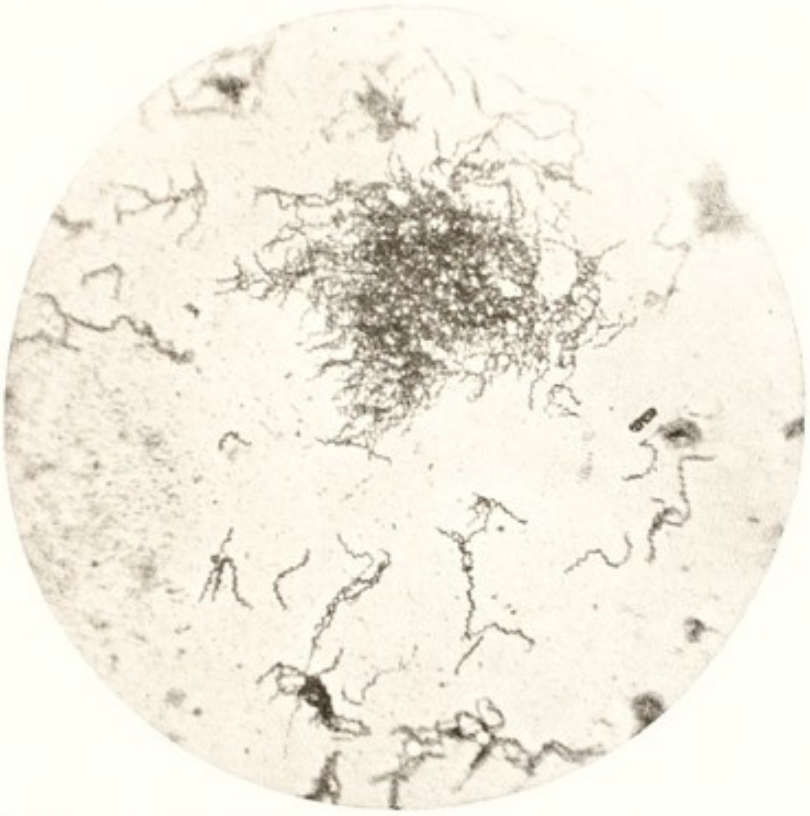


Fig. 296.—Treponema pallidum impregnated with silver. Film prepared from the skin of a macerated, congenitally syphilitic fetus. × 750 diameters (Flexner). The dense aggregation of organisms may indicate agglutination.

II. Section.—Staining the organism in the tissues is a more difficult matter, for the Giemsa stain scarcely shows it at all. Bertarelli and Volpino† tried a modification of the van Ermengen method for flagella with some success, but there was no real success until Levaditi‡ devised his methods of silver impregnation.

This consists in hardening pieces of tissue about 1 mm. in thickness in 10 per cent. formol for twenty-four hours, rinsing in water, and immersing in 95 per cent. alcohol for twenty-four hours. The block is then placed in diluted water until it sinks to the bottom of the container, and then transferred to a 1.5 to 3 per cent. aqueous solution of nitrate of silver in a blue or amber bottle and kept in a dark

* "Wiener klin. Wochenschrift," July 1, 1909.
† "Centralbl. f. Bakt. u. Parasitenk.," Orig., 1905, XL, p. 56.
‡ "Compt.-rendu de la Soc. de Biol. de Paris," 1905, LIX, p. 326.

incubating oven at 37°C. for from three to five days. Finally, it is washed in water and placed in a solution of pyrogallic acid, 2 to 4 grams; formol, 5 cc.; distilled water, 100 cc., and kept in the dark, at room temperature, from twenty-four to seventy-two hours, then washed in distilled water, embedded in paraffin, and cut. The treponemata are intensely black, the tissue yellow brown. The sections are finally stained with—(*a*) Giemsa's stain for a few minutes, then washed in water, differentiated with absolute alcohol containing a few drops of oil of cloves, cleared with oil of bergamot or xylol, or (*b*) concentrated solution of toluidin blue, differentiated in alcohol containing a few drops of Unna's glycerin-ether mixture, cleared in oil of bergamot, then in xylol, and mounted in Canada balsam.

This method was later improved by Levaditi and Manouelian* by the addition of 10 per cent. of pyridin to the silver bath just before the block of tissue is put in, and by using for the reducing bath a mixture of pyrogallic acid, acetone, and pyridin.

The details are as follows: Fragments of organs or tissues 1 to 2 mm. in thickness are fixed for twenty-four to forty-eight hours in a solution of formalin 10: 100, then washed in 96 per cent. alcohol for twelve to sixteen hours, then in distilled water until the blocks fall to the bottom of the container. They are then impregnated by immersion in a bath composed of a 1 per cent. solution of nitrate of silver, to which, at the moment of employment, 10 per cent. of pyridin is added. Keep the blocks immersed in this solution at room temperature for two or three hours, and at 50°C. for four or six hours, then wash rapidly in a 10 per cent. solution of pyridin, and reduce in a bath composed of 4 per cent. pyrogallic acid, to which, at the moment of using, 10 per cent. of pure acetone and 15 per cent. (total volume) of pyridin are added. The reduction bath must be continued for several hours, after which the tissue goes through 70 per cent. alcohol, xylol, paraffin, and sections are cut. The sections, fastened to the slide, are stained with Unna's blue or toluidin blue, differentiated with glycerin-ether, and finally mounted in Canada balsam.

Distribution.—The Treponema pallidum is not known in nature apart from the lesions of syphilis. It has now been found in all the lesions of this disease and in the blood of syphilitics in larger or smaller numbers. The discovery has greatly modified our ideas of the tertiary stage, for the demonstration of the organisms in its lesions shows them to be undoubtedly contagious. The greatest number of the organisms are found in the tissues—especially the liver—of still-born infants with congenital syphilis.

Cultivation.—The cultivation of the treponema was first attempted by Levaditi and McIntosh,† who, deriving the organism from an experimental primary lesion in a monkey (Macacus rhesus), carried it through several generations in collodion sacs inclosed in the peritoneal cavity of other monkeys (Macacus cynomolgus) and in the peritoneal cavity of rabbits. They were unable, however, to secure the treponema in pure culture, having it continually mixed with other organisms from the primary lesion. In the mixture, however, they were able to maintain it for generations and study its morphology and behavior. During cultivation its virulence was lost.

Schereschewsky‡ endeavored to cultivate the treponema by

* "Compt.-rendu de la Soc. de Biol. de Paris," 1906, LVIII, p. 134.
† "Ann. de l'Inst. Pasteur," 1907, p. 784.
‡ "Deutsche med. Wochenschrift," 1909, XXXV, 835, 1260, 1652.

placing a fragment of human tissue, containing it, deep down into gelatinized horse-serum. The treponema grew together with the contaminating organism and no pure culture was secured. Muhlens* and Hoffmann,† using the same method, succeeded in securing pure cultures of the treponema, but found them avirulent.

Noguchi,‡ taking advantage of the observations of Bruckner and Galasesco§ and Sowade,‖ that an enormous multiplication of treponema occurred when material containing it was inoculated into the rabbit's testis, performed a lengthy series of cultivation experiments with the enriched material thus obtained. The culture-medium used in these experiments was a "serum water," composed of 1 part of the serum of the sheep, horse, or rabbit and 3 parts of distilled water; 16 cc. of this mixture was placed in test-tubes 20 cm. long and 1.5 cm. in diameter and sterilized for fifteen minutes at 100°C. each day for three days.

To each of a series of such tubes a carefully removed fragment of sterile rabbit's testis was added, after which the tubes were incubated at 37°C. for two days to determine their sterility. To each tube the material from the inoculated rabbit's testis, rich in the treponema, is added, after which the surface of the medium in each receives a thick layer of sterile paraffin oil. As the most strict anaërobiosis is necessary, the tubes are placed in a Novy jar, the bottom of which contains pyrogallic acid. Noguchi first passes H gas through the jar, permitting it to bubble through the pyrogallic acid solution for ten minutes. He then uses a vacuum pump to exhaust the atmosphere in the jar, and lastly permits the alkaline solution (KOH) to flow down one of the tubes and mix with the pyrogallic acid.

In these cultures the pallidum grows together with such bacteria as may have been simultaneously introduced. To secure the cultures free from these bacteria Noguchi permitted the treponema to grow through a Berkefeld filter, which for a long time held back the other organisms. Later it was found that both bacteria and treponema grow side by side in a deep stab in a serum-agar-tissue medium, but that the bacteria grow only in the stab or puncture, whereas the treponemata grow out into the medium as a hazy cloud. By cautiously breaking the tube and securing material for transplantation from the scarcely visible cloud, the organisms may be transplanted to new media and pure cultures obtained.

In a later paper, Noguchi** details the cultivation of the treponema from fragments of human chancres, mucous patches, and other cutaneous lesions. The medium employed is a mixture of 2 per cent. slightly alkaline agar and 1 part of ascitic or hydrocele fluid, at the bottom of which a fragment of rabbit kidney or testis is placed. The medium is prepared in the tubes, after the addition of the tissue, by mixing 2 parts of the melted agar at 50°C. with

* Ibid., 1909, XXXV, 1261.
† "Zeitschrift für Hygiene und Infektionsk.," 1911, LXVIII, 27.
‡ "Journal of Experimental Medicine," 1911, XIV, 99.
§ "Compt.-rendu de la Soc. de Biol. de Paris," 1910, LXVIII, 648.
‖ "Deutsche med. Wochenschrift," 1911, XXXVII, 682.
** "Journal of Experimental Medicine," 1912, XV, 1, p. 90.

1 part of the ascitic or hydrocele fluid. After solidification a layer of paraffin oil 3 cm. deep is added.

A considerable number of tubes should be prepared at the same time and incubated for a few days prior to use to determine sterility. The bits of human tissue are snipped up with sterile scissors in salt solution containing 1 per cent. of sodium citrate and should be kept immersed in this fluid from the time of securing to the time of planting, so as not to become dried. A bit of the tissue should be emulsified in a mortar with citrate solution and examined with a dark field illuminator to make sure that the organisms to be cultivated are present.

If they are found, and the material shown to be adapted to cultivation, each of the remaining bits of tissue is taken up by a thin blunt glass rod and pushed to the bottom of a culture-tube and into each tube several drops of the emulsion examined are introduced by means of a capillary pipet, also inserted deeply into the medium. The tubes are next incubated at 37°C. for two or three weeks. In successful tubes, in which the medium has not been broken up by gas-producing bacteria, there is a dense opaque growth of bacteria along the line of puncture, and a diffuse opalescence of the agar-agar caused by the extension into it of the growing treponemata. A capillary tube cautiously inserted into the opalescent medium withdraws a particle that can be examined with the dark field illuminator. When such observation shows the cause of the opalescence to be, in fact, the treponema, the tube can be cautiously broken at some appropriate part and the transplantation made from the opalescent part of the medium to fresh appropriate culture-media. By these means, after a few trials, pure cultures of treponema were secured.

The colonies were said never to be sharp, but always faintly visible. There is no color and no odor.

By inoculating the organisms recently secured from human lesions (by the method given) into monkeys (Macacus rhesus and Cereopithicus callitrichus) Noguchi was able to produce typical syphilis of the monkey, thus showing that the virulence of the organisms was not lost in the cultivation.

Zinsser, Hopkins and Gilbert* found it possible to grow Treponema pallidum in massive cultures in fluid media. They employed a flask with a long slender neck like a "specific gravity flask." The flask was filled with slightly acid (0.2 to 0.8 per cent. acidity) broth containing sheep-serum, ascitic fluid, horse-serum or rabbit-serum, with an addition of autoclaved and hence thoroughly sterilized tissue (kidney, liver, brain, lung or heart muscle) and covered with sterile neutral paraffine oil. The culture contains the greatest number of organisms after three weeks. To collect them for making *luetin*, etc., the fluid in the flasks was poured into tubes and

*"Journal of Experimental Medicine," 1915, XXI, No. 3, p. 213.

centrifugated for a short time to throw down scraps of the nutrient tissue, the fluid then decanted and recentrifugated rapidly and for a longer time to throw down the micro-organisms.

Pathogenesis and Specificity.—There can be no doubt about the causal relation of Treponema pallidum to syphilis. It is unknown in every other relation; it has appeared in every required relation, and thus has completely fulfilled the laws of specificity laid down by Koch. Treponema pallidum is not only pathogenic for man, but, as has already been shown, can also be successfully implanted into chimpanzees, macaques, rabbits, guinea-pigs, and other experiment animals. As syphilis is, however, unknown under natural conditions, except in man, it may be looked upon as a human disease.

The organism enters the body through a local breach of continuity of the superficial tissues, except in experimental and congenital infections, where it may immediately reach the blood.

In ordinary acquired syphilis the point of entrance shows the first manifestations of the disease after a period of *primary incubation* about three weeks long, in what is known as the *primary lesion* or *chancre*. This appears as a papule, grows larger, undergoes superficial indolent ulceration, and eventually heals with the formation of an indurated cicatrix. It is in this lesion that the treponema first makes its appearance. From this lesion, where it multiplies slowly, it enters the lymphatics and soon reaches the lymph-nodes, which swell one by one as its invasion progresses. During this stage of glandular enlargement the organisms can be found in small numbers in juice secured from a puncture made in the gland with a hollow needle. This period of *primary symptoms* (chancre and adenitis) includes part of what is known as the period of *secondary incubation*, which intervenes between the appearance of the chancre and that of the *secondary symptoms*. It usually lasts about six weeks. During this time the organisms are multiplying in the lymph-nodes and occasionally entering the blood. What fate the organisms meet when they reach the blood in small numbers is not yet known, but the slow invasion suggests that those first entering are destroyed, and that it is only when their numbers are great and their virulence increased that they suddenly become able to overcome the defenses and permit the development of the *secondary symptoms*. The period of secondary symptoms corresponds to the invasion of the blood by the parasite. It may continue from one to three years, during which time the patient suffers from general symptoms, fever, etc., probably due to intoxication and local symptoms, such as alopecia, exanthemata, etc., due to local colonization of the organisms. At the end of this period a partial immunity, such as is seen in other infectious diseases (malaria), develops, the organisms disappear from the blood, the general local and constitutional disturbances recover, and the patient may

be well. Should he continue to harbor some of the micro-parasites, however, there may be an insidious sclerosis of the blood-vessels and parenchymatous organs consequent upon the growth and multiplication of the parasites, or there may be after many years a period of *tertiary symptoms* characterized by the sudden appearance of severe lesions in which the parasites are very few in number.

The specific organisms are present in juice expressed from the primary lesion, in juice from the buboes and enlarged lymph-nodes; in the blood, in the roseola, and all of the secondary lesions, and sparingly in the tertiary lesions.

In congenital syphilis they reach the fetus from the ovum, the spermatozoön, or the blood of the mother. Prenatal death from syphilis is accompanied by lesions in which enormous numbers of the organisms can be found, and furnishes the best tissues for their experimental demonstration and study.

Lesions.—The lesions of syphilis are so numerous that the reader is referred to works on pathology and dermatology for satisfactory descriptions. Here it may suffice to say that though diverse in appearance and location, they have certain features in common. The first of these, and that which naturally places syphilis among the infectious granulomata, is the lymphocytic infiltration of the tissues, with which all of the lesions begin. The second is a peculiar form of necrosis—slimy when superficial, gummy when deep—with which they terminate. The third is a tendency toward excessive cicatrization.

Diagnosis.—It is now possible to make a certain and early diagnosis of syphilis by the recognition of the specific organisms, and as the difficulty of treatment is in proportion to the stage at which the disease arrives before treatment, it should never be neglected.

I. *Staining.*—The expressed lymph from a carefully cleaned freshly abraded primary lesion can be stained by Giemsa's method, or, as is much better and more certain, by Stern's method, with nitrate of silver, or by the use of India ink.

II. *Dark-field Examination.*—For those who possess the "dark-field illuminator" or some similar apparatus with the proper lamp, direct examination of the fluid expressed from the lesions can be made, and the living, moving organisms recognized. This should be the quickest method of diagnosis, though it takes practice.

III. *Serum Diagnosis.*—Wassermann and Bruck have devised a laboratory method of making the diagnosis of syphilis by testing the complement fixing power of the patient's serum. This method, now known as the "Wassermann reaction," (*q.v.*) is given elsewhere in complete detail.

The success of the von Pirquet cutaneous tuberculin reaction in assisting the diagnosis of tuberculosis led to experiments on the part of a number of investigators—Meirowsky, Wolff-Eisner, Tedeschi, Nobe, Ciuffo, Nicholas, Favre, and Gauthier and Jodas-

shon—to obtain analogous reaction in syphilis by applying extracts of syphilitic tissues to the scarified epiderm of syphilitics. Some reactions were observed, but Neisser and Bruck found that similar reactions occurred when a concentrated extract of normal liver was applied, and to such reactions which could not be looked upon as specific, Neisser applied the term "Umstimmung."

After having successfully achieved the cultivation of Treponema pallidum, Noguchi* resolved to try the effect of an application of an extract of the organisms applied to the skin, in the hope that it might provoke a reaction useful for diagnosis. To this end he prepared two cultures, one in ascitic fluid containing a piece of sterile placenta, the other in ascitic fluid agar also containing a piece of placenta. After permitting them to grow under strictly anaërobic conditions at 37°C. until luxuriant development occurred, the lower part of the solid culture was carefully cut off, the tissue fragment removed, and the rich culture carefully ground in a sterile mortar, the thick paste being diluted from time to time by adding a little of the fluid culture. The grinding was continued until the emulsion became perfectly clear, when it was heated to 60°C. for one hour upon a water-bath and 0.5 per cent. of carbolic acid added. When examined with the dark-field illuminator, 40 to 100 dead treponemata could be seen in every field. Cultures made from the suspension remained sterile and inoculation into rabbits' testicles was without result.

This extract of the treponema culture he calls *luetin*. When it was applied to the ear of a normal rabbit, by means of an endermic injection with a fine needle, an erythema appeared, but faded within forty-eight hours, the skin resuming its normal appearance, but when it was applied to the ear of a syphilized rabbit, at the end of the forty-eight hours the redness developed into an induration the size of a pea and persisted from four to six days, disappearing in ten days. In one case a sterile pustule developed.

Luetin was tested by Noguchi and his colleagues upon 400 cases: 146 of these were controls, 177 syphilitics, and 77 parasyphilitics. In the controls there was erythema without pain or itching, which disappeared without induration within forty-eight hours. In the syphilitics at the end of forty-eight hours there was an induration in the form of a papule 5 to 10 mm. in diameter, surrounded by a zone of redness and telangiectasis. This slowly increased for three or four days and became dark bluish red. It usually disappeared in about a week. Sometimes the papule underwent vesiculation and sometimes pustulation. It always healed kindly without induration. In certain cases described as *torpid*, the erythema cleared away and a negative result was supposed to have resulted, when suddenly the spots lighted up again and progressed to vesiculation or pustulation. In 3 cases there were constitutional symptoms—

* "Journal of Experimental Medicine," 1911, XIII, p. 557.

malaise, loss of appetite, and diarrhea. Noguchi found that the reaction is specific, that it is most striking and most constantly present in tertiary, latent tertiary, and congenital syphilis. It, therefore, forms a valuable adjunct to diagnosis, seeing that it is most evident in precisely those cases in which the Wassermann reaction is most apt to fail. A few early cases energetically treated with mercury and salvarsan give marked reactions. A few old cases fail to give it.

Spirochæta Refringens (Schaudinn and Hoffmann)

This spiral organism, though given the name by which it is now known by Schaudinn and Hoffmann, was probably first described by Donné under the name Vibrio lineola. It is probably a frequent organism of the skin and mucous membranes, and occurs in greatest numbers in lesions of the genitalia because of the smegma upon which it customarily lives. It is present in most primary lesions of syphilis, but is no less frequently found in non-syphilitic lesions, such as balanitis, venereal warts, and genital carcinoma. It is also found in the mouth and on the tonsils. According to Hoffmann and Prowazek* it is not entirely harmless, but has a pathogenic action, and some of the complicating lesions of syphilis as well as some of the destructive diseases of the genitals may be intensified by it. They found it pathogenic for apes.

Morphologically, it is much broader than Treponema pallidum, its spiral waves are much coarser and less regular. It is easy to stain by all methods and is hence easily found. It has been cultivated by Noguchi.†

* "Centralbl. f. Bakt.," etc., 1906, XLI.

† "Journal of Experimental Medicine," May 1, 1912, XV.

CHAPTER XXXIV

FRAMBESIA TROPICA (YAWS)

Treponema Pertenue (Pallidulum) (Castellani)

This peculiar, specific, infectious, contagious, chronic febrile disease of the tropics is characterized by the appearance upon the skin of one or more primary papular lesions—the yaws—bearing some semblance to raspberries, and by subsequent malaise, fever, and other constitutional disturbances. These are later followed by the appearance of a second crop of small papules which grow to the size of a pea or a small nut or may grow to be as large as apples, which become covered with firm scabs and gradually cicatrize. The patient either recovers or suffers from relapses and the appearance of further crops of the lesions. The duration of the disease varies from a few weeks to several years. In most cases the constitutional disturbances occur only at the period preceding the development of the eruptions and for a short time afterward. Little children frequently die; older children and adults may die of exhaustion in case extensive lesions with marked ulcerations develop.

The patients usually recover and pigmented areas remain for some time where the lesions have occurred.

The disease appears to have been known since 1525, when Oviedo became acquainted with it in St. Domingo. It has always been very puzzling because it bears so many resemblances to syphilis; but the peculiar raspberry-like character of the primary lesion, its disposition to occur upon the face, mouth, nose, eyes, neck, limbs, fingers, and toes, as well as upon the genitals, seem to point in another direction, and all authorities now admit that it is not syphilis, but an independent disease.

It occurs only in tropical countries, and is most frequent in equatorial Africa on the west coast, from Senegambia to Angola. It also occurs in West Soudan, Algeria, the Nile Valley, and in the islands about the east coast of Africa. It has been seen rarely in South Africa. In Asia it occurs in Malabar, Assam, Ceylon, Burmah, Siam, Malay Peninsula, the Indian Archipelago, Moluccas, and China. It is also endemic in many of the islands and archipelagos of the southern Pacific.

The disease rarely makes its appearance in the United States, and it is of interest to know that Wood* has been able to collect nine such cases from the literature.

*"American Journal of Tropical Medicine," 1915, II, No. 7, p. 431.

Specific Organism.—The cause of the disease was unknown until the discovery of Treponema pallidum, which opened a way for its investigation. Castellani* was quick to seize the opportunity, and in the same year in which Schaudinn and Hoffmann discovered the cause of syphilis, announced a similar organism as the cause of yaws. At the time of discovery he called it Spirochæta pertenuis and Spirochæta pallidula, but it is now recognized as a treponema and is called *Treponema pertenue*.

Morphology.—The organism so closely resembles Treponema pallidum that it is rather by knowing the source from which the organism was derived than by any morphologic distinctions that the two are separated. It is said to be a little shorter than T.

Fig. 297.—Yaws (photograph by P. B. Cousland, M. B., Swatow, China).

pallidum, measures 7 to 20 μ in length, is closely and regularly coiled, and is said to have rounded ends.

Staining.—It stains like its close relative, palely with most of the dyes. The silver nitrate, the India ink methods, and the other methods of staining Treponema are all appropriate, both for demonstrating it in smears from the lesions or in sections of tissue.

Cultivation.—The organism seems not yet to have been cultivated.

Pathogenesis.—Castellani† has succeeded in infecting monkeys with the scrapings from yaws papules. The infection usually resulted in a local lesion, though there was also a generalized infection, for he found treponemata everywhere in the lymph-nodes. When the inoculation material was filtered and all of the organisms removed, the infectivity was destroyed. Blood and splenic substance from the infected monkey, containing no organisms other than the

* "Brit. Med. Jour.," 1905, II, 282, 1280, 1330.
† "Jour. of Hygiene," 1907, VII, p. 558.

treponemata, was infective for other monkeys. When monkeys successfully inoculated with yaws are afterward infected with syphilitic virus they are not immune. On the other hand, monkeys that have successfully been inoculated with syphilis are not immune against yaws. Levaditi and Nattan-Larrier* differ from Castellani in this particular, and found that monkeys infected with syphilis are refractory to yaws. Castellani was able, by means of complement-fixation tests, to detect different specific antibodies for syphilis and yaws. Halberstadter† has successfully infected orang-outangs.

There is no doubt but that in their clinical manifestations and in their etiology frambesia and syphilis are closely related.

Diagnosis.—In addition to the clinical manifestations which are usually quite sufficient for diagnosis, the discovery of the Treponema pertenue is of assistance. It can usually be found without difficulty by expressing the serum from a lesion and staining it by any of the methods recommended for Treponema pallidum, the India-ink method being the most simple.

The Wassermann reaction is always positive in yaws, hence is of no use for purposes of differential diagnosis.

* "Ann. de l'Inst. Pasteur," 1908, XXII, 260.
† "Arbeiten a. d. Kaiserl. Gesund.," 1907, XXVI, 48.

CHAPTER XXXV

ACTINOMYCOSIS

Actinomyces Bovis (Bollinger)

General Characteristics.—A parasitic, pathogenic, aërobic and optionally anaërobic, non-motile, non-flagellate, non-sporogenous (?), liquefying, pathogenic, branched micro-organism, belonging to the higher bacteria, staining by ordinary methods and by Gram's method.

In 1845 Langenbeck discovered that an infectious disease of cattle known as "wooden tongue" and "lumpy jaw," and later as actinomycosis, could be communicated to man. The observation, however, was not published until 1878, one year after Bollinger* had discovered the *actinomyces*, the specific cause of the disease.

Israel† wrote the first important paper upon actinomycosis as a disease of man, though the best paper on the subject is probably

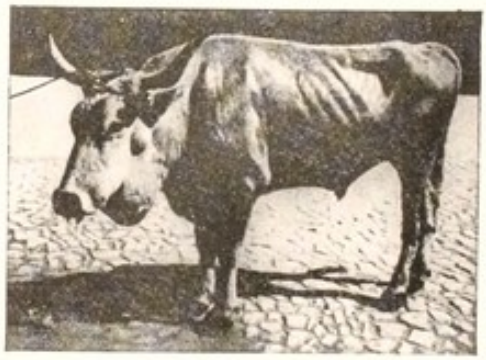


Fig. 298.—Bovine actinomycosis.

that by Boström,‡ who made a careful study of the microscopic lesions of the disease.

Its first manifestations are usually found either about the jaw or in the tongue, and consist of considerable sized enlargements which are sometimes dense and fibrous (wooden tongue), sometimes suppurative in character. In sections of tissue containing these nodular formations, small yellowish granules surrounded by some pus can usually be found. These granules, when examined beneath the microscope, consist of peculiar rosette-like bodies—the "ray-fungi" or *actinomyces*.

Distribution.—The actinomyces is best known as a parasitic organism associated with actinomycosis. That it occurs rather

* "Deutsche Zeitschrift für Thiermedizin," 1877.

† "Virchow's Archives," 1874–78.

‡ "Berl. klin. Wochenschrift," 1885. "Beiträge zur Path. Anat. und zur Allg. Path.," 1890, IX.

widely in nature seems to be indicated by the fact that cases of infection have been known to occur from the spines of barley and other cereals. Berestnew* succeeded in isolating the organisms from hay and straw.

Morphology.—A complete ray-fungus consists of several distinct zones composed of different elements. The center is composed of a granular mass containing numerous bodies resembling micrococci or spores. Extending from this center into the neighboring tissue is a radiating, branched, tangled mass of mycelial threads.

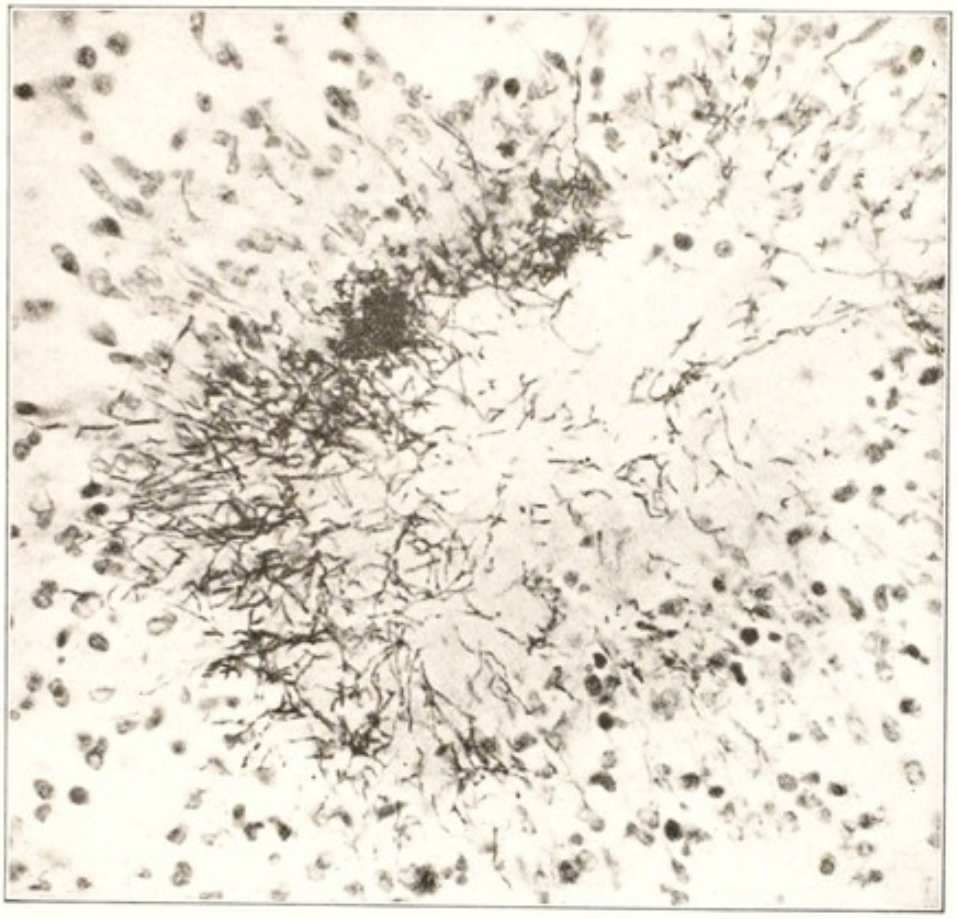


Fig. 299.—Colony or granule of actinomyces in a section through a lesion showing the Gram-stained filaments and hyaline material and also the pus-cells surrounding the colony (Wright and Brown).

In an outer zone these threads are seen to terminate in conspicuous, club-shaped, radiating forms which give the colonies their rosette-like appearance. The clubs are inconspicuous in the human lesions of the disease.

The pleomorphism of the organism and the branched network it forms class it among the higher bacteria in the genus *Actinomyces*. When the clumps formed in artificial cultivations of the parasite are properly crushed, spread out, and stained, the long mycelial threads, 0.3–0.5 μ in thickness, occasionally show flask- or bottle-like expansions—the clubs—at the ends. These probably depend

* "Centralbl. f. Bact.," etc., Ref., 1898, No. 24.

upon gelatinization of the cell-membrane of the degenerating parasite. The club is one of the chief characteristics of the organism. In sections of tissue the radiating filaments are very distinct, and the terminal clubs are all directed outward, closely packed together, and making the whole mass form a rounded little body often spoken of as an "actinomyces grain." When tissues are stained first with carmin and then by Gram's method, the fungous threads appear blue-black, the clubs red. The cells of the tissues affected and a larger or smaller collection of leukocytes form the surrounding resisting tissue-zone.

The fungus is of sufficient size to be detected in pus, etc., by the naked eye. As it usually has a bright yellow color it is not infrequently spoken of as a "sulphur grain."

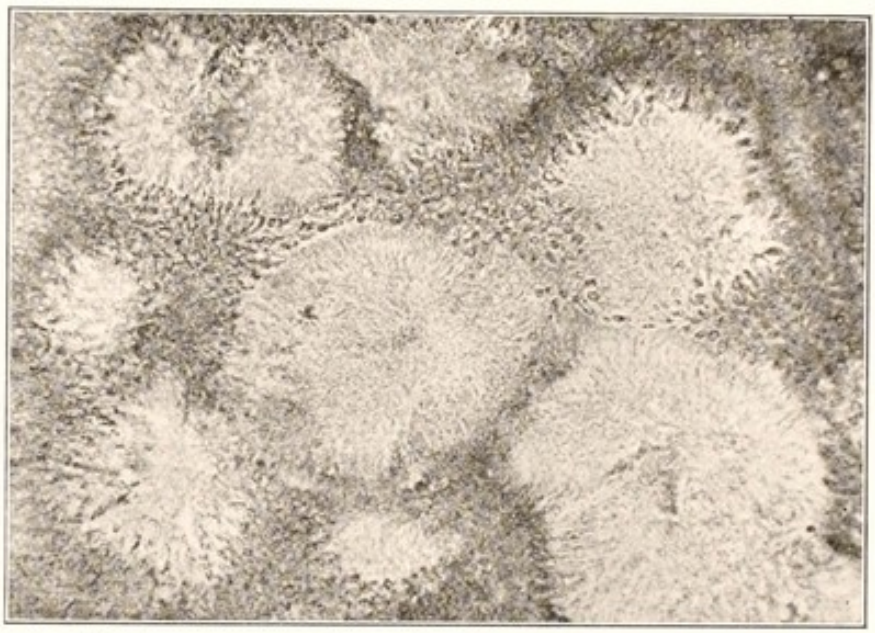


Fig. 300.—Actinomyces granule crushed beneath a cover-glass, showing radial striations in the hyaline masses. Preparation not stained; low magnifying power (Wright and Brown).

Cultivation.— The actinomyces fungus may be grown upon artificial culture media, as has been shown by Israel,* Wolff, and others.

"The granules, preferably obtained from closed lesions, are first thoroughly washed in sterile water or bouillon and then crushed and disintegrated between two sterile slides. If one is working with a bovine case it is well to examine microscopically the disintegrated material, after mixing it with a drop or two of bouillon under a cover-glass, to see if filamentous masses are present. If they are not, or if they are very few, proceed no further, but begin again with another granule, because the granules in bovine lesions sometimes contain no living filaments at all, but may be composed entirely of degenerated structures from which no growth of micro-organisms can be

* "Virchow's Archives," cxv.

generated. If filaments and filamentous masses are found to be present in the granule, then the disintegrated products of the granule are to be transferred by means of the platinum loop to melted 1 per cent. dextrose agar-agar contained in test-tubes filled to a depth of 7 or 8 centimeters which have been cooled to about 40°C. The material is to be thoroughly distributed throughout the melted agar-agar by means of the loop, and the tube then placed in the incubator. Several tubes should be prepared. At the same time a number of granules, after washing in sterile water or bouillon, should be placed on the sides of sterile test-tubes plugged with cotton and kept at room temperature in the dark. The sugar-agar tubes inoculated as above described should be examined from day to day for the presence of the characteristic colonies in the depths of the agar-agar. If very many colonies of contaminating bacteria have developed in the tubes, it will probably be very difficult or impossible to isolate the specific micro-organism. If there are few or no contaminating colonies, then the colonies of the specific organism should be expected to develop in the course of two or three days to a week. If a good number of living filaments of the micro-organism have been distributed throughout the agar, the specific colonies that develop will be very numerous in the depths of the agar, especially throughout a shallow zone situated about 5 to 12 mm. below the surface of the agar-agar. When the presence of the characteristic colonies has been determined, slices or pieces of the agar containing colonies are to be cut out of the tube by means of a stiff platinum wire with a flattened and bent extremity. A piece of the agar-agar is to be placed upon a clean slide and covered with a clean cover-glass. It is to be examined under a low power of the microscope, and an isolated colony selected for transplantation. By obvious manipulations, under continuous control of microscopic observation, the selected colony, together with a small amount of the surrounding agar-agar is to be cut out, care being taken to be sure that no other colony is present in the small piece of agar-agar containing the colony. The small piece of agar-agar thus cut out should not have a greatest dimension of more than 2 mm. The piece of agar-agar is then transferred from the slide by means of a platinum loop to a tube of sterile bouillon where it is thoroughly shaken up to free it from any adherent bacteria. If there be any reason to believe that the small piece of agar has been very much contaminated with bacteria, it should be washed in a second tube of bouillon, then the piece of agar-agar is to be transferred by means of the platinum loop to a tube of melted sugar-agar cooled to 40°C. It should be immersed deeply in the agar and the tube placed in the incubator. If the colony thus transferred to the agar-agar is capable of growth, in the course of some days it will have formed a good-sized colony from which transplants in various culture-media may be made."

Fig. 301.—Colony of actinomyces with well-developed "clubs" at the periphery in a nodule in the peritoneal cavity of a guinea-pig inoculated with a culture from another guinea-pig. Paraffin section. Low magnification (Wright). (Photograph by Mr. L. S. Brown.)

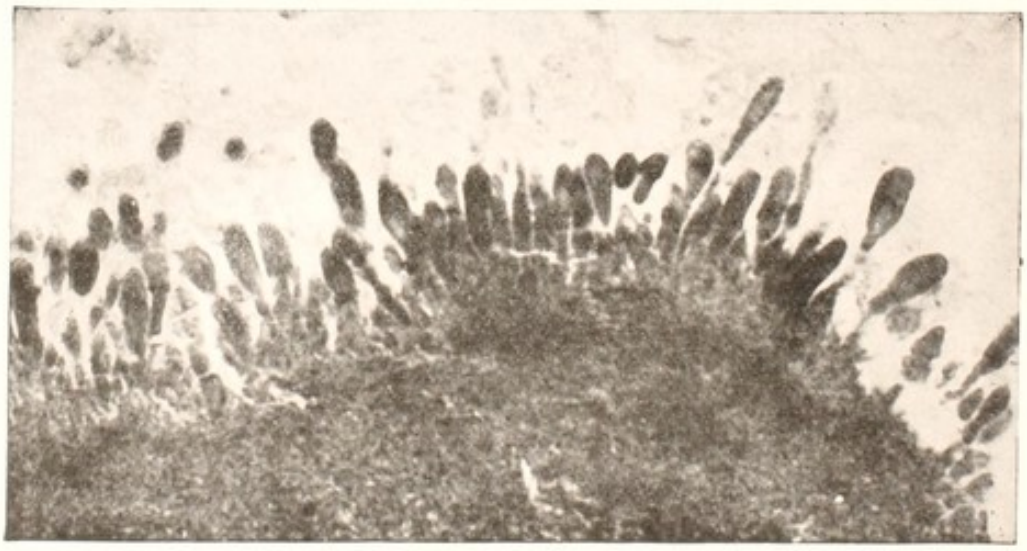
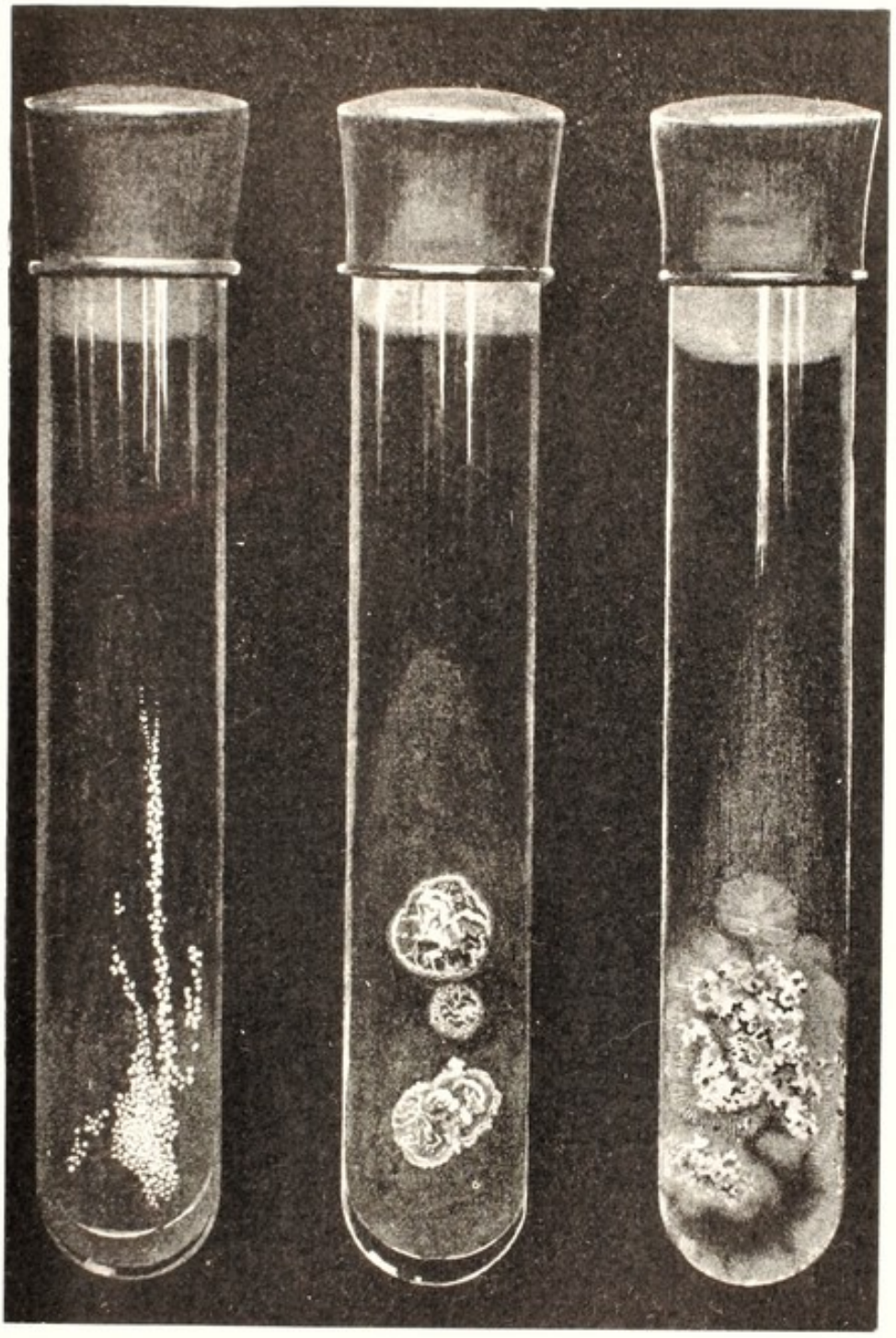


Fig. 302.—A colony of actinomyces in a nodule twenty-eight days old in the peritoneal cavity of a guinea-pig inoculated with a culture from another guinea-pig (Bovine case). The "clubs" are well developed and show some indications of stratification. Paraffin section. × 750 approx. (Wright). (Photograph by Mr. L. S. Brown.)

From such anaërobic cultures the micro-organism can, after a few generations, be made to grow upon the surface of solid media, where it invariably forms rounded nodular, elevated masses.



A *B* *C*

Fig. 303.—Actinomycosis; glycerin-agar cultures: *A*, Discrete rounded colonies after about ten days' incubation at 37°C.; *B*, limpet-shaped colonies three and a half months old; *C*, lichen-like appearance frequently seen; the growth is three and a half months old (Curtis).

Blood-serum.—Upon blood-serum the nodular growths present a yellowish or rust-red color, and are surrounded with a whitish down

of fine threads. The colonies adhere closely to the culture-media and are so firm that they crush with difficulty. If the surface be scraped, spores and fine threads may be secured. If the mass be crushed, branched filaments may be secured. The colonies become confluent in the course of time, and a thick wrinkled membrane is produced. The growth liquefies blood-serum.

Gelatin.—In gelatin puncture cultures an arborescent growth occurs and the gelatin is liquefied.

Agar-agar.—Upon agar-agar and glycerin agar-agar the growth is similar to that upon blood-serum. The agar-agar turns brown as the culture ages.

Bouillon.—In bouillon the growth occurs in the form of large granules if allowed to stand quietly; of numerous small granules if frequently shaken up. The granules are similar in structure to those formed upon the dense media. The bouillon does not become clouded.

Potato.—Upon potato the growth resembles that upon blood-serum, but is slower in developing. The color is reddish-yellow and the white down early makes its appearance.

Eggs.—The organism can also be grown in raw eggs, into which it is carefully introduced through a small opening made under aseptic precautions. In the eggs long, branched mycelial threads are formed.

The characteristic rosettes so constantly found in the tissues are never seen in artificial cultures.

Metabolism.—There seems to be some difference of opinion as to the oxygen requirement of actinomyces. Israel, Boström and others state that it grows best when provided with a free oxygen supply. Wright found it to grow best under anaërobic conditions.

It does not ferment sugar, and does not evolve gas. It liquefies gelatin and blood-serum but does not coagulate milk. Some strains seem to produce a small quantity of orange-red pigment.

A small amount of soluble toxin appears in culture-filtrates.

Temperature.—In well established strains accustomed to saprophytic life, growth progresses slowly but continuously at 20°C. (room temperature). Freshly isolated cultures just being started will only grow at 37°C. Growth ceases at a point between 45°C. and 50°C. Wright found the organism killed after an hour at 60°C.

Virulence.—When the actinomyces is grown upon artificial media the virulence is retained for a considerable time. Different strains show varying degrees of pathogenesis, some being almost or quite non-pathogenic, others virulent. The difficulty of making successful injections of the laboratory animals limits our power to accurately gauge the virulence.

Pathogenesis.—Actinomycosis is almost peculiar to bovine animals, but sometimes occurs in hogs, horses, and other animals, and rarely in human beings. The disease can with difficulty be

inoculated into experiment animals, the introduced fungi either becoming absorbed or encapsulated by connective tissue and not growing. In the abdominal cavities of rabbits the peritoneum, mesentery and omentum show typical nodules containing the actinomyces rays in cases of successful inoculation.

Mode of Infection.—The manner by which the organism enters the body is not positively known. In some cases it may be by direct

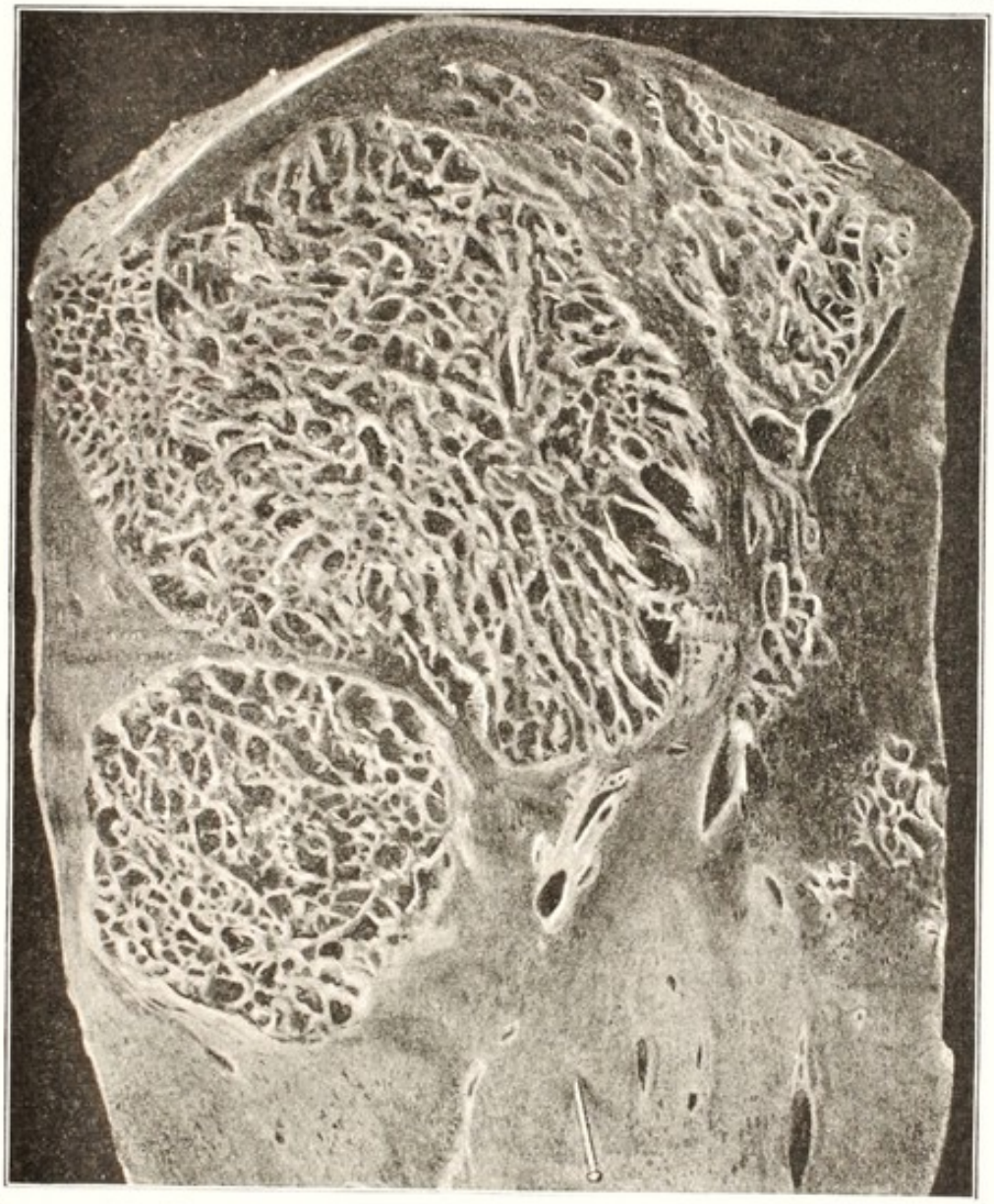


Fig. 304.—Section of liver from a case of actinomycosis in man (Crookshank).

inoculation with infectious pus, but there is some reason to believe that the organism occurs in nature as a saprophyte, or as an epiphyte upon the hulls of certain grains, especially barley. Woodhead has recorded a case where a primary mediastinal actinomycosis in the human subject was apparently traced to perforation of the posterior pharyngeal wall by a barley spikelet accidentally swallowed by the patient.

Cases of actinomycosis are fortunately somewhat rare in human medicine, and do not always occur in those brought in contact with the lower animals. The fungi may enter the organism through the mouth and pharynx, through the respiratory tract, through the digestive tract, or through wounds.

The invasion has been known to take place at the roots of carious teeth, and is more liable to occur in the lower than in the upper jaw. Israel reported a case in which the primary lesion seemed to occur external to the bone of the lower jaw, as a tumor about the size of a cherry, with an external opening. Two cases of the disease observed by Murphy, of Chicago, began with toothache and swelling of the jaw. A few cases of dermal infection are recorded. Elsching* has seen a case in which calcified actinomyces grains were observed in the tear duct.

When inhaled, the organisms enter the deeper portions of the lung and cause a suppurative broncho-pneumonia with adhesive inflammation of the contiguous pleura. After the formation of the pleuritic adhesions the disease may penetrate the newly formed tissue, extend to the chest-wall, and ultimately form external sinuses; or, it may penetrate the diaphragm and invade the abdominal organs, causing interesting and characteristic lesions in the liver and other large viscera.

Lesions.—The degree of chemotactic influence exerted by the organism seems to depend upon the tissue affected, upon the peculiarity of the animal, and upon the virulence of the organism. When an animal is but slightly susceptible, and especially when the tongue is affected, the disease is characterized by the formation of cicatricial tissue—"wooden tongue." If, on the other hand, the animal be highly susceptible and the jaw-bone affected, suppuration, with the formation of abscesses, osteoporotic cavities, and sinuses, are apt to be noticed. This form of the disease is called "lumpy jaw" in cattle.

Before the nature of the affection was understood it was confounded with diseases of the bones, especially osteosarcoma.

From the tissues primarily affected the disease spreads to the lymphatic glands, and eventually to the lungs. Israel has pointed out that certain cases of human actinomycosis begin in the peribronchial tissues, probably from inhalation of the fungi.

But few cases recover, the disease terminating in death from exhaustion or from complicating pneumonia or other organic lesions.

* "Centralbl. f. Bakt. u. Parasitenk.," XVIII, p. 7.

CHAPTER XXXVI

MYCETOMA, OR MADURA-FOOT

ACTINOMYCES MADURÆ (VINCENT)

General Characteristics.—A non-motile, non-flagellate, sporogenous (?), non-liquefying, non-aërogenic, chromogenic, aërobic and optionally anaërobic, branched, parasitic organism belonging to the higher bacteria, staining by ordinary methods and by Gram's method, and pathogenic for man.

A curious disease of not infrequent occurrence in the Indian province of Scinde and of rare occurrence in other countries is known as mycetoma, Madura-foot, or *pied de Madura*. Although described as peculiar to Scinde, the disease is not limited to that province, but has been met with in Madura, Hissar, Bicanir, Delhi, Bombay, Baratpur, Morocco, Algeria, and in Italy. In America less than a dozen cases of the disease have been placed on record. In India it almost invariably affects natives of the agricultural class, and in nearly all cases is referred by the patient to the prick of a thorn. It usually affects the foot, more rarely the hand, and in one instance was seen by Boyce to affect the shoulder and hip. It is more common in men than in women, individuals between twenty and forty years of age suffering most frequently, though persons of any age may suffer from the disease. It is insidious in onset, no symptoms being observed in what might be called the incubation stage of a couple of weeks' duration, except the formation of a nodular growth which gradually attains the size of a marble. Its deep attachments are indistinct and diffuse. The skin over it becomes purplish, thickened, indurated, and adherent. The ball of the great toe and the pads of the fingers and toes are the points most frequently invaded. The lesions progress very slowly, and in the course of a few months form distinct inflammatory nodes. After a year or two the nodes begin to soften, break down, discharge necrotic and purulent material, occasioning the formation of ulcers and sinuses. The matter discharged from the lesions at this stage of the disease is a thin serum, and contains occasional fine round pink or black bodies, similar to actinomyces "grains," described, when pink, as resembling fish-roe; when black, as resembling gunpowder. It is upon the detection of these particles that the diagnosis rests. According to the color of the bodies found, cases are divided into the *pale* or *ochroid* and *melanoid* varieties.

The progress of the disease causes an enormous enlargement of the affected part. The malady is usually painless.

The micro-organismal nature of the disease was early suspected. In spite of the confusion caused by some who confounded the disease with "guinea-worm," Carter held that it was due to some indigenous fungus as early as 1874. Boyce and Surveyor found that the black particles of the melanoid variety consisted of a large branching septate fungus.

Pale Variety.—Kanthack was the first to prove the identity of the fungus with the well-known actinomyces, but there seems to be considerable doubt about the identity of the species.

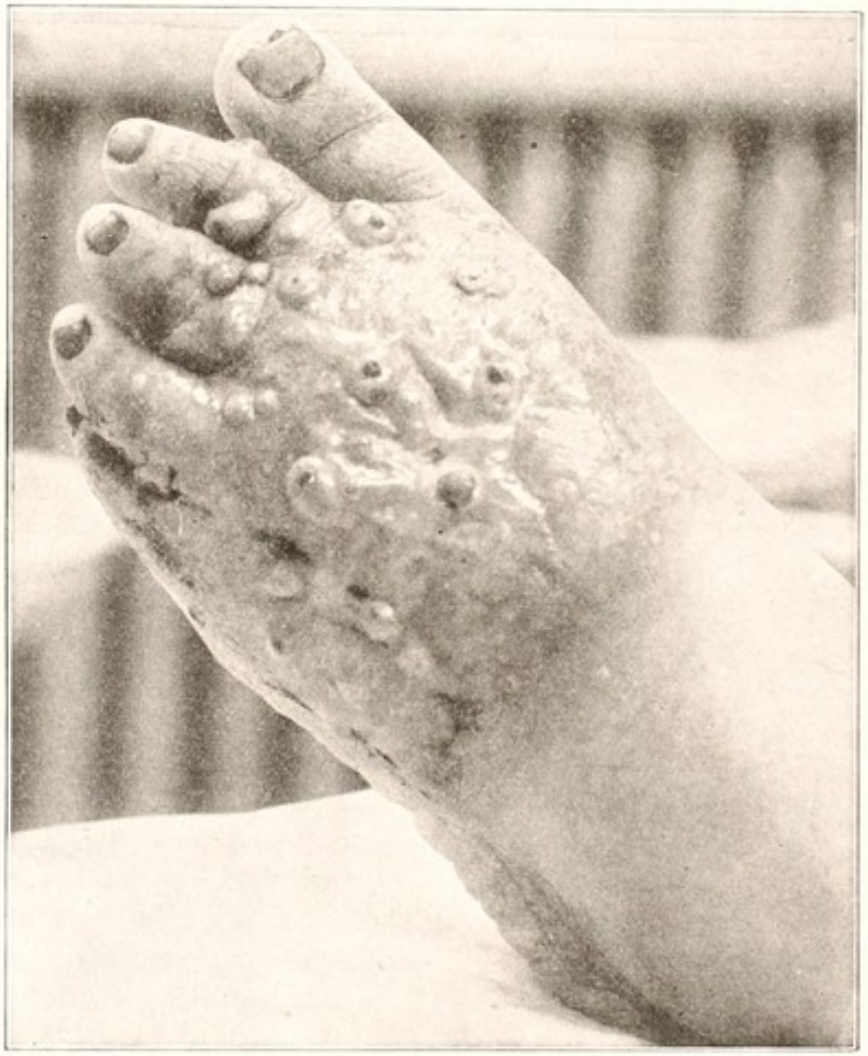


Fig. 305.—Mycetoma. Dorsum of foot showing sinuses, some of which are covered by hard brownish crusts (courtesy of Dr. John W. Perkins).

Morphology.—Under the microscope the organism was found by Vincent* to be branched and belong to the higher bacteria. It consists of long, branched bacillary threads forming a tangled mass. In many of the threads spores could be made out. He was unable to communicate the disease to animals by inoculation.

Cultivation.—Vincent succeeded in isolating the specific micro-organism by puncturing one of the nodes with a sterile pipette, and

* "Ann. de l'Inst. Pasteur," 1894, VIII, 30.

cultivated it upon artificial media, acid vegetable infusions seeming best adapted to its growth. It develops scantily at the room temperature, better at 37°C.—in from four to five days. In twenty to thirty days a colony attains the size of a little pea.

Bouillon.—In bouillon and other liquid media the organisms form little clumps resembling those of actinomyces. They cling to the glass, remain near the surface of the medium, and develop a rose- or bright-red color. Those which sink to the bottom form spheric balls devoid of the color.

Gelatin.—The growth in gelatin is not very abundant, and forms dense, slightly reddish, rounded clumps. Sometimes there is no color. There is no liquefaction.

Agar-agar.—Upon the surface of agar-agar beautiful rounded, glazed colonies are formed. They are at first colorless, but later become rose-colored or bright red. The majority of the clusters remain isolated, some of them attaining the size of a small pea. They are usually umbilicated like a variola pustule, and present a curious appearance when the central part is pale and the periphery red. As the colony ages the red color is lost and it becomes dull white or downy from the formation of aerial hyphæ. The colonies are very adherent to the surface of the medium, and are of almost cartilaginous consistence.

Fig. 306.—Actinomyces maduræ in a section of diseased tissue (Vincent).

Milk.—The organism grows in milk without causing coagulation.

Potato.—Upon potato the growth of the organism is meager and slow, with very little chromogenesis. The color-production is more marked if the potato be acid in reaction. Some of the colonies upon agar-agar and potato have a powdery surface, either from the formation of spores or of aerial hyphæ.

Lesions.—Microscopic study of the diseased tissues in mycetoma is not without interest. The healthy tissue is sharply separated from the diseased areas, which appear like large degenerated tubercles, except that they are extremely vascular. The mycelial or filamentous mass occupies the center of an area of degeneration, where it can be beautifully demonstrated by the use of appropriate stains, Gram's and Weigert's methods being excellent for the purpose. The tissue surrounding the nodes is infiltrated with small

round cells. The youngest nodules consist of granulation-tissue, whose development is checked by early coagulation-necrosis. Giant-cells are few.

Not infrequently small hemorrhages occur from the ulcers and sinuses of the diseased tissues; the hemorrhages can be explained by the abundance of small blood-vessels in the diseased tissue.

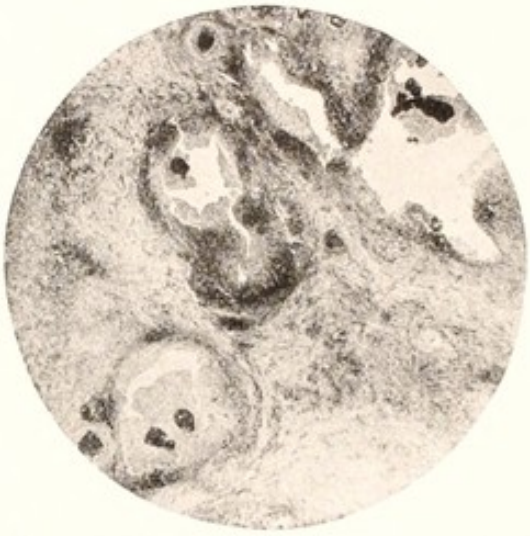


Fig. 307.—Melanoid form of mycetoma. Section showing black granules and general features of the lesions as they appear under a low-magnifying power. Zeiss a_2 (James H. Wright).

Fig. 308.—Melanoid form of mycetoma, showing structure and appearance of the hyphæ of the mycelium obtained from the granules. Zeiss apochromat; 4 mm. (James H. Wright).

The Melanoid Form of mycetoma has been carefully investigated by Wright* and appears to depend upon an entirely different micro-organism properly classed among the hyphomycetes. It is probably identical with the organism described by Boyce and Surveyor.

In the case studied, Wright found the diseased tissues, consisting

* "Journal of Experimental Medicine," 1898, vol. III, p. 421.

of several of the pads of the toes, to be either translucent and myxomatous or yellowish and necrotic in appearance. The black granules were embedded in the tissue and appeared mulberry-like and less than 1 mm. in diameter. They were firm, and when enucleated and pressed between cover and slide did not crush. Only after digestion with a solution of caustic potash and careful teasing could the

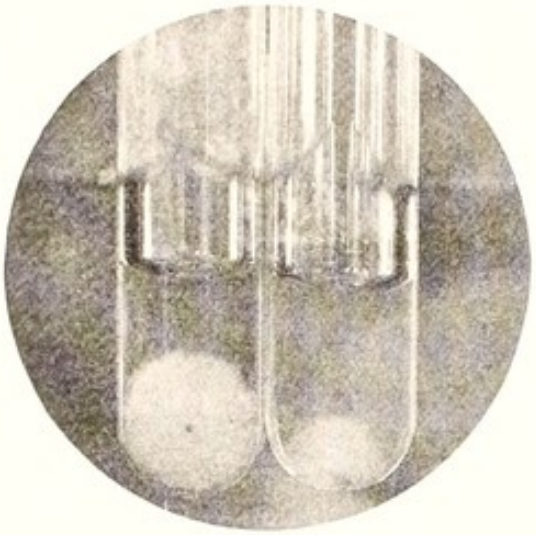


Fig. 309.—Melanoid form of mycetoma. Two bouillon cultures showing the powder-puff ball appearance. In one the black granule is seen in the center of the growth (James H. Wright).

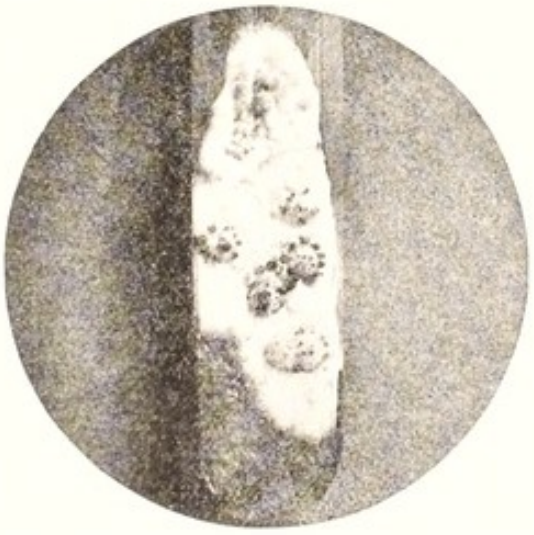


Fig. 310.—Melanoid form of mycetoma. Potato culture of the hyphomycete obtained from the granules. The black globules are composed of a dark brown fluid (James H. Wright).

granules be resolved into the hyphæ of the mold. The central part of the granule formed a reticulum, with radiating, somewhat clavate elements projecting from it.

In sections of tissue it was found possible to stain the fungus with Gram's and Weigert's stains, though prolonged washing removed most of the dye.

Cultural Characteristics.—Enucleated granules carefully washed in sterile bouillon and then planted upon agar-agar afforded cultures of the mold in 25 out of 65 attempts.

The growth began in five or six days, appearing on solid media as a tuft of delicate whitish filaments, springing from the black grain, and in a few days covering the entire surface of the medium with a whitish or pale brown felt-work. Upon potato this felt-work supports drops of brownish fluid. The long branched hyphæ thus formed were from 3 to 8 μ in diameter, with transverse septa in the younger ones. The older hyphæ were swollen at the ends. No buds were observed. No fruit organs were detected. In fluid media the filaments radiated from the central grain with the formation of a kind of puff-ball. Eventually the whole medium becomes filled with mycelia and a definite surface growth forms.

The general characteristics of the fungus are well shown in the accompanying illustrations from Wright's paper.

CHAPTER XXXVII

BLASTOMYCOSIS

Blastomyces Dermatitidis (Gilchrist and Stokes)

The first case in which yeasts or blastomycetes were definitely connected with disease seems to have been published by Busse.* He observed a case of tibial abscess in a woman thirty-one years of age, who died about a year after coming under observation. Post-mortem examination showed numbers of broken-down nodular formations upon the bones, and in the spleen, kidneys, and lungs. In all of these lesions he found, and from them he cultivated, an yeast, which, when introduced in pure culture into animals—mice and rats—proved infective for them. He called the organism Saccharomyces hominis, and the affection in which it was found "Saccharomycosis hominis."

In May, 1904, three months before the appearance of Busse's paper, Gilchrist exhibited to the American Dermatological Association in Washington, microscopic sections from a case of cutaneous disease, in which peculiar bodies, recognized as plant forms, were found. After the appearance of Busse's papers, Gilchrist† more fully described and illustrated his findings, calling the lesions "blastomycetic dermatitis." Though much work upon pathogenic blastomycetes has been published and pathogenic forms of these micro-organisms have been described by Sanfelice,‡ Rabinowitsch,§ and others, the chief and almost the sole form in which these infections make their appearance is a dermal infection known as "blastomycetic dermatitis."

The infection usually begins with the formation of a papule upon the face or one of the extremities, which suppurates and evacuates minute quantities of viscid pus. The lesion crusts and begins to heal, but at the periphery new and usually minute foci of suppuration occur, so that while the original lesion tends to heal very slowly, with much cicatricial formation, it is always spreading. The progress is usually slow, and Gilchrist's first case spread only 2 inches in four years.

Though the progress is slow, it is sure, and there is no tendency to spontaneous recovery in most cases, nor is the condition modified by treatment. The patients may die from intercurrent disease or

* "Centralbl. f. Bakt. u. Parasitenk.," 1894, XVI, 175.
† "Johns Hopkins Hospital Reports," 1, 269, 291.
‡ "Centralbl. f. Bakt. u. Parasitenk.," 1895, XVII, 113, 625; XVIII, 521; XX, 219
§ "Zeitschrift für Hygiene," etc., 1896, XXI, 11.

from a generalization of the blastomycetic infection, which not infrequently happens.

After the work of Gilchrist had made clear the symptomatology and parasitology of the disease, a number of other cases were reported, and Ricketts* published an excellent and lengthy summary of all the cases with references to all of the literature up to that date. Another very interesting paper by Montgomery,† published in 1902, contains a splendid atlas of photographs of the various lesions and of the cultures.

In addition to the cutaneous blastomycosis, a second form is also occasionally seen, and is known as *Coccidioidal granuloma*. It seems to have been first observed by Posadas and Wernicke‡ and has been carefully studied by Ophüls.§ In this form of the disease the lesions are in the internal organs, macroscopically and

Fig. 311.—Cutaneous blastomycosis (Montgomery).

microscopically resemble tubercles, and can only be differentiated from them by the presence of the blastomyces and the absence of tubercle bacilli. The lungs may be affected, and Walker and Montgomery‖ mistook a case for miliary tuberculosis of the lungs. According to Evans** the disease seems to have a predilection for the central nervous system.

There seems to be little reason for believing that there is any other difference than that of distribution between the blastomycetic dermatitis and the blastomycetic granuloma, or that they are caused by different micro-organisms. Regarding the organisms, however, we are by no means sure that there are not several species.

* "Jour. Med. Research," 1901, I, 373.
† "Jour. Amer. Med. Assoc.," June 7, 1902, I, 1486.
‡ "Jour. de Micro-organismen," 1891, XV, 14.
§ "Jour. Experimental Medicine," 1905, VI, 443. Ophüls and Moffit, "Phila. Med. Jour.," 1900, V, 1471.
‖ "Jour. Amer. Med. Assoc.," 1902, XXXVIII, 867.
** "Jour. of Infectious Diseases," 1909, VI, 535.

Specific Organism.—The organism presents a variety of appearances which may be thus brought together: First, there are round and elliptical disk-like bodies that some regard as spores, others as the primitive or yeast form. These measure 10 to 30 μ in greatest diameter, are distinctly doubly contoured, highly refracting, and, though sometimes clear and transparent, are frequently granular and vacuolated. From them buds may grow, as in the yeasts, or hypha may form, as in oïdium. In artificial cultivations the hypha may form a tangled mycelium.

Staining.—The organisms are usually better found without staining. They do not stain with aqueous anilin dyes, but are penetrated by warm thionin, alkaline methylene-blue, and polychrome methylene-blue. In sections of tissue stained with hematoxylon

Fig. 312.—Giant cell from a cutaneous lesion in blastomycosis, showing a group of blastomyces (Montgomery).

and eosin they show as uncolored circles; with thionin and alkaline methylene-blue they may take a blue color.

Cultivation.—The organism grows readily upon artificial media when once started, but the primitive culture is difficult to secure, because the cocci and other associated organisms are more numerous than the blastomyces and outgrow it. It seems most satisfactory to first infect a guinea-pig with the organism from the skin, and then start the cultivation from its lesions than to attempt it directly from the pus from human dermal lesions. When the human lesions are internal, pure cultures are easily started.

Gilchrist and Stokes* were able to start cultures directly from the dermal lesions. Hiss and Zinsser recommended that this be done

* "Journal of Experimental Medicine," 1898, III, 53.

by greatly diluting the culture material, so as to separate the contained organisms widely.

Many culture-media prove appropriate, glycerin agar-agar and agar-agar containing 1 per cent. of dextrose being excellent. When once isolated the organism is easily kept growing by transplanting every month or two.

The **colonies** appear in a few days as small round hemispheric dots with numerous prickles about the surfaces. Later they have a moldy appearance from the development of aërial hypha. They are almost purely aërobic, those on the surface growing well, those deeply seated in the medium scarcely at all.

Agar-agar Slants.—These at first show a creamy white layer that becomes quite thick, and is moldy and fluffy on the surface. After

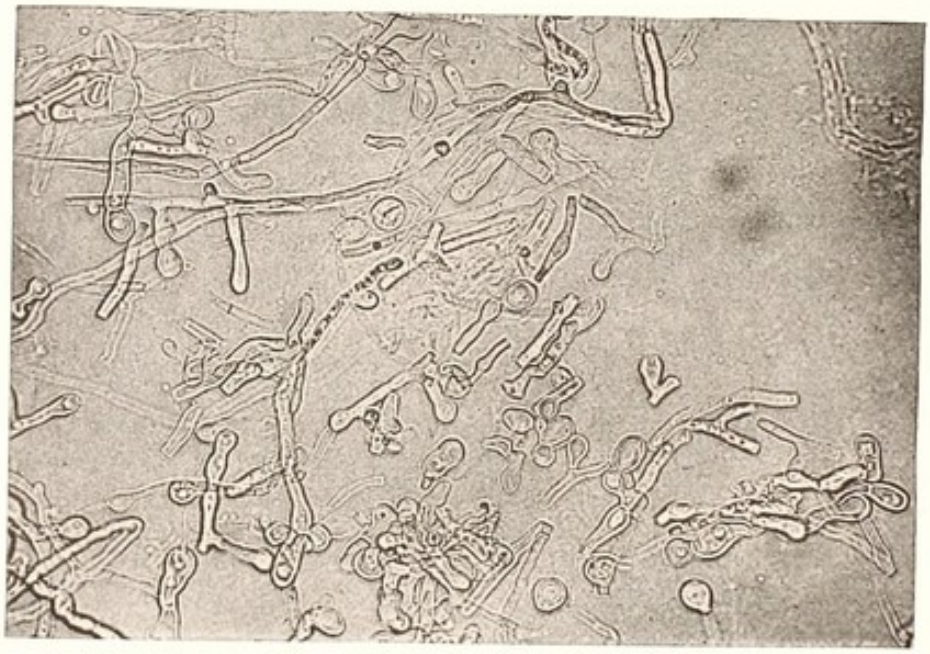


Fig. 313.—Blastomyces dermatitidis. Budding forms and mycelial growths from glucose agar (Irons and Graham, in "Journal of Infectious Diseases").

a few weeks the agar-agar begins to turn yellow and later may become brown, though the growth itself remains white and unchanged. The growth is firmly attached to the agar. When old, the growth wrinkles.

Bouillon.—The growth is not luxuriant. The medium is not clouded and contains fluffy flocculi of stringy viscid material. Sugars added to the medium may be fermented.

Gelatin.—Growth takes place with aërial hypha. Liquefaction does not occur or is very slow.

Potato.—Abundant growth with aërial hypha.

Milk.—Not coagulated, not acidified, slowly digested.

There is some difficulty in describing the cultures, as different authors describe them quite differently, evidently having different organisms or different strains under observation.

Pathogenesis.—The organisms are pathogenic for guinea-pigs, rabbits, and dogs, in which an abscess, not infrequently followed by a generalized infection, takes place.

Lesions.—The human lesions vary somewhat. Gilchrist's first case resembled lupus vulgaris, other cases present an exaggeration of the ulcerative element. Cases have also been mistaken for

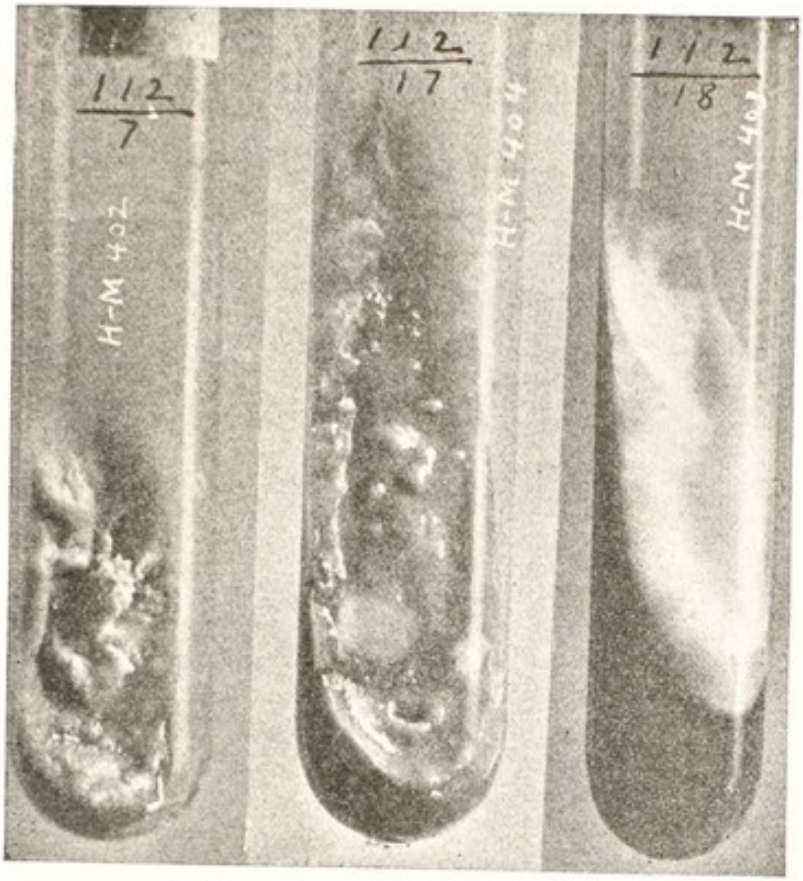


Fig. 314.—Cultures of Blastomyces dermatitidis upon solid culture-media (Montgomery).

syphilis. The intractable character of the lesions is suggestive, and the finding of the micro-organisms in the viscid pus is pathognomonic.

Upon section the lesions still resemble lupus and other tuberculous lesions, but here again the absence of tubercle bacilli and the presence of the blastomyces enable diagnosis to be made.

Transmission.—The disease is transmissible. The source of infection is not known.

CHAPTER XXXVIII

RINGWORM

Trichophyton Tonsurans (Malmsten)

Tinea trichophytina, ringworm of the scalp, herpes tonsurans, tinea circinata, ringworm of the body, herpes circinatus, tinea unguium, onychomycosis, tinea imbricata, herpes desquamans, tinea versicolor, pityriasis versicolor, erythrasma, etc., are diseases with well-marked clinical manifestations and differences, all of which may be comprehended under the general term dermatomycosis, and are caused by closely related forms of parasitic fungi, whose generic and specific differences are matters of considerable uncertainty.

That certain of the diseases affect hairy parts and others hairless parts of the body, that still others occur about the nails, and that some are superficial and practically saprophytic, while others penetrate more deeply and are undoubtedly parasitic, do not necessarily point any more conclusively to essential differences in the infecting organisms than to accidents of infection and variations in resisting power. A review of the literature leaves the student with a deplorable confusion of ideas, and a feeling that the synonomy is too complicated and the use of terms too loose to permit of systematic reconstruction.

The discovery of micro-organisms in these lesions seems to have been made in 1842 by Gruby,* who found mycelial threads and spores on and in the hairs, and in 1860 by Hebra,† between the epithelial cells. The organism appears to have been called Trichophyton tonsurans in 1845 by Malmsten. The parasitology of all of the trichophyton infections was thoroughly studied by Sabouraud,‡ and the old species, Trichophyton tonsurans, divided into eleven new species, to which four others have since been added, so that there are now described, with or without justification, Trichophyton crateriforme, T. acuminatum, T. violaceum, T. effractum, T. fulmatum, T. umbilicatum, T. regulare, T. pilosum, T. glabrum, T. sulphureum, T. polygonum, T. exsiccatum, T. circonvulatum, T. flavum, and T. plicatili.

In general it is customary to divide the organisms into two groups, Trichophyton microsporon and T. megalosporon, the former having large, the latter small, spores.

* "Compt.-rendu," Paris, 1842, xv.

† "Handbuch der spezulien Path. u. Therapie von Virchow," III, 1860.

‡ "Ann. de dermat. et de syphilis," 1892, III; 1893, IV; 1894, V; "Monatshefte," 1896, 576; "La Practique dermatologique. Trichophytie," 1900.

Morphology.—The trichophyton parasites form delicate mycelia composed of somewhat slender septate hypha. They can best be observed by extracting one of the hairs, including its root, from the diseased area, or if the affection be upon a hairless part of the body, by scraping off some of the epiderm, and mounting the material between a slide and cover in a drop of caustic potash solution (20 per cent.). Under these circumstances the spores are conspicuous and so numerous as to give the impression that they occur in rows in a kind of structureless zoöglea upon the outside of the hair. In some cases, however, especially in Trichophyton megalosporon, the hypha may be observed with the spores inside. The hypha measure from 2 to 8 μ in diameter, are usually simple, and rarely divide. The spores are from 2 to 3 μ in diameter in the Trichophyton microsporon and 7 to 8 μ in T. megalosporon. The former is the more common upon the hairless, the latter upon the hairy, portions of the skin.

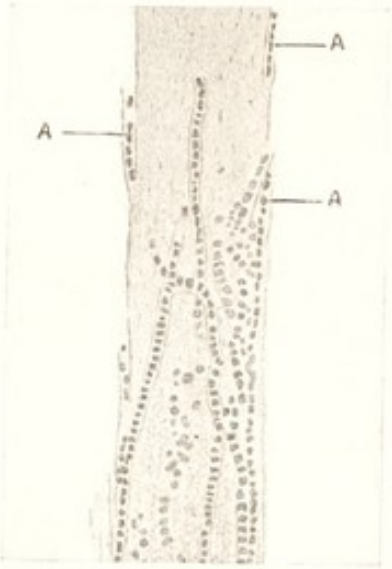


Fig. 315.—Invasion of a human hair by trichophyton: A, Points at which the parasitic fungi coming from the epidermis are elevating the cuticle of the hair and entering into its substance. Magnified 200 diameters (Sabouraud).

Cultivation.—The organisms may be secured in pure culture without much difficulty, except for the annoying and almost constant presence of the associated bacteria of the skin. By crushing the hairs and scales in a mortar with some dilute KOH solution, and then, after thoroughly distributing the spores through the alkaline medium which dissolves many of the bacteria, plates can be made with high dilutions, or drops of the fluid may be spread over potato, which is an excellent medium for the culture.

The culture, whether upon agar-agar, glycerin agar-agar, glucose agar-agar, gelatin, or potato, occurs in the form of a tuft of white mycelial filaments with aërial hypha, looking like a tiny white powder-puff. Upon the surface of liquid culture-media the growth appears as a thick wrinkled pellicle with aërial hypha of velvety appearance. As the cultures grow older the lower mycelial growth becomes yellowish and wrinkled, but the aërial hypha maintain the velvety white appearance. Some of the colonies are mammillated, some are crateriform. Gelatin is liquefied, the growth floating upon the surface of the fluid. As the cultures become very old and dry, the velvety appearance is lost and the surface becomes powdery. The powder detaches only when the growth is touched, and does not shake off.

Pathogenesis.—The trichophytons are pathogenic for man and for the lower animals. They spread from animal to animal by contact and by inoculation. Men, dogs, cats, horses, sheep, goats, and swine all suffer from the infection. The growth of the hypha between the epidermal layers causes a chronic inflammation, with hyperemia, desquamation, the formation of some papules, and oc-

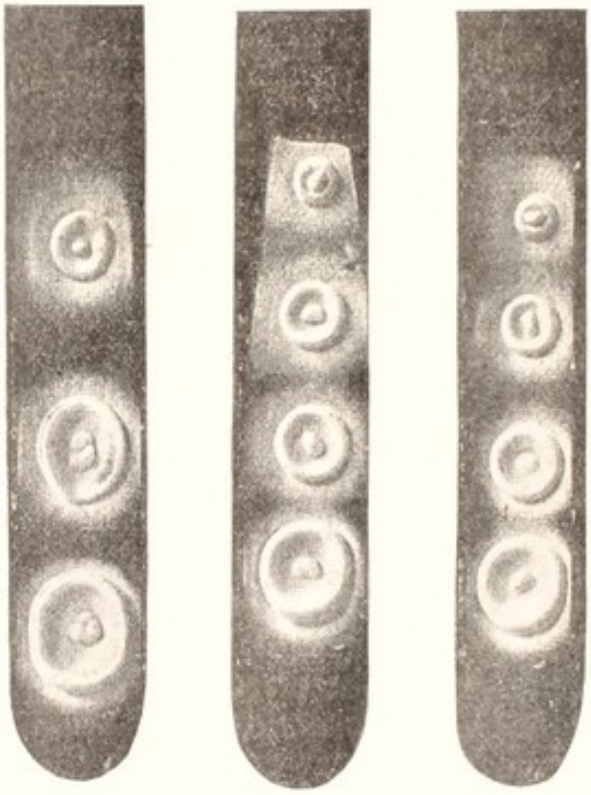


Fig. 316.—Trichophyton tonsurans. Primary cultures twenty days old on maltose agar-agar. Natural size (Sabouraud).

casional pustules. The invasion of the hair-follicles and the growth of the fungi into the hairs cause them to become fragile and break off, as well as to loosen and drop out.

The name "barber's itch" results from the frequent transmission of the infection by the barber's razors. The disease is easily transmissible and precautions should always be taken to prevent its dissemination.

CHAPTER XXXIX

FAVUS

Achorion Schönleinii (Remak)

Favus, or tinea favosa, is a chronic and destructive form of dermatomycosis occurring in man and animals, caused by a fungus discovered in 1839 by Schönlein,* and called in his honor Achorion schönleinii by Remak in 1845. This fungus is widely distributed and affects mice, cats, dogs, rabbits, fowls, and men. Among human beings it usually occurs upon the scalp and other hairy parts of the body, though it may also affect the hairless portions and even attack the roots of the nails. It is more frequent in children than in adults. The fungus grows vigorously and usually forms a small sulphur yellow disk about the base of a hair. The edges of this detach, become everted, and the whole eventually separates, forming the "scutulum," or characteristic lesion of the disease. The reaction is more marked, the damage done greater, and the disease less tractable than in other forms of dermatomycosis.

The infection seems to take place in most cases by way of the hair-follicles, and the mycelia of the fungi grow into and about the hairs, invading the epiderm, and causing atrophy of the hair-follicles by pressure. Beneath and around the scutulum, which consists chiefly of the fungi, an inflammatory reaction takes place, and leukocytic invasion and ulceration cause the scutulum to separate.

Although usually confined to the skin, the favus infection may extend to the mucous membranes, and Kaposi and Kundrat† have reported a case in which favus fungi were found to have invaded the stomach and intestines.

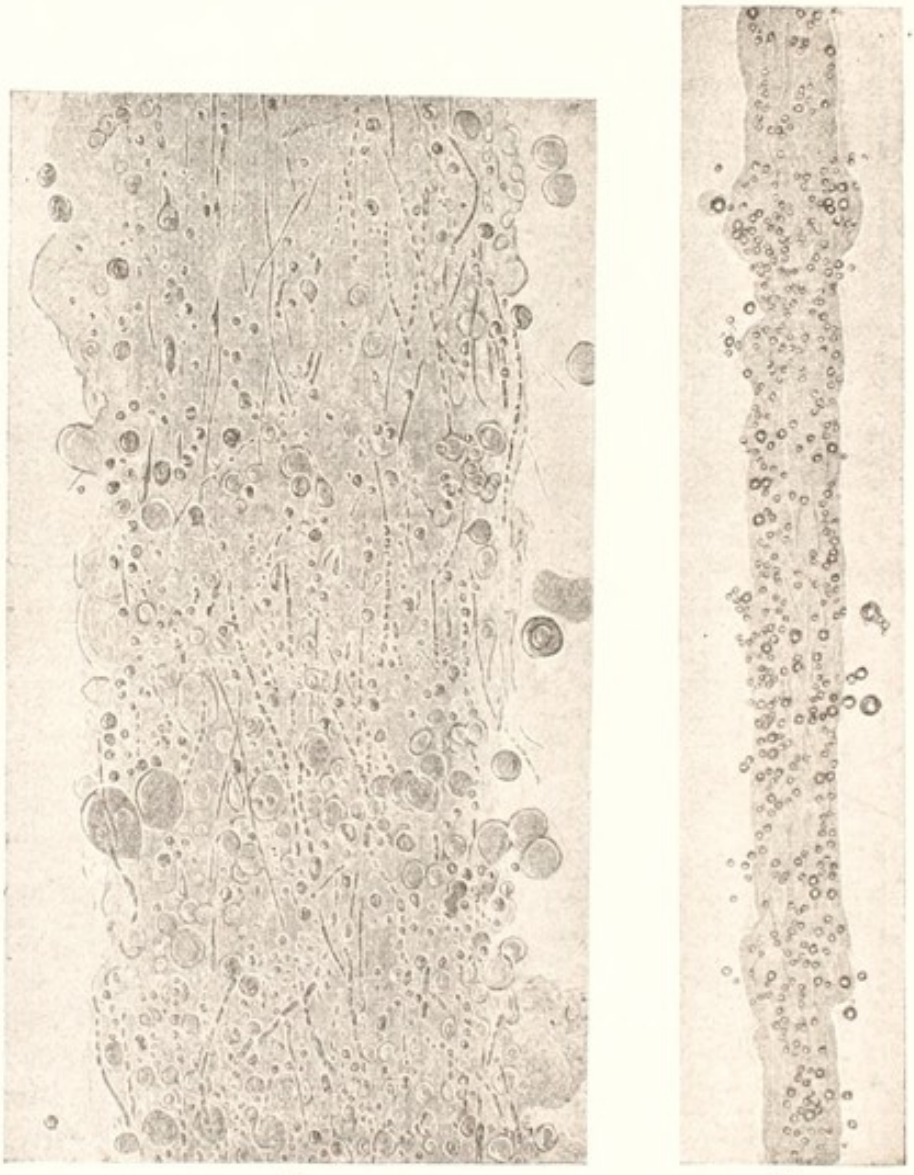
The disease runs a course sometimes extending over many years. Crocker‡ mentions a case that recovered after thirteen years. It may remain localized upon the scalp or may spread itself over much of the skin surface. When the lesions are large they give off an odor suggesting that peculiar to white mice. In recovering, the lesions leave considerable cicatricial scarring, and atrophy of hair-follicles, sweat, and sebaceous glands is inevitable.

The Specific Organism.—The Achorion schönleinii is probably better regarded as a group of closely related organisms than as a single one. Indeed, Quincke has described three species, though they are not yet generally accepted.

* Müller's "Archiv," 1839.
† "Ann. de Dermat. et de Syph.," 1895, p. 104.
‡ "Diseases of the Skin," Phila., 1903, p. 1276.

The organism can be studied by extracting a hair and examining it in KOH or NaOH solution (20 per cent.), or by teasing a scutulum in the same medium and examining with a low power. Sections of the skin may also be made when possible.

The fungus resolves itself into mycelial threads, and spores. The



A B

Fig. 317.—Favus. Hairs of a child infected with Achorion schönleinii. A, Magnified 260 diameters; B, 75 diameters. The large rounded bodies are droplets of air which always appear after treatment with 40 per cent. potash solution. The linear threads are the fungi. Some are without spores, others contain rows of spores (Sabouraud).

scutulum consists of masses of spores at the center and about the hair, with mycelia containing spores at the edges. From the mycelium hypha are given off, the ends being knobbed or clavate.

The mycelial threads are highly refractile, contain granular protoplasm, and are of varying thickness. Sometimes the terminal

hypha are simple, sometimes they fork, the ends are always clavate. The hypha give off buds at right angles along their course.

The spores are oval, doubly contoured, as a rule, but may be round or pointed and more or less polyhedral. They measure 3 to 8 μ in length and 3 to 4 μ in breadth. They form the great central mass of the scutulum, which is the oldest part. Together with them one finds a number of detritus granules, fat-droplets, and occasional swollen epidermal cells.

Cultivation.—The cultivation of the achorion is quite easy if care be used, for the central part of each scutulum contains pure cultures of the organism. The best method is probably that of Kral,* which is as follows: "A good deal of the material from the scutula is rubbed up in a porcelain mortar dish with previously heated diatomaceous earth, with a porcelain pestle, without exerting too much pressure. Melted agar-agar tubes are then inoculated with

Fig. 318.—Achorion schönleinii. Four weeks old culture upon beerwort agar-agar (Kolle and Wassermann).

Fig. 319.—Achorion schönleinii. Pure culture, four weeks old, on beerwort agar-agar (Kolle and Wassermann).

two or three loopfuls of the crushed material and poured into Petri dishes. Greater dilution can be made if desired. The plates are examined after forty-eight hours.

Cultures may, however, be directly made with material from the center of a scutulum. Agar-agar should be used, as the cultures grow best at the body temperature. The young colonies that appear in forty-eight hours can easily be transplanted by fishing under a lens.

The best medium was found by Sabouraud to consist of maltose, 4; peptone, 2; fucus crispi, 1.5; water, 100.

As the colonies eventually become quite large it is recommended that, instead of tubes, they be made in Erlenmeyer flasks, the transplanted little colonies being placed at the center of the medium congealed upon the bottom of the flask.

The appearance of the cultures varies considerably. Plaut gives

* See Plaut, in Kolle and Wassermann's "Pathogene Mikroörganismen," 1, p. 608.

two principal varieties: (1) The waxy type—a yellowish mass of a waxy character with radiating folds and a central elevation. As a rule no aërial hyphæ, but occasionally short aërial hypha.

(2) The downy type—this forms a white disk with a velvety or plush-like covering of white aërial hypha. Sometimes instead of white the color is yellowish or reddish. A marked dimple with a smaller elevation usually occurs in the middle, and there may be radial folds.

Pathogenesis.—The micro-organism is pathogenic for mice, rabbits, cats, dogs, hens, and men, in all of whom typical scutula form. Scutulum formation has not been observed in guinea-pigs. The disease readily spreads from animal to animal by direct contact and by indirect contact by the use of combs, hair-brushes, and similar objects. On account of its chronicity, its obstinacy, its disfigurement, and its transmissibility it is a dangerous disease, and one that requires prompt isolation of the patient and the utmost care for the prevention of contagion.

CHAPTER XL

SPOROTRICHOSIS

Sporotrichosis is a somewhat rare disease of man, caused by various members of a genus of fungi known as *Sporotrichum* (Link-Saccardo). The first occurrence of human sporotrichosis seems to have been reported by B. R. Schenck.* The isolated micro-organism in this case was carefully studied and later was found to be identical with a micro-organism isolated from another case of somewhat similar character studied by Hektoen and Perkins,† who described it as Sporotrichum schenckii. In 1903 de Beurmann‡ and his associates took up the subject in France, and Lutz and Splendore§ in Brazil, and new cases were reported. On Aug. 8, 1908, the writer of an editorial in the Journal of the American Medical Association was able to give references to 14 cases of the disease. In 1912 Ruediger‖ was able to collect 57 cases that had occurred in the United States. In 1912 de Beurmann** reported that more than 200 cases had been put on record since the beginning of his work in 1903. It will thus be seen that the recognition of the cause of the disease and the improvement in diagnosis that followed it have made possible the detection of many cases of a disease not recognized until 1900.

According to de Beurmann who has shown great interest in the affection and prosecuted its study with much industry, the known organisms of the Sporotrichum group comprise the following:

Sporotrichum schencki.
Sporotrichum beurmanni.
Sporotrichum beurmanni var. asteroides (Splendore).
Sporotrichum beurmanni var. indicum (Castellani).
Sporotrichum jeanselmei.
Sporotrichum gougerati.

Specific Organism.—The sporotrichum is characterized by a filamentous spore-bearing mycelium. The filaments are fine, measuring about 2 μ in diameter, partitioned, colorless, much branched and tangled. The chief feature is the occurrence of the spores which are situated along the length of the recumbent filaments either on

* "Bulletin of the Johns Hopkins Hospital," Dec., 1898.
† "Journal of Experimental Medicine," 1900, 1901, V, 77.
‡ Ann. de Dermatologie et Syphilographie, 1906, 538.
§ "Centralbl. f. Bakt., etc.," 1907, XLV, Orig., 632.
‖ "Jour. of Infectious Diseases," 1912, XI, 193.
** "British Medical Journal," Aug. 10, 1912, II, 2900.

their extremities or on branches. They are arranged in cylindrical cuffs about 10 μ in size and in glomeruli. As a matter of fact the spores are readily isolated from one another. They arise one by one in variable numbers along the mycelium, but as a rule in very large quantity in each segment of the thallus. There is no apparent order in their arrangement. So long as it remains on the filament the spore appears pear-shaped. It is attached by a very fine sterigma, from 1–2 μ in length and from 0.5 μ in width. When shed, the spore is oval. Its dimensions vary from 3–5–6 μ in length and from 2–3–4 μ in breadth. The form, the distribution and the brown color of the spores and their fructification in the form of cylindrical cuffs, arranged in branches at the extremities of the filaments, constitute together

Fig. 320.—Sporothrix schenckii. Margin of living hanging-drop culture (gelatin) × about 150 (Hektoen and Perkins in "Jour. of Exper. Med.").

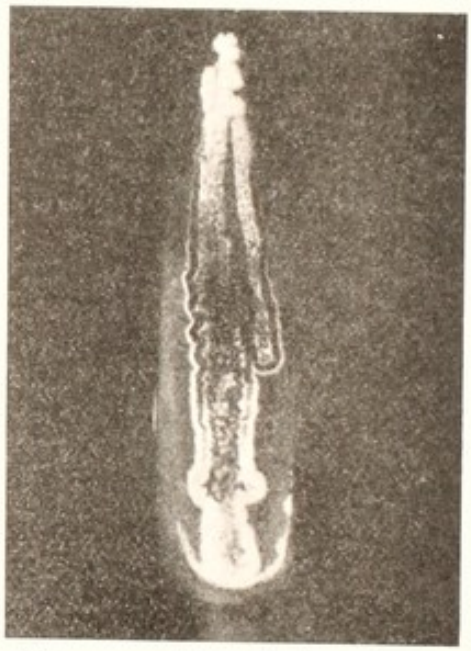


Fig. 321.—Sporothrix schenckii. Slant culture on glucose agar, eight days old (Hektoen and Perkins, in "Jour. of Exper. Med.").

with the original substratum of the fungus, a group of characters which differentiates Sporotrichum beurmanni sharply from all other sporotrichs (Matruchat).

Hektoen and Perkins thus describe Sporotrichum schenckii: The threads of the mycelium are seen to be doubly contoured; the protoplasm is somewhat granular and interrupted at fairly regular intervals by transverse septa; the diameter of the threads varies somewhat, the average being about 2 μ; the branches are not frequent and do not bear any fixed relations to the septa. In the hanging-drop cultures the relations of the conidia to the mycelium are very nicely shown. The spore-bearing branches which grow out in a radiating manner from the central feltwork, are commonly tipped by a cluster

of from three to six or more conidia, which, in the case of the larger cluster, are attached by the smaller end to the slightly expanded extremity of the branch. Similar ovate buds also arise from the sides of the hyphæ at shorter or longer intervals. The spores are also doubly contoured and granular, resembling very much yeast cells. These various features are well shown in the photographs on the accompanying plate. The attachment, by means of the short pedicles of the spores to the threads, is very easily severed as shown by the difficulty in obtaining stained preparations with the spores *in situ*. When placed in the hanging drop, the conidia grow out into one or more straight germ tubes which spring from either or both ends or from the side. These embryonal threads again give rise to lateral or terminal buds, which in all particulars resembles the spores and some of which form branching spore-producing threads, so that in the early stages very peculiar-looking bodies are produced.

In the tissues and in the pus from the lesions of the disease the parasites have quite a different appearance, assuming a short oblong form like a thick short bacillus 3–5 μ in length and 2–3 μ broad, basophilic, finely granular and surrounded by a very delicate, colorless membrane. de Beurmann has watched the growth of this degraded form of the parasite into the filamentous and spore-bearing form, in artificial culture.

Fig. 322.—Abscesses caused by sporothrix schenckii. Arm of patient showing ulcers and scars, at a late stage of the lesions (Hektoen and Perkins, in "Jour. of Exper. Med.").

Staining.—The micro-organism is much better examined in the fresh and living condition than dried and stained as it greatly changes in appearance through shrinking. It does stain, however, with the usual dyes, and retains Gram's stain except when the alcohol washing is unduly prolonged.

Cultivation. Colonies.—Upon agar-agar, at the end of about forty-eight hours, the colonies appear elevated, whitish, with feathery fringes and some filamentous downgrowths into the medium. Upon gelatin the downward growth results in liquefaction and the growing colonies sink below the surface.

Agar-agar.—Along the needle track made by a stroke culture, a grayish granular slightly elevated line with feathery edges forms in forty-eight hours and in seventy-two hours assumes the form of a

band with numerous transverse wrinkles; in a couple of days more "the surface becomes more markedly corrugated and looks like a chain of mountains on a map." About the seventh day, the growth, which has increased in thickness, becomes light brownish in color, the margins being smooth and wavy and marked by shallow transverse grooves. Still later the growth becomes dark brown, wrinkled and covered by a delicate fuzz. The agar-agar becomes brown.

Gelatin.—In gelatin punctures the growth is confined to the upper strata. Lateral branches are sent out from the needle track. A surface felt-like mass of mycelial threads forms beneath which the gelatin liquefies. The surface growth sinks into the liquid medium.

Blood-serum.—The growth is somewhat like that on agar-agar but not so massive. It is apt to be covered by a white down.

Bouillon.—The growth which is fairly abundant, is in flakes and tufts, shreds and filaments that settle to the bottom or cling to the sides. A white surface film is apt to cover the liquid. No fermentation occurs in sugar bouillon.

Potato.—Upon potato tufts form in twenty-four hours. These have a brownish-gray color and soon become raised, wrinkled, and frosted. The potato is darkened.

Milk.—The growth is scanty and owing to the opacity of the medium, difficult to see. Litmus milk is not acidified. There is no coagulation.

Vital Resistance.—The optimum temperature is about 37°C. The organism grows slowly at room temperature but in the end attains pretty much the same magnitude as those kept in the thermostat. The death point is 55°C. for one hour. Hektoen and Perkins found S. schenckii killed in four and one-half minutes at 60°C.

Metabolic Products.—The organism produces no curdling or proteolytic ferments for milk or blood-serum. It does, however, liquefy gelatin. It grows aërobically or anaërobically, but under the latter conditions it does not produce acid or ferment sugars, or evolve gas. No indol is formed. It has a remarkable tolerance for acid media. Page, Frothingham and Paige* found that it grew well in media at least six times as acid as those ordinarily employed for bacteria. They also found that the organism does produce acid in media containing dextrose.

Distribution in Nature.—According to de Beurmann, the sporotrichum is a widely distributed micro-organism in nature. It has been found on green vegetables, upon bark, thorns, potatoes, various implements, in the soil, and in infected insects.

Pathogenesis.—The sporotrichum is pathogenic for men, horses, rats, dogs, and white mice.

It would seem as though the rarity of its occurrence as a pathogenic agent signified that it was by no means easy for it to effect the

* "Jour. Med. Research," 1910, XXIII, p. 129.

invasion of the animal body. However, de Beurmann mentions a man wounded in the forehead by a coster's awl whom he believed to have been infected by a cap, used to conceal the untreated wound, that usually lay on the fruit and vegetables that filled his barrow; a market woman infected by the salad that she was in the habit of handling all day. Dominici and Duval report a case following a cut inflicted while peeling a potato; Saint-Girons, a case following the prick of a thorn of a barberry bush. A patient of Lutz's was inoculated through the bite of a cat; one of Wyse-Lauzun's through the bite of a parrot. Perkins' case was that of a child that had abraded a finger with a hammer. de Beurmann found the organism in the pharynx of healthy persons "carriers," whose saliva might, therefore, be infectious. He believes that infection may take place through the hair-follicles; that the healthy skin may be penetrated, and that the healthy gastro-intestinal mucosa may be penetrated.

Lesions.—The seat of primary disturbance is the seat of a chronic and destructive ulceration from which the disease spreads to numerous secondary foci chiefly by lymphatic metastasis. Hektoen and Perkins describe the appearance of the primary lesion in Perkins' case of infection by S. schenckii, thus: "the finger from the first to the third joints is swollen to twice its original size, presenting in the center a deep, well-defined, sharp, undermined ulceration, the size of a ten-cent piece. The base of the ulceration is rough and covered with grayish-looking pus. This, when sponged away, leaves a bright red surface; the ulcer extends through the whole thickness of the skin. Surrounding the ulcer over about one-half of the infiltrated area, are a large number of vesicles and a few pustules. The dorsal surface of the hand and the extensor surface of the forearm present a chain of swollen lymphatics along which are about twenty nodules the size of a small pea to a large hazel nut. . . . This little patient does not complain of much pain." In the course of two months Perkins opened and treated more than twenty abscesses resulting from the enlargement and softening of the nodes.

De Beurmann and Gougerot found that the most characteristic lesion of the skin is a nodule in which three processes are found, sometimes mixed up in an irregular manner, but most frequently arranged concentrically. "In the center an abscess containing polymorphonuclear leukocytes and macrophages; in the intermediate zone an area of degenerated epithelioid giant cells and tuberculous follicles, and at the periphery a proliferation of basophile lymph and connective-tissue cells or a fibro-cellular infiltration." "The structure of the sporotrichoma is, therefore, very closely allied to that of the lesions caused by syphilis, tuberculosis, and by the agents of chronic suppuration, and it resembles sometimes the one, sometimes the other."

De Beurmann and Raymond, 1903, and de Beurmann and Gougerot, 1906, describe three clinical varieties of the disease.

1. **Disseminated Gummatous Sporotrichosis.**—The onset is insidious. An accident usually leads to the discovery of the first gummata. The number of gummata may vary up to 100. The first takes origin from any point in the subcutaneous tissue. Others disseminate themselves over the whole body. Each gumma has an autonomous evolution which is the same for all. At first it is a little rounded mass, hard, elastic, painless and invariably in the subcutaneous tissue. The mass evolves rapidly in the direction of softening and in four or six weeks terminates in a characteristic cold abscess. When it undergoes liquefaction, it contains a fluid which is at first transparent, viscid, gummy, and with purulent streaks and later becomes opaque, thick and purulent. It does not undergo complete softening, and when it becomes fluctuating we find a central cup-shaped depression surrounded by a firm and resisting zone, and when its contents are evacuated, there remains round the empty pocket a persistent and indurated ring.

2. **Disseminated Subcutaneous, Gummatous Sporotrichosis with Ulceration.**—In this variety, the subcutaneous gummata after having passed through the phases described above, become hypodermo-dermic and destroy the skin by ulceration more or less rapidly, sometimes in twenty days, sometimes in two or three months. As a rule the ulcers are tuberculous in appearance. Frequently the ulceration is at first no more than a narrow fistula from which oozes a viscid, colorless and sometimes reddish pus or a yellowish serous fluid.

3. **Mixed forms** are frequent. When the disease has existed for a long time it presents a complete clinical picture. Side by side are lesions of different age with different tendencies and different appearances; tuberculous looking, syphilitic looking, ecthymous, rupial and furuncular. There may be associated lesions of the lymphatics, and lesions of the dermis, epidermis, mucous membranes, muscles, osseous tissues, synovial membranes, eyes, epididymis, etc.

4. **Localized Sporotrichosis.**—The sporotrichum penetrates by a cutaneous lesion at the site of which it produces an initial lesion, which may be called the "sporotrichotic chancre." Then it gradually invades the lymphatics and a hard lymphatic cord studded with gummata—centripetal gummatous sporotrichosis—makes its appearance. Sometimes the lymph-nodes of the region react, but this is not constant. The disease remains localized to the region primarily affected.

Sporotrichosis of the mucous membranes, of the muscles, of the bones and joints, of the synovial membranes, of the eye, of the epididymis, of the kidney, and of the lung are described by de Beurmann.*

Bacteriologic Diagnosis.—Diagnosis by immediate and direct examination of the pus either stained or unstained is difficult because the parasites are few in number, and are present in the bacillary form that is so difficult to recognize.

The approved method is to carefully cleanse the skin over one of the closed lesions, disinfect it with iodine, and then puncture the abscess with a hollow needle. The pus obtained is spread plentifully over the surface of culture-media in a number of tubes and stood in the incubating oven. The characteristic colonies should appear in from four to twelve days.

Should cultures be on hand in the laboratory at the time a case presents itself for diagnosis, two other methods may be employed.

1. **The Agglutination Test.**—A suspension of the spores from cultures of the sporotrichum will be agglutinated by the patient's serum in dilutions of 1–400 to 1–500 on the average.

2. **The Complement-fixation Test.**—The entire culture is used as an antigen, the serum of the patient and guinea-pig complement employed as usual. As, however, oïdium, actinomyces, discomyces

* "Brit. Med. Jour.," 1912, II, 293.

and other fungi give the same degree of fixation, the method lacks precision.

Bloch has also employed an intra-dermic injection of a sterilized emulsion of the sporotrichum for purposes of diagnosis. In twenty-four hours, patients with sporotrichosis give a marked reaction in the form of an indurated nodule with a broad reddish surrounding areola.

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