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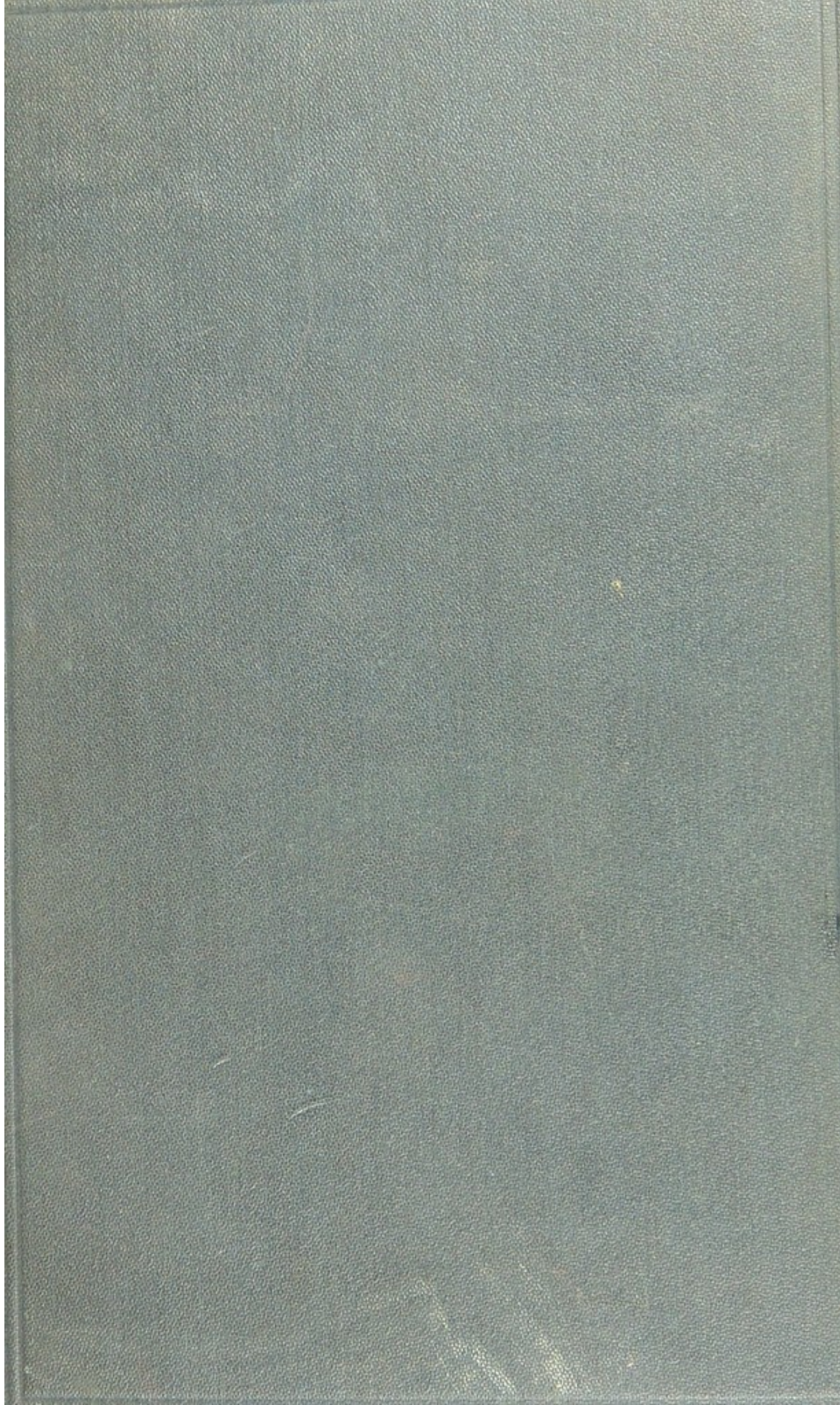
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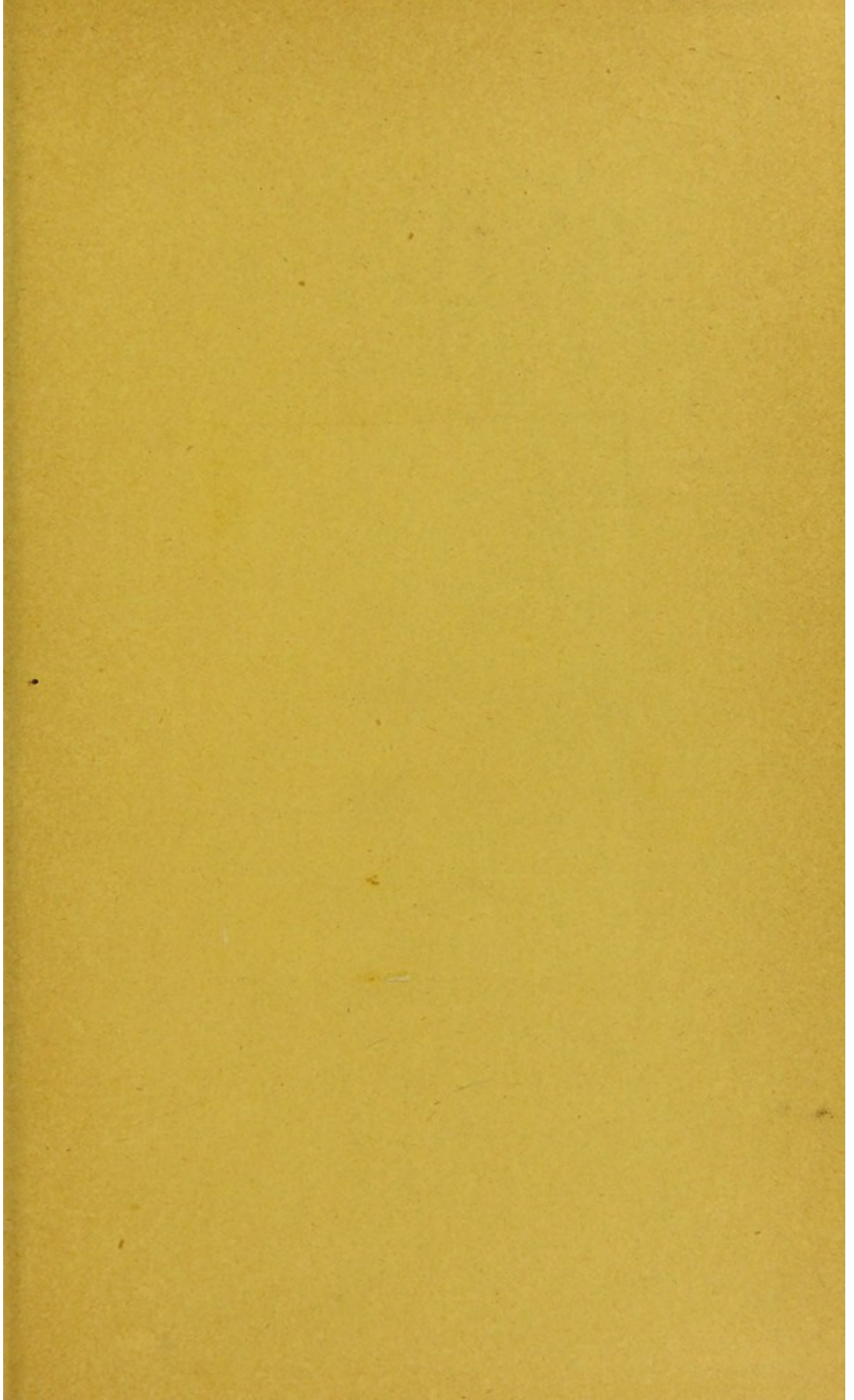
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Culture of glanders bacillus upon cooked potato (Löffler).

A TEXT-BOOK
UPON THE
PATHOGENIC BACTERIA

FOR
STUDENTS OF MEDICINE AND PHYSICIANS

BY
JOSEPH MCFARLAND, M.D.

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WITH 113 ILLUSTRATIONS

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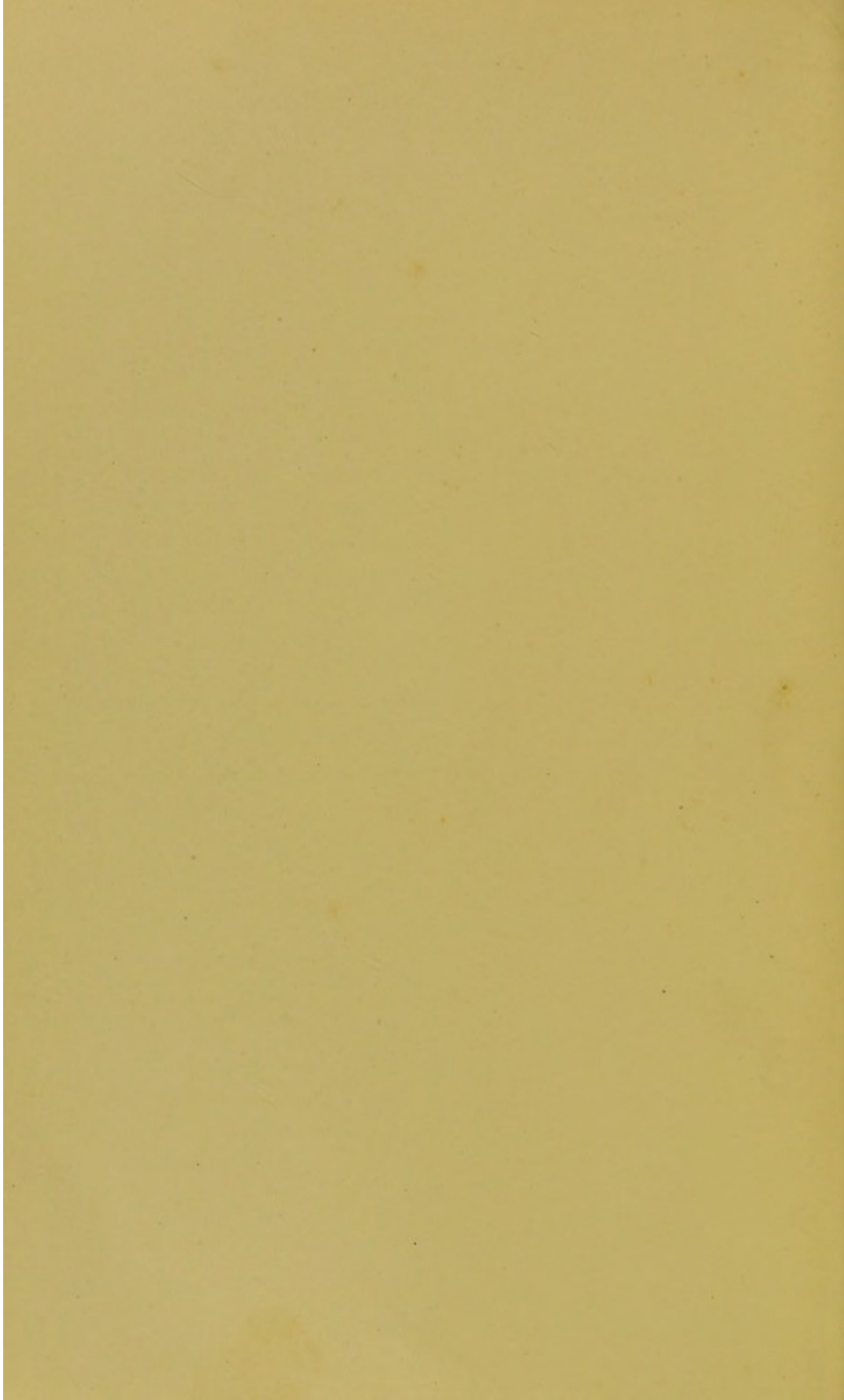
TO

MY HONORED AND BELOVED GRANDFATHER

MR. JACOB GRIM

WHOSE PARENTAL LOVE AND LIBERALITY HAVE ENABLED ME TO PURSUE
MY MEDICAL EDUCATION

THIS BOOK IS AFFECTIONATELY DEDICATED



PREFACE.

THE following pages are intended to convey to the reader a concise account of the technical procedures necessary in the study of bacteriology, a brief description of the life-history of the important pathogenic bacteria, and sufficient description of the pathological lesions accompanying the micro-organismal invasions to give an idea of the origin of symptoms and the causes of death.

The work being upon Pathogenic Bacteria, it does not cover the whole scope of parasitology, and the parasites of higher orders are all omitted. Malaria and amebic dysentery are omitted as logically as tape-worms and pediculi. The higher fungi are also omitted, both because they are not bacteria and because their proper consideration would make a small book in itself.

In leaving out the non-pathogenic bacteria of course a stumbling-block was encountered. The *Sarcina ventriculi*, for instance, may be a cause of dyspepsia, yet can scarcely be regarded as pathogenic, and, together with other similar bacteria of questionable deleterious operation, has been omitted; on the other hand, it has been thought advisable to include and describe somewhat at length a long list of spirilla similar to, and probably closely allied with, the spirillum of cholera, yet not the cause of any particular diseased condition.

The aim has been to describe only such bacteria as can be proven pathogenic by the lesions or toxins which they engender, and, while considering them, to mention as fully as is necessary the species with which they may be confounded.

The book, of course, will find its proper sphere of usefulness in the hands of medical students; its pages, however, will be found to contain much that will be of interest and profit to those practitioners of medicine who graduated before modern science had thrown its light upon the etiology of disease.

In writing this work the popular text-books have been drawn upon. Hüppe, Flügge, Sternberg, Fränkel, Günther, Thoinot and Masselin, and others have been freely consulted.

The illustrations are mainly reproductions of the best the world affords, and, being taken from the great standards, are surely superior to anything new covering the same ground. Credit has carefully been given for each illustration.

J. McF.

PHILADELPHIA, Feb. 1, 1896.

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PATHOGENIC BACTERIA.

PART I. GENERAL CONSIDERATIONS.

INTRODUCTION.

THE unrecognized inception of the department of science which we are about to study had its latent germs in the thought of antiquity.

It is folly to begin the consideration of bacteria with their probable discoverer, Leeuwenhoek, or with the so-called "Father of bacteriology," Henle. The controversies and ideas which stimulated the investigations and researches which have brought us to our present state of knowledge were begun hundreds of years before the beginning of the Christian era.

Excepting such as taught and believed that "in six days the Lord made heaven and earth, the sea and all that in them is," or a kindred theory of the origin of things, the thinkers of antiquity never seem to have doubted that under favorable conditions life, both animal and vegetable, might arise spontaneously.

Among the early Greeks we find that Anaximander (43d Olympiad, 610 B. C.) of Miletus held the theory that animals were formed from moisture—an idea that would stamp him a disciple of Thales if we did not know that his doctrine was that "the Infinite is the substance of all things." Empedocles of Agrigentum (450 B. C.) attributed to spontaneous generation all the living beings which he found peopling the earth. Aristotle (B. C. 384) 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." He also formulated a principle that "every dry substance which becomes moist, and every moist body which becomes dried, produces living creatures, provided it is fit to nourish them."

Three centuries later, in his disquisition upon the Pythagorean philosophy, we find Ovid defending the same doctrine:¹

"By this sure experiment we know
That living creatures from corruption grow :
Hide in a hollow pit a slaughter'd steer,
Bees from his putrid bowels will appear,
Who, like their parents, haunt the fields and bring
Their honey-harvest home, and hope another spring
The warlike steed is multiplied, we find,
To wasps and hornets of the warrior kind.
Cut from a crab his crooked claws, and hide
The rest in earth, a scorpion thence will glide,
And shoot his sting ; his tail in circles toss'd
Refers the limbs his backward father lost ;
And worms that stretch on leaves their filmy loom
Crawl from their bags and butterflies become.
The slime begets the frog's loquacious race ;
Short of their feet at first, in little space,
With arms and legs endued, long leaps they take,
Raised on their hinder part, and swim the lake,
And waves repel ; for nature gives their kind,
To that intent, a length of legs behind."

Not only was the doctrine of spontaneous generation of life current among the ancients, but we find it persisting through the Middle Ages, and descending to our own generation to be an accidental but important factor in the development of a new branch of science. 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,

¹ Ovid's *Metamorphoses*, translated by Mr. Dryden, published by Sir Samuel Garth, London, 1794.

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.¹

About 1668, Francesco Redi seems to have been the first to doubt that the maggots familiar in putrid meat arose *de novo*: "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 these organisms. 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."

It was not long before Leeuwenhoek, Vallisneri, Swammerdan, and others, following the trend of Redi's work, contributed additional facts in favor of his view, and it may safely be asserted that ever since the time of this eminent man the tide of scientific opinion has turned more and more strongly against the idea that life is spontaneously generated.

About this time (1675) one whose name has been already mentioned, Anthony van Leeuwenhoek, and who is justly called the "Father of microscopy," came into prominence. An optician by trade, Leeuwenhoek devoted much time to the perfection of the compound microscope, which was just coming into use. The science of

¹ See Tyndall: *Floating Matter in the Air*.

optics, however, was not sufficiently developed to enable him to overcome the errors of refraction, and after the loss of much time he turned to the simple lens, using it in so careful and remarkable a manner as to be able to record his observations in one hundred and twelve contributions to the *Philosophical Transactions*. Leeuwenhoek, among other things, 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 the rotifers, and also the bacteria, seeing them first in saliva.

Although one of those who contributed to the support of Redi's arguments against the spontaneous generation of maggots, 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 these minute organisms to exist in such numbers that putrescent infusions, both animal and vegetable, literally teemed with them, one drop of such a liquid furnishing a banquet for millions.

Much hostility arose in the scientific world as years went on until two schools attained prominence—one headed by Buffon, whose doctrine was that of "organic molecules;" the other championed by Needham, whose doctrine was the existence of a "vegetative force" which drew the molecules together.

Experimentation was begun and attracted much attention. Among the pioneers was Abbé Lazzaro Spallanzani (1777), who 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 the 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 had 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.

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

Cagniard Latour and Schwann in the year 1837 succeeded in proving that the minute oval bodies which had been observed in yeast since the the time of Leeuwenhoek were living organisms—vegetable forms—capable of growth; and when Boehm succeeded a year later in demonstrating their occurrence in the stools of cholera, and conjectured that the process of fermentation was concerned in the causation of that disease, the study of these low forms of life received an immense impetus from the important position which they began to assume in relation to medical science.

The experiments of Schwann, by proving that the free admission of calcined air to closed vessels containing putrescible infusions was without effect, while the admission of ordinary air brought about decomposition, suggested that the causes of putrefaction which were in the air were living entities.

In 1862, Pasteur published a paper "On the Organized Corpuscles existing in the Atmosphere," in which he showed that many of the floating particles which he had been able to collect from the atmosphere of his

laboratory were organized bodies. If these were planted in sterile infusions, abundant crops of micro-organisms were obtainable. By the use of more refined methods he repeated the experiments of Schwann and 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 enclosed in hermetically-sealed flasks.

Chevreul and Pasteur (about 1836) proved that animal solids did not putrefy or decompose if kept free from the access of germs, and thus suggested to surgeons that the putrefaction which occurred in wounds was due rather to the entrance of something from without than to some change within. The deadly nature of the discharges from these 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.

Examinations of the blood of diseased animals were now begun, and Pollender (1849) and Davaine (1850) succeeded in demonstrating the presence of the anthrax bacillus in that disease. Several years later (1863) Davaine, having made numerous inoculation-experiments, demonstrated that this bacillus was the *materies morbi* of the disease.

Tyndall enlarged upon the experiments of Pasteur, and very conclusively proved that the micro-organismal germs were in the dust suspended in the atmosphere, not ubiquitous in their distribution. His experiments were very ingenious and are of interest to medical men. First preparing light wooden chambers, with one large glass window in the front and one smaller window in each

side, he arranged a series of empty test-tubes in the bottom and a pipette in the top, so that when desired the tubes, one by one, could be filled through it. The chamber was first submitted to an optical test to determine the purity of its atmosphere, and was allowed to stand undisturbed and unused until a powerful ray of light passed through the side windows failed to reflect rays from suspended particles of dust when viewed from the front. When the dust had settled so as to allow the optical test of its purity, the tubes were filled with urine, beef-broth, and a variety of animal and vegetable broths, boiled by submergence in a pan of hot brine; the tubes were then allowed to remain undisturbed for days, weeks, or months. In nearly every case life failed to develop after the purity of the atmosphere was established.

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.

Thus evidence slowly accumulated to establish the theory for which Henle had labored as early as 1821, that for many diseases at least there was a distinct and specific *contagium vivum*, and the "GERM THEORY" was propounded.

Is it not strange that the very idea which was to be the outcome of all this investigation and discussion—an idea which would form a new era in scientific medicine and become a fundamental principle of pathology—was one which had been conceived and taught by a philosopher who lived nearly two thousand years ago? Among the numerous works of Varro¹ is one entitled *Rerum Rusticarum libri tres*, from which the following is quoted: "Animadvertendum etiam, si qua erunt loca palustria—quod crescunt animalia quaedam minuta, quae non possunt oculi consequi et per aëra intus in corpus per os ac nares perveniunt atque efficiunt difficilis morbus" (I., xii. 2).—"It is also to be noticed, if there be any marshy

¹ *Univ. Med. Mag.*, vol. iii., No. 3, Dec., 1890, p. 152.

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)"—a doctrine which, as Prof. Lamberton, to whom the writer is indebted for the extract, points out, is handed down to us from "the days of Cicero and Cæsar," yet corresponds closely to the ideas of malaria which we entertain at present.

Pasteur had long before suggested that for the different kinds of fermentation there must be specific ferments, and by fractional cultures had succeeded in roughly separating them.

Klebs, who was one of the pioneers of the germ theory, published in 1872 his work upon septicemia and pyemia, in which he expressed himself convinced that the causes of these diseases must come from without the body. Billroth 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," or through inflammation a "phlogistischezymoid," supplying the necessary feeding-grounds, was produced.

Klebs was not alone in the opposition aroused. Davaine no sooner announced the contagium of anthrax than critics declared that inasmuch as he introduced blood from the diseased animal into the other animal to whom the disease was to be communicated, it was altogether unreasonable to believe the bacilli which were in all probability accidentally present in that blood were the cause of the disease.

In 1875 the number of scientific men who had 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, retained the old unquestioning faith that the bacteria, whose presence in putrescent wounds as well as in artificially prepared media was unquestionable, were spontaneously generated there.

The following extracts from Tyndall's work¹ will illustrate the slow growth of the germ theory even among men of eminence :

“At a meeting of the Pathological Society of London, held April 6, 1875, the ‘germ theory’ of disease was formally introduced as a subject for discussion, the debate being continued with great ability and earnestness at subsequent meetings. The conference was attended by many distinguished medical men, some of whom were profoundly influenced by the arguments, and none of whom disputed the facts brought forward against the theory on that occasion.”

“The leader of the debate, and the most prominent speaker, was Dr. Bastian, to whom also fell the task of replying on all the questions raised.”

“The coexistence of bacteria and contagious disease was admitted; but, instead of considering these organisms as probably the essence, or an inseparable part of the essence, of the contagium, Dr. Bastian contended that *they were pathological products spontaneously generated in the body after it had been rendered diseased by the real contagium.*”

“The grouping of the ultimate particles of matter to form living organisms Dr. Bastian considered to be an operation as little requiring the action of antecedent life as their grouping to form any of the less complex chemical compounds.” “Such a position must, of course, stand or fall by the evidence which its supporter is able to produce, and accordingly Dr. Bastian appeals to the law and testimony of experiment as demonstrating the soundness of his view.” “He seems quite aware of the

¹ *Op. cit.*

gravity of the matter at hand ; this is his deliberate and almost solemn appeal : ' With the view of settling these questions, therefore, we may carefully prepare an infusion from some animal tissue, be it muscle, kidney, or liver ; we may place it in a flask whose neck is drawn out and narrowed in the blowpipe flame ; we may boil the fluid, seal the vessel during ebullition, and, keeping it in a warm place, may await the result, as I have often done. . . . After a variable time the previously heated fluid within the hermetically-sealed flask swarms more or less plentifully with bacteria and allied organisms, even though the fluids have been so much degraded in quality by exposure to the temperature of 212° F., and have in all probability been rendered far less prone to engender independent living units than the unheated fluids in the tissues would be.' "

These somewhat lengthy quotations are of great interest, for they show exactly the state of the scientific mind at a period as recent as twenty years ago.

In 1877 the introduction of the anilin dyes by Weigert made possible a much more thorough investigation of the bacteria by enabling the observers to color them intensely, and thus detect their presence in tissues and organs where their transparency had caused them to be overlooked.

Rapid strides were immediately made, and before another decade had passed discoveries were so numerous and convincing that it was impossible to doubt that bacteria were causes of disease.

Before the publication of the discoveries of which we speak, however, there was suggested a practical application of the little known about bacteria which produced greater agitation and incited more observation and experimentation than anything suggested in surgery since the introduction of anesthetics—namely, *antisepsis*.

"The seminal thought of antiseptic surgery may perhaps be traced to John Colbach, a member of the College of Physicians, England, whose collection of tracts, printed

1704, contained a description of a new and secret method of treating wounds, by which healing took place quickly without inflammation or suppuration; but 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."¹

Lister was not the discoverer of carbolic acid nor of the fact that it would kill bacteria; but, convinced that inflammation and suppuration were due to the entrance of germs from the air, instruments, fingers, etc. into wounds, he suggested the antiseptic which would insist upon the use of sterile instruments and clean hands and towels; which would keep the surface of the wound moist with a germicidal solution to kill such germs as accidentally entered; and which would conclude an operation by a protective dressing to exclude the entrance of germs at a subsequent period.

Listerism, originated (1875) a few years before Koch published his famous work on the *Wundinfektionskrankheiten* (traumatic infectious diseases) (1878), spread slowly at first, but surely in the end, to all departments of surgery and obstetrics.

The discovery of the yeast-plant by Latour and Schwann as the cause of fermentation, and the later discovery by Bassi of the yeast-like plant causing the miasmatic contagious disease of silkworms, had led Henle (1840) to believe that the cause of miasmatic, infective, and contagious diseases must be looked for in fungi or in other minute living organisms. Unfortunately, the methods of study employed in Henle's time prevented him from demonstrating the accuracy of his belief.

"It would indeed have been difficult at that period to satisfy every condition that he required to be fulfilled: the methods now in use were then unknown, and have

¹ Agnew's *Surgery*, vol. i. chap. ii.

only been perfected by workers as it has been found necessary from time to time to comply in the most minute detail with Henle's conditions, and as, one point being carried, it was found necessary to advance on others. The first of these was that a specific organism should always be associated with the disease under consideration. As such presence, however, might be accidental, these organisms were not only to be found in pus, etc., but actually in the living body. As they might be, even then, merely parasitic, and not associated directly with the causation of the disease, it would be necessary to isolate the germs, the contagium organisms, and the contagium fluids, and to experiment with these separately with special reference to their power of producing similar diseases in other animals. We now know that it has only been by strict compliance with all these conditions, again postulated by Koch, that the most brilliant scientific observers and experimentalists in Germany, France, England, [and America] have been able to determine the causal connection between micro-organisms and disease." ¹

The refined methods of Pasteur, but more especially of Koch, by making possible the fulfilment of the postulates of Henle caused an enormous increase in the rapidity with which data upon disease-germs were gathered. Almost within a decade the causes of the most important specific diseases were isolated and cultivated.

In 1879, Hausen announced the discovery of bacilli in the cells of leprous nodules. The same year Neisser discovered the gonococcus to be specific for gonorrhoea.

In 1880 the bacillus of typhoid fever was first observed by Eberth, and independently by Koch.

In 1880, Pasteur published his work upon "chicken-cholera." In the same year Sternberg described the pneumococcus, calling it the *micrococcus Pasteuri*.

In 1882, Koch made himself immortal by his discovery of and work upon the tubercle bacillus. The same

¹ Woodhead: *Bacteria and their Products*, p. 65.

year Pasteur published a work upon *Rouget du Porc*, and Löffler and Schütz reported the discovery of the bacillus of glanders.

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

In 1892, Canon and Pfeiffer discovered the bacillus of influenza.

In 1892, Canon and Pielicke first found the bacillus now thought to be specific for measles.

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

Between the years 1884 and 1892 few new bacteria were discovered, attention being directed toward perfecting the methods of technical procedure, investigating interesting subjects relating to the biology of the bacteria, and the study of immunity.

CHAPTER I.

BACTERIA.

A BACTERIUM is a minute vegetable organism consisting of a single cell principally composed of an albuminous substance, which Nencki has called *mycoprotein*. Nencki found the chemical analysis of bacteria in the active state to consist of 82.42 per cent. of water. In 100 parts of the dried constituents he found 84.20 parts of mycoprotein; 6.04 of fat; 4.72 of ash; 5.04 of undetermined substances.

Mycoprotein, which has the composition C 52.32, H 7.55, N 14.75, is a perfectly transparent, generally homogeneous body, which probably varies somewhat according to the species from which it is obtained, the culture-medium in which it is grown, and the vital products which the organism produces by its growth. Sometimes the mycoprotein is granular, as in bacillus megatherium; sometimes it contains fine granules of chlorophyl, sulphur, fat, or pigment. Each cell is surrounded by a cell-wall, which in some species shows the cellulose reaction with iodine.

When subjected to the influence of nuclear stains the bacteria not only take the stain faintly, but in such a manner as to show the existence of a large nucleus situated in the centre of the cell and constituting its great bulk. The cell-wall generally is not stained, but when it does tinge, a delicate line of unstained material can sometimes be made out between the nucleus and the cell-wall, showing the existence of a protoplasm.

The anilin dyes, which possess a great penetrating power, color the organisms so intensely as to preclude the differentiation of the cellular constituents. Under

these conditions the bacteria appear as solidly-colored spheres, rods, or spirals, as the case may be.

The cell-walls of some of the bacteria seem at times to undergo a peculiar gelatinous change or to allow the exudation of gelatinous material from the protoplasm, so that the individuals appear surrounded by a distinct halo. This is not only a peculiarity of certain individuals, but one which only takes place when they develop under certain conditions; thus, Friedländer points out that the capsule of his pneumonia bacillus, when it was found in the lung or in the "prune-juice" sputum, was very distinct, while it could not be demonstrated at all when the organisms grew in gelatin.

From the cell-walls of many bacteria numerous delicate straight or wavy filaments project. These are called *cilia* or *flagella*, and seem to be organs of locomotion. Sometimes they are only observed projecting from the ends or from one end; sometimes they are so numerous and so regular in their distribution as to give the organisms a woolly appearance.

Many of the bacteria which are thus supplied with flagella are actively motile and swim about like microscopic serpents. In all probability the locomotory powers of the bacteria are not entirely dependent upon the presence of the flagella, but may sometimes be due to contractility of the protoplasm within an elastic cell-wall. The micro-organisms most plentifully supplied with them are those of the rod and spiral shape. Only one of the spherical forms, *Micrococcus agilis* of Ali-Cohen, has been shown to have flagella. This and one other species are probably the only motile cocci. Observing that the organisms known to be most active are those best supplied with flagella, it is reasonable to conclude that the motility is dependent upon the flagella.

The presence of flagella, however, does not necessarily imply motility, for some of the bacilli amply provided with these appendages are not motile (*bacillus coli communis*). The flagella may not only serve as organs of

locomotion, and be of use to the organism by conveying it from an area where the nutrition is less to one where it is greater, but, as Woodhead points out, may, in the non-motile species, serve to stimulate the passage of currents of nutrient material past the organism, so as to increase the food-supply. The flagellate bacteria have a greater number of representatives among those whose lives are spent in water and in fermenting and decaying materials than among those inhabiting the bodies of animals. This is an additional fact in favor of the view that locomotion and flagella are provisions favorable to the maintenance of the species by keeping the individuals supplied with food.

In carrying the argument a little farther it may be added that such parasitic disease-producing bacteria as do not habitually gain access to the tissues, but inhabit the intestine, as the bacillus of typhoid fever and the spirillum of cholera, are actively motile, like the saprophytes. Of course this example is open to criticism, because the spirillum of relapsing fever, which has never been found elsewhere than in the blood and spleen of affected animals, is actively motile, while the *Bacterium coli communis*, which is always present in the intestine, is non-motile.

One of the linear organisms known as the *Bacillus megatherium* has a distinct but limited ameboid movement.

The commonly observed dancing movement of the spherical forms seems to be the well-known Brownian movement, which is simply a physical phenomenon. It is sometimes difficult to determine whether an organism is really motile or whether it is only vibrating. In the latter case it does not change its relative position to surrounding objects.

The bacteria are so minute that a special unit of measurement has been adopted by bacteriologists for their estimation. This is the *micro-millimeter* (μ), or one-thousandth part of a millimeter, and about equivalent to the one-twenty-five-thousandth of an inch.

As a rule, the spherical organisms are the smallest and the spiral organisms the longest, except the chains of bacilli called *leptothrix*. Their measurements vary from 0.15μ (micrococcus of progressive abscess-formation in rabbits) to 2.8μ (*Diplococcus albicans amplius*) for cocci, and from $1 \times 0.2 \mu$ (bacillus of mouse-septicemia) to $5 \times 1.5 \mu$ (anthrax bacillus) for bacilli. Some of the spirilla are very long, that of relapsing fever measuring 40μ at times.

This estimation of size almost prepares one for the estimation of weight given by Nägeli, who found that an average bacterium under ordinary conditions weighed $\frac{1}{1000000000000}$ of a milligram.

The bacteria multiply in two ways : by direct division (fission) and by the development of spores, seeds, or eggs (sporulation). The more common mode is by binary division. The bacterium which is about to divide appears a little larger than normal, and, if a spherical organism, more or less ovoid. No karyokinetic changes have been observed in the nuclei, though they may occur. When the conditions of nutrition are good, the process of 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, or, if reckoned, could scarcely be imagined—four thousand seven hundred and seventy-two billions. If we reduce this number to weight, we find that the mass arising from this single germ would in three days weigh no less than seventy-five hundred tons." "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.”

When the conditions for rapid multiplication are no longer good, the organism assumes a protective attitude and develops in its interior small oval eggs, seeds, or, as they are more correctly called, *spores* (Fig. 1). Such



FIG. 1.—Diagram illustrating sporulation: *a*, bacillus enclosing 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.

spores developed within the bacteria are called *endospores*. When the formation of such a spore is about to commence, a small bright point appears in the protoplasm, and increases in size until its diameter is nearly or quite as great as that of the bacterium. As it nears perfection a dark, highly-refracting capsule is formed about it. As soon as the spore arrives at perfection the bacterium seems to die, as if its vitality were exhausted in the development of the permanent form.

Endospores are generally formed in the elongate bacteria—bacillus and spirillum—but Zopf has described similar bodies as occurring in micrococci. Escherich also claims to have found undoubted spores in a form of sarcina.

The spores found in the bacilli are either round or oval. As a rule, each bacillus produces a single spore, which is situated either at its centre or at its end. When, as sometimes happens, the diameter of the spore is greater than the diameter of the bacillus, it causes a bulging of the organism, with a peculiar appearance described as *clostridium*. When the distending spore is in the centre of the bacillus, it produces a barrel-shaped organism; when situated at the end, a “Trommelschläger,” or drumstick-shaped one. As the degeneration of the protoplasm of the bacillus sets the spore free, it appears as a clear,

highly-refracting sphere or ovoid situated in a little collection of granular matter.

Spores differ from the bacteria in that their capsules seem to prevent evaporation and to enable them to withstand drying and the application of a considerable amount of heat. Ordinarily, bacteria are unable to resist a temperature above 60° C. for any considerable length of time, only a few resistant forms tolerating a temperature of 70° C. The spores, however, are uninjured by such temperatures, and can even successfully resist that of boiling water (100° C.) for a short time. The extreme desiccation caused by a protracted exposure to a temperature of 150° C. will, however, destroy them. Not only can the spores resist a considerable degree of heat, but they are also unaffected by cold of almost any intensity.

While the cell-wall of the bacterium is easily penetrated by solutions of the anilin dyes, it is a matter of much difficulty to accomplish the staining of spores, so that we see they are probably more resistant to the action of chemical agents than the bacteria themselves.

When a spore is accidentally dropped into some nutrient medium a change is shortly observed. The protoplasm, which has been clear, becomes somewhat granular, the capsule a little less distinct; the body increases slightly in size, and in the course of time splits open to allow the escape of the young organism. The direction in which the escape of the young bacillus takes place is of interest, as varying in the different species. The *Bacillus subtilis* escapes from the end of the spore, where a longitudinal fissure occurs; the bacillus of anthrax escapes from the side, sometimes leaving the capsule of the spore in the shape of two small cups.

As soon as the young bacillus escapes it begins to increase in size, develops around its soft protoplasm a characteristic capsule, and, having once established itself, presently begins the propagation of its species by fission.

In addition to the endospores, of which we have just been speaking, there are *arthrospores*. The formation

of these is much less clear. It seems to be an effort to convert the entire microbe into a permanent form. This process is observed particularly in the micrococci, where the substance of a cell is said to break up into segments, each of which becomes a resisting body fruitful in propagating its species. Of the arthrospores little has, so far, been learned. It is not improbable that among the micrococci, and also among some of the smaller bacilli in whom no spores have been observed, the maintenance of the species when conditions of life become unfavorable is due to the assumption of a permanent form by some of the individuals, without the formation of any spore-like bodies. This is at present largely a matter of conjecture, but the indications pointing in that direction are numerous.

It is believed by Fränkel and others that sporulation in the bacteria is not a sign of the exhaustion of nutrition, but a sign of the vital perfection of the organism. These observers regard spore-formation as analogous to the flowering of higher plants, which takes place only when the conditions and development are best.

Morphology.—The morphology of the bacteria is quite varied. Three principal forms, however, exist, from which the others seem to be but variations.

The most simple appear as minute spheres, and from

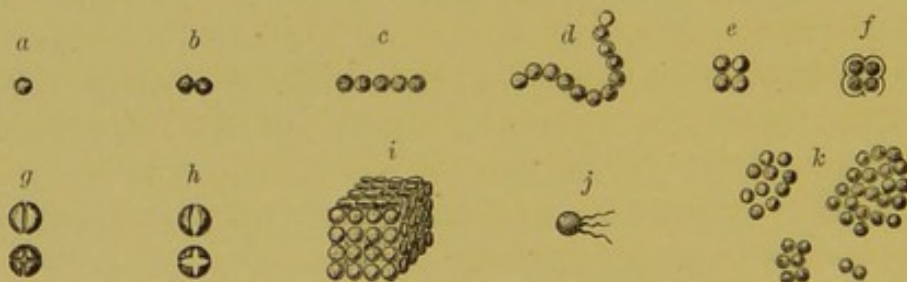


FIG. 2.—Diagram illustrating the morphology of the cocci: *a*, coccus or micrococcus; *b*, diplococcus; *c*, *d*, streptococci; *e*, *f*, tetragenococci or merismopedia; *g*, *h*, modes of division of cocci; *i*, sarcina; *j*, coccus with flagella; *k*, staphylococci.

their fancied resemblance to little berries are called *cocci* or *micrococci* (Fig. 2, *a*). When the bacteria of this form

multiply by fission the resulting two organisms not infrequently remain attached to each other, producing what is called a *diplococcus* (Fig. 2, *b*). The diplococci sometimes consist of two perfect spheres, but more often show a flattening of the contiguous surfaces, which are not in absolute apposition (Fig. 2, *g*). In a few cases, as the gonococcus, the approximated surfaces are slightly concave, causing the organism to somewhat resemble the German biscuit called a "semmel," hence biscuit- or semmel-cocci (Fig. 2, *h*). Frequently a second binary division occurs, causing four individuals to remain closely approximated, without disturbing the arrangement of the first two. When division of this kind produces a distinct tetrad, the organism is described as a *tetragenococcus*, while to the entire class of cocci dividing so as to produce fours, eights, twelves, etc. on the same plane the name *merismopedia* is given (Fig. 2, *e* and *f*).

If, as sometimes happens, the divisions take place in three directions, so as to produce cubical masses or "packages" of cocci, the resulting aggregation is described as a *sarcina* (Fig. 2, *i*). This form slightly resembles a dice or a bale of cotton in miniature.

If the divisions always take place in the same direction, so as to produce a chain or string of beads, the organism is described as *streptococcus* (Fig. 2, *d*). When there are diplococci joined in this manner a *strepto-diplococcus* is of course formed.

More common than any of the forms already described is one in which, without any definite arrangement, the cocci occur in irregular groups having a fancied resemblance to bunches of grapes. These are called *staphylococci*, and, as it is very unusual to find cocci habitually occurring isolated, most cocci not classified under one of the above heads are called staphylococci.

When cocci are associated in globular or lobulated clusters encased in a resisting glutinous, homogeneous mass, the name *ascococcus* has been used in describing them. A modified form of this, in which the cocci are

in chains or solitary and are surrounded by an encasement almost cartilaginous in consistence, has been called *leuconostoc*.

Certain bacteria, constituting a better-known if not more important group, are not spherical, but elongate or "rod-shaped," and bear the name *bacillus* (Fig. 3).

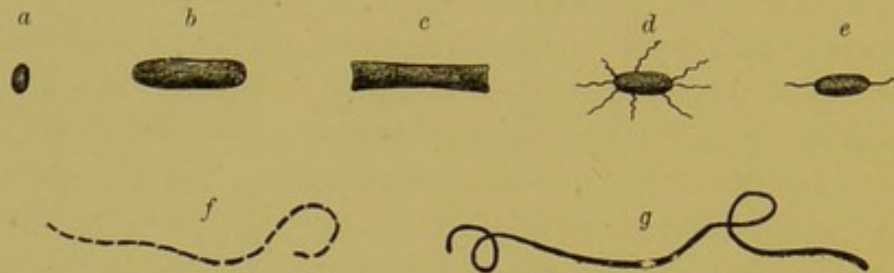


FIG. 3.—Diagram illustrating the morphology of the bacilli: *a, b, c*, various forms of bacilli; *d, e*, bacilli with flagella; *f*, chain of bacilli, individuals distinct; *g*, chain of bacilli, individuals not separated.

I would remark that the absence of a standard by which to separate the cocci from the bacilli is the cause of much confusion. In the judgment of the author, it would be well to place all individuals having one diameter greater than the other among the bacilli. This would prevent the error of describing one species as "oval cocci" and another as "nearly round bacilli," and by giving a definite standard would greatly aid in the formation of a differential table.

The bacilli present a considerable variety of forms. Some are quite short, with rounded ends, so as to appear elliptical; some are long and delicate. Some have rounded ends, as *subtilis*; others have square ends, as anthrax. Some are enormously large, some exceedingly small. Some are always isolated, never forming threads or chains; others nearly always occur in these forms.

The bacilli always divide by transverse fission, so that the only peculiarity of arrangement is the formation of threads or chains.

In the older writings the short, stout bacilli were all described under the generic term *bacterium*. This genus, like some of the species it comprehended, has now passed

out of use. Some of the flexile bacilli, whose movements are sinuous, much resembling the swimming of a snake or an eel, were described as *vibrio*, but this name also has passed into disuse.

The long filaments formed by the division of bacilli without their distinct separation are sometimes called *leptothrix*, and when these long threads form distinct masses surrounded by a jelly-like material, the name *myconostoc* is sometimes applied to them.

Certain forms much resembling bacilli in their isolated state, characterized by the formation of long filaments with a peculiar grouping which gives the appearance of a false branching, are described as *cladothrix*; others in which true branchings are seen, as *streptothrix*. One other bacillus-like form, consisting of long, thick, not distinctly segmented, straight threads, is called *beggiatoa*. The only important difference between it and *leptothrix* is that its filaments are thick and coarse, while those of *leptothrix* are very delicate.

Some of the elongate bacteria have a remarkably twisted form and bear some resemblance to a corkscrew. These are called *spirilla* (Fig. 4). A subdivision

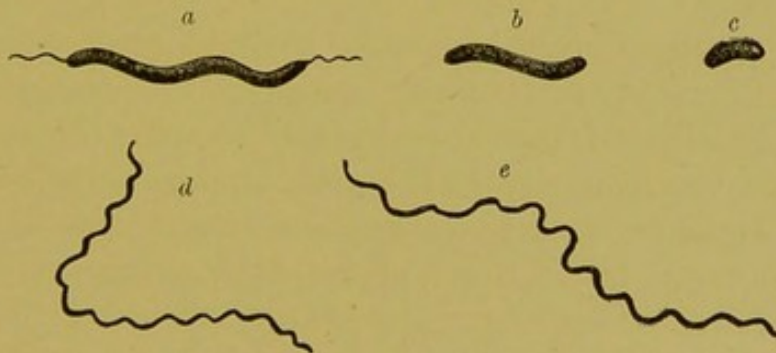


FIG. 4.—Diagram illustrating the morphology of the spirilla: *a, b, c*, spirilla; *d, e*, spirochæta.

of them, whose individuals are not only twisted but are also very flexible, is called *spirochæta*. Though not formerly differentiated from *vibrio*, these forms are quite distinct.

A spiral organism of a ribbon shape is called *spiro-*

monas, while a similar organism of spindle shape is called a *spirulina*. One species of spiral bacteria in whose protoplasm sulphur-grounds have been detected has been called *ophidiomonas*.

Some of the spirilla are exceedingly long and delicate, as the spirochæta of relapsing fever; others which are stouter, like the spirillum of cholera, habitually occur in such short individuals as to be easily mistaken for slightly-bent bacilli.

Classification.—Leeuwenhoek, when he first saw the bacteria—and his successors even to so recent a date as to include Ehrenberg and Dujardin—did not doubt that they belonged to the infusoria.

It was not until biologists had concluded that organisms which take into their bodies particles of solid or semi-solid material, digest that which is useful, and extrude the remainder, are animals, and that those which live purely by osmosis and exosmosis are vegetables, that the bacteria, which we have seen provided with a resistant cell-wall, allowing of no possibility of nutrition except by osmosis and exosmosis, could be finally and correctly classed among the members of the vegetable kingdom.

The extremely simple organization of bacteria naturally places them among the lowest members of the vegetable kingdom, in that class of the Cryptogamia known as Thallophytæ, comprising the algæ, lichens, and fungi.

The algæ are mostly water-plants, containing chlorophyl and obtaining their nourishment from inorganic substances.

The lichens are plants, some of which contain chlorophyl. They live upon inorganic matter, which they generally absorb from the air. According to the new view of the subject, some, if not all, of these plants are regarded as fungi growing parasitically upon algæ.

The fungi, the lowest group of all, are minute or large plants, mostly devoid of chlorophyl, living upon organic matter, which they obtain as saprophytes from decom-

posing animal and vegetable matters, or as parasites upon the tissues or juices of living animals or plants.

This lowest family, the fungi, are divisible into the—

Hyphomycetes or Mucorini, or moulds;
Saccharomycetes, or yeasts; and
Schizomycetes, or bacteria.

Cohn divided the bacteria, according to their morphology, into—

Sphero-bacteria, or cocci;
Micro-bacteria—short rods;
Desmo-bacteria—bacilli;
Spiro-bacteria—spirilla.

Davaine suggested a classification based upon motility, making four classes—Bacterium, Vibrio, Bacteridium, and Spirillum, neglecting to provide for the cocci.

Zoph arranged them, according to his theory of pleomorphism, into the COCCACEÆ, comprising those known only in the coccus form, and comprehending the *streptococci*, *merismopedia*, *sarcina*, *micrococcus*, and *ascococcus*; the BACTERIACEÆ, comprehending the genera *bacterium*, *spirillum*, *vibrio*, *leuconostoc*, *bacillus*, and *clostridium* (chiefly coccus, rod, and thread forms; the former may be absent; in the latter there is no distinction between base and apex; threads straight or screw-like); and the LEPTOTHRICHEÆ, comprehending *crenqthrix*, *beggiatoa*, *phragmidiothrix*, and *leptothrix* (coccus, rod, and thread forms; the latter show a distinction between base and apex; threads straight or screw-like; spore-formation not demonstrated).

This classification is, however, based upon what is probably an erroneous principle, the pleomorphism of the bacteria.

Van Tieghem, DeBary, and Hüppe formed classifications the main feature of which was the formation of endospores or arthrospores, but, as the sporulation of many species is as yet unknown, they cannot be properly placed in it.

It has even been suggested to classify the bacteria by the size and number of their flagella, of which so little is known.

The most convenient classification, though it cannot be purely scientific, seems to be the morphological one given by Cohn. Baumgarten, recognizing the relative pleomorphism of certain of the species, has modified it as follows, and thus made it answer all the needs of the pathologist at least:

- | | | |
|-----------------|---|----------------------------------|
| I. Cocci, | } | species relatively monomorphous. |
| II. Bacilli, | | |
| III. Spirilla, | | |
| IV. Spirulina, | } | species relatively pleomorphous. |
| V. Leptothrix, | | |
| VI. Cladothrix, | | |

The members of the first group, the cocci, bacilli, and spirilla, are practically the only ones which are of pathological significance.

CHAPTER II.

BIOLOGY OF BACTERIA.

THE distribution of bacteria is wellnigh universal. They and their spores float in the atmosphere we breathe, swim in the water we drink, grow upon the food we eat, and luxuriate in the soil beneath our feet. Nor is this all, for, entering the palpebral fissures, they develop upon the conjunctiva; entering the nares, they establish themselves in the nose; the mouth is always replete with them; and, as many are swallowed, the digestive apparatus always contains them. The surface of the body never escapes their establishment, and so deeply are some individuals situated beneath the epithelial cells that the most careful washing and scrubbing and the use of the most powerful germicides are required to rid the surgeon's hands of what may prove to be dangerous hindrances to the healing of wounds. The ear is not without its microscopic flora; special varieties live beneath the finger-nails, and especially the toe-nails, in the vagina, and beneath the prepuce.

While so general, however, they are not ubiquitous. Tyndall succeeded in proving that the atmosphere of high Alpine altitudes was free from them, and likewise that the glacier ice contained none. Wherever man, animals, or even plants, live, die, and decompose, bacteria are sure to be present.

Notwithstanding their extreme familiarity with the animal body, there are certain parts of it into which bacteria do not enter, for *the body-juices and tissues of normal animals are constantly free from them, and their occurrence there may be accepted as a sign of disease.*

The presence of bacteria in the air is generally de-

pendent upon their previous existence in the soil, its pulverization, and its 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 bacteria can live only upon organic matter, this is readily understandable. 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, the bacteria may occur much farther from the surface, yet they are rarely found at any great depth, because the majority of the known species require oxygen.

The water of stagnant pools always teems with bacteria, but that of deep wells rarely contains many unless it is polluted from the surface of the earth.

Being generally present in the soil, which the feet of men and animals grind to powder, the bacteria, together with the pulverized earth, are blown from place to place into every nook and cranny, until it is impossible to escape them. It has been suggested by Soyka that the currents of air passing over the surface of liquids might take up bacteria, 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 becomes roughened or broken.

Most of the bacteria which are carried about by the air are what are called saprophytes, and are perfectly harmless to the human being; but not all belong to this class, nor will they do so while tuberculous patients are allowed to expectorate upon the sidewalks, and typhoid patients' wash to dry upon the clothes-line, and their dejecta to be spread upon the ground.

The growth of bacteria is profoundly influenced by environment, so that a consideration of the conditions

favorable or detrimental to their existence becomes a necessity.

Conditions influencing the Growth of Bacteria.—

(a) *Oxygen*.—The majority of bacteria grow best when exposed to the air. Some develop better when the air is withheld; some will not grow at all where the least amount of oxygen is present. Because of these peculiarities bacteria are divisible into the

Aërobic bacteria, those growing in oxygen.

Anaërobic bacteria, those not growing in the presence of oxygen.

As, however, some of the aërobic forms will grow almost as well without as with oxygen, the term *optional* (facultative) *anaërobics* has been applied to the special class made to include them.

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

(b) *Nutrient*.—The bacteria do not seem able to derive their nourishment from purely inorganic matter. Proskauer and Beck, however, 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., glycerin 1.5 per cent. They grow best where diffusible albumins are present. The ammonium salts are rather less fitted to support them than their organic compounds. The individual bacterium varies very widely in the nutrient which it requires. 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. 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 transplanted organism. Sometimes the addition of such substances as glucose and glycerin has a peculiarly favorable influence upon bacteria, causing, for example, the tubercle bacillus to grow upon agar-agar.

(c) *Moisture*.—A certain amount of water is always necessary for the growth of bacteria. The amount can be exceedingly small, however, so that the *Bacillus prodigiosus* is able to develop successfully upon crackers and dried bread. Materials used as culture-media should not be too concentrated; at least 80 per cent. of water should be present. Most bacteria grow best in liquid media; that is, they form the longest threads, and diffuse themselves throughout the liquid so as to be present in far greater numbers than when on solid media.

The statement that certain forms of bacteria can flourish in clean distilled water seems to be untrue. When transferred to such a medium the organisms soon die and undergo a granular degeneration of their substance. If, however, in their introduction a good-sized drop of culture-material is carried with them, the distilled water ceases to be such, and becomes a dilute bouillon fitted to support life for a time.

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

(e) *Light*.—Most species of bacteria are not influenced in their growth by the presence or absence of light. The

direct rays of the sun, and to a less degree the intense rays of the electric arc-light, retard and in numerous instances kill bacteria. Some colors are distinctly inhibitory to their growth, blue being especially prejudicial. Some of the chromogenic forms will only produce their colors when exposed to the ordinary light of the room. The *Bacillus mycoides roseus* will not produce its red pigment except in the absence of light. The pathogenic bacteria have their virulence gradually attenuated if grown in the light.

(*f*) *Electricity*.—Very little is known about the action of electric currents upon bacteria. Very powerful discharges of electricity through culture-media are said to kill the organisms.

(*g*) *Movement*.—When bacteria are growing in a liquid medium perfect rest seems to be the condition best adapted for their development. A slow-flowing movement does not have much inhibitory action, but violent agitation, as by shaking a culture in a machine, greatly hinders or prevents their growth. The practical application of this will show that rapidly-flowing streams, whose currents are interrupted by falls and rapids, will, other things being equal, furnish a better drinking-water than a deep, still-flowing river.

(*h*) *Temperature*.—The question of temperature is of importance from its bearing upon sterilization. According to Fränkel, bacteria will scarcely grow at all below 16° and above 40° C.

The researches of Flügge show that the *Bacillus subtilis* will grow very slowly at 6° C., and as the temperature is elevated it is said that until 12.5° C. is reached fission does not occur oftener than every four or five hours. When 25° C. is reached the fission occurs every three-quarters of an hour, and at 30° C. about every half hour.

Most bacteria die at a higher temperature than 60–75° C. The spores can resist boiling water, but are killed by dry heat if exposed to 150° C. for an hour or to

175° C. for five to ten minutes. Freezing kills many, but not all bacteria, but does not affect the spores at all.

Most bacteria grow best at the ordinary temperature of a comfortably heated room, and are not affected by its occasional slight changes. Some, chiefly the pathogenic forms, are not cultivable except at the temperature of the animal body (37° C.); others, like the tubercle bacillus, grow best at a temperature a little above that of the body—40° C.

Some forms of the bacteria are never found except in the tissues of diseased animals. Such organisms are called *parasites*. The parasitic group really is divisible into the *purely parasitic* and the *occasionally parasitic* bacteria. Of the first division the tubercle bacillus may be used as an illustration, for, so far as is known, it is never found in other places than the bodies and dejecta of diseased animals. The cholera spirillum illustrates the second group, for, while it produces the disease known as Asiatic cholera when admitted to the digestive tract, it is a constant inhabitant of certain waters, where it multiplies with luxuriance.

Bacteria which do not enter the animal economy, or if accidentally admitted do no harm, but live upon decaying animal and vegetable materials, are called *saprophytes*. The parasitic organisms alone possess much interest to the physician, but as in their growth the saprophytes exhibit many interesting vital manifestations, it is not well to exclude them or their products from the following consideration of the

Results of Vital Activity in Bacteria.—I. *Fermentation.*—The alcoholic fermentation, which is a familiar phenomenon to the layman as well as to the brewer and the chemist, is not the work of a bacterium, but of a yeast-plant, one of the *saccharomyces* fungi. The acetic-acid, lactic-acid, and butyric-acid fermentations are, however, caused by bacilli. A considerable number of bacilli seem capable of converting milk-sugar into lactic acid, sometimes associating this with coagulation of milk, some-

times not. The production of coagulation in milk is not always associated with acid-production, but with the production of a curdling ferment similar to that belonging to the gastric juice. There seems to be no real specific micro-organism for the lactic-acid fermentation, although the *Bacillus acidi lactici* seems to be the most powerful generator of the acid. There may also be several bacteria which produce the acetic fermentation, though it is generally attributed to a special common form, the *Mycoderma aceti* or *Bacillus aceticus*. The butyric fermentation is generally due to the *Bacillus butyricus*, though it also may be caused by other bacilli, the one named simply being the most common. (For an exact description of the chemistry of the fermentations reference must be made to text-books upon that subject, as their consideration here would occupy too much space.)

2. *Putrefaction*.—This process is in many respects similar to the preceding, except that instead of occurring in carbohydrates it takes place in nitrogenous bodies. The first step seems to be the transformation of the albumins into peptones, then the splitting up of the peptones into a large number of gases, acids, bases, and salts. In the process the innocuous albumins are frequently changed to toxalbumins, and sometimes to distinct animal alkaloids known as *ptomaines*. Vaughan and Novy declare the term "animal alkaloid" to be a misnomer, as ptomaines are sometimes produced from vegetable substances in the process of decomposition; they suggest the term "putrefactive alkaloids" as preferable. The definition of a ptomaine given by these observers is "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 book¹ is brief and excellent. Among the ptomaines the following appear to be important: Methylamin (CH_3NH_2), the simplest organic base formed in the process of putrefaction; dime-

¹ *Ptomaines and Leucomaines*.

thylamin ($(\text{CH}_3)_2\text{NH}$); trimethylamin ($\text{C}_3\text{H}_9\text{N} = (\text{CH}_3)_3\text{N}$); ethylamin ($\text{C}_2\text{H}_5\cdot\text{NH}_2$); diethylamin ($\text{C}_4\text{H}_{11}\text{N} = (\text{C}_2\text{H}_5)_2\text{NH}$); triethylamin ($\text{C}_6\text{H}_{15}\text{N} = (\text{C}_2\text{H}_5)_3\text{N}$); propylamin ($\text{C}_3\text{H}_7\cdot\text{NH}_2$); butylamin ($\text{C}_4\text{H}_{11}\text{N}$); iso-amylamin; caproylamin; tetanotoxin; spasmotoxin; dihydrolutidin; putrescin; cadaverin; neuridin; saprin; pyocyanin; and tyrotoxon. Numerous others have been described, some toxic, others harmless.

3. *Chromogenesis*.—Those bacteria which produce colored colonies or impart color to the medium in which they grow are called *chromogenic*; those with which no color is associated, *non-chromogenic*. Most chromogenic bacteria are saprophytic and non-pathogenic. Some of the pathogenic forms, as the *Staphylococcus pyogenes aureus* and *citreus*, are, however, color-producers. It seems likely that the bacteria do not form the actual pigments, but certain chromogenetic substances which, uniting with substances in the culture-medium, produce the colors.

Galleotti has described two kinds of pigment, one of which, being soluble, readily penetrates all neighboring portions of the culture-medium, like the colors of *Bacillus pyocyaneus*, and an insoluble pigment which does not tinge the solid culture-media at all, but is constantly found associated with the colonies, like the pigment of *Bacillus prodigiosus*. The pigments are found in their greatest intensity near the surface of the colony. The coloring matter never occupies the protoplasm of the bacteria (except the *Bacillus prodigiosus*, in whose cells occasional pigment-granules may be seen), but occurs in an intercellular excrementitious substance.

The pigments are so varied as to give almost every known color. It sometimes happens that a bacterium will elaborate two or more colors. The *Bacillus pyocyaneus* thus produces pyocyanin and fluorescin, both being soluble pigments—one blue, the other green. Gessard has shown that when the *Bacillus pyocyaneus* is cultivated upon white of egg, it produces only the

green fluorescent pigment, while in pure peptone solution it grows with the production of blue pyocyanin alone. His experiments prove a 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 one pigment is soluble, the other insoluble, so that the colony will appear one color, the medium upon which it grows another. Some organisms will only produce their colors in the light; others, as the *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. Colored lights seem to have no modifying influence upon the pigment-production. Even if for successive generations the bacterium be grown so as to be colorless, it speedily recovers its primitive color when restored to its old environment, no matter what the color of the light thrown upon it. Bacteria which have been robbed of their color by incubation, when placed in the normal environment produce the original color, no matter what color the light they receive. Some of the pigments—perhaps most of them—are formed only in the presence of oxygen.

4. *Liquefaction of Gelatin.*—When certain forms of bacteria are grown in gelatin the culture-medium is partly or entirely liquefied. This characteristic is entirely independent of any other property of the bacterium, and is one manifested alike by pathogenic and non-pathogenic individuals. Sternberg and Bitter 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 that the property is not resident in the bacteria, but in some substance in solution in their excreted products. These products are 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. As some of the bacteria not only liquefy the gelatin, but do so in a peculiar and constantly similar manner, the presence or absence of the change becomes extremely useful for the separation of different species.

5. *Production of Acids and Alkalies.*—Under the head of "Fermentation" the formation of acetic, lactic, and butyric acids has been discussed. These, however, are by no means all the acids resulting from microbial metabolism. Ziegler mentions formic, propionic, baldricianic, palmitic, and margaric as being among those produced, and even this list may not comprehend them all. As the acidity due to the microbial metabolism progresses, it impedes, and ultimately completely inhibits, the development of the bacteria. The addition of litmus to the culture-medium is one of the best methods for detecting the acids. Milk to which litmus is added is particularly convenient. Rosalic acid may also be used, the acid converting its red into an orange color. The same tests will also determine the alkali-production, which occurs rather less frequently than acid-formation, and depends chiefly upon the salts of ammonium.

6. *Production of Gases.*—This seems, in reality, to be a part of the process of decomposition and fermentation. Among the gases due to bacterial action, CO_2 , H_2S , NH_4 , CH_4 , and others have been described. If the bacterium be anaërobic and develop at the lower part of a tube of gelatin, not infrequently a bubble of gas will be formed about the colonies. This is almost constant in tetanus and malignant edema. Ordinarily, the production or liberation of gases passes undetected, the vapors escaping from the surface of the culture-medium.

7. *Production of Odors.*—Of course such gases as H_2S and NH_3 are sufficiently characteristic to be described as odors. There are, however, a considerable number of pungent odors which seem dependent purely upon odor-

iferous principles dissociated from gases. Many of the odors are extremely unpleasant, as the fetid one caused by *Bacillus pyogenes foetidus*. The odor does not have any direct relation to decomposition, but, like the colors and acids, seems to be a peculiar individual characteristic of the metabolism of the organism.

8. *Production of Phosphorescence*.—A *Bacillus phosphorescens* and numerous other organisms have a distinct phosphorescence associated with their growth. It is said that so much illumination is sometimes caused by a gelatin culture of some of these as to enable one to tell the time by a watch. Most of them are found in sea-water, and are best grown in sea-water gelatin.

9. *Production of Aromatics*.—The most important of these is *indol*, which was at one time thought to be peculiar to the cholera spirillum. At present we know that a variety of organisms produce it, and that it and phenol, kresol, hydrochinon, hydroparacumaric acid, and paroxyphenylic-acetic acid are by no means uncommon.

10. *Reduction of Nitrites*.—A considerable number of bacteria are able to reduce nitrites present in the soil or in culture-media prepared for them into ammonia and nitrogen. To the horticulturist this is a matter of much interest. Winogradsky has found a specific nitrifying bacillus in soil, and asserts that the presence of ordinary bacteria in the soil causes the reduction of no nitrites so long as his special bacillus is withheld.

11. *Production of Disease*.—Bacteria which produce diseases are known as *pathogenic*; those which 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 will be entirely lost, while at times bacteria ordinarily harmless may be made toxic by certain manipulations or by introducing them into animals in certain combinations. The diseases produced are the result of the sum of numerous activities exhibited by the bacteria. For example, it may be that a microbe, having effected its

entrance into an animal, grows with great rapidity, completely blocking up the blood- and lymph-channels, so that the proper circulation of these fluids is stopped and disease and death must result. Perhaps more common than this is a local establishment of the organisms, with a resulting inflammation, due partly to the presence of the foreign organisms, and partly to their toxic metabolic products. More often, however, the pathogenic bacteria produce powerful metabolic poisons—toxins, ptomaines, etc.—which either cause widespread destruction of the tissues immediately acted upon, or, circulating throughout the organism, produce fever, nervous excitation, and a general overthrow of the normal physiological equilibrium. These peculiarities serve to divide the bacteria into

Septic bacteria,
Phlogistic bacteria,
Toxic bacteria.

The bacteria of suppuration probably act in several ways. Their products may be of a violently chemotactic nature, or their virulence, exerted upon the surrounding tissue, may destroy large numbers of the cells, whose dead bodies may be chemotactic. When the suppuration is violent the toxic product of the bacterium is itself most probably strongly chemotactic.

How the disease-producing bacteria effect their entrance into the tissues is an interesting question. The channels naturally open to them are those leading into the interior of the organism, and must be separately considered.

(a) *The Digestive Tract.*—Attention has already been called to the facility with which the bacteria enter the digestive tract in foods and drinks. Once their metabolism is in active progress, the poisons which they produce are ready for absorption. It seems probable that the absorption of the toxic substances by reducing the vitality of the individual predisposes to the formation of local lesions through which the bacteria may enter the intes-

tinal walls to continue their existence and produce greater damage than before. Some such theory may explain the activity of such organisms as those of typhoid and cholera, but it is not true that all bacteria can be admitted into the intestinal structure in this way. Before reaching the intestine the bacteria pass through the stomach, and must resist the deleterious action of the acid gastric juice, which few are able to do. Eichhorst has reported an epidemic of typhoid fever that occurred in a military barracks. In this epidemic the infection seemed to take place through the rectum, and was traced to the wearing of underclothing previously worn by patients and improperly washed.

(b) *The Respiratory Tract.*—Notwithstanding the moist interiors of the mouth and nose and the lashing cilia of the pharyngeal and tracheal mucous membrane, large numbers of bacteria enter the smaller bronchioles, and sometimes penetrate as deeply as the air-cells. It is unusual to find a section of healthy or diseased lung in which no bacteria can be found. It seems to have been proven by Buchner that micro-organismal infection may take place through the lungs without definite breach of continuity of the alveolar walls. He mixed anthrax spores and lycopodium powder together, and caused mice and guinea-pigs to inhale them. Out of the 66 animals used in his experiments, 50 died of anthrax and 9 of pneumonia. Our knowledge of the disposition of foreign particles in the lung probably explains such infection by assuming that the presence of the lycopodium attracted numerous leucocytes to the affected air-cells, that these took up the powder, and with it the spores, and that the leucocytes, being cells of very susceptible animals, were unable to resist the growth into bacilli of the spores which they had carried into the lymph-channels.

On the other hand, it has been shown that when the entering spores are unaccompanied by a mechanical irritant like the lycopodium powder, but are inspired

in a pulverized liquid, infection takes place much less readily.

Tuberculosis and pneumonia are in all probability generally the result of the inspiration of the specific organisms.

(c) *The Skin and the Superficial Mucous Membranes.*—The entrance of bacteria into the tissues by way of the skin is probably extremely rare if the skin is sound. Numerous experimenters have caused infection by rubbing bacteria or their spores upon the skin. It would seem probable that in these cases there must have been some microscopic lesions into which the bacteria were forced. My own investigations have shown virulent staphylococci of suppuration upon the conjunctivæ in health. It is very improbable that the bacteria habitually present upon the skin, and ready to enter the least abrasion, can penetrate the outer coverings of the body, except when disease or accident has rendered them abnormally thin or macerated.

Turro seems to have shown that the gonococcus can enter the tissues without any pre-existing lesion, for he asserts that if a virulent culture simply be touched to the meatus urinarius, the disease will be established.

(d) *Wounds.*—The results of the entrance of bacteria into unprotected wounds are now so familiar that no one deserving of the name of surgeon dares to allow a wound to go undressed.

(e) *The Placenta.*—Very frequently the occurrence of specific diseases during pregnancy causes abortion of the product of conception. In certain cases the specific contagion passes through the placenta and infects the fetus. This has been pretty clearly demonstrated for variola, malaria, syphilis, measles, anthrax, symptomatic anthrax, glanders, relapsing fever, typhoid, and in rare cases for tuberculosis.

Seeing that the channels by which bacteria can enter the body are so numerous, and that there is scarce a moment when some part of us is not in contact with

them, how is it that we are not constantly subject to disease? The consideration of this question, together with the closely-related questions why we should be subject to certain diseases only, and to these diseases at certain times only, must be reserved for another chapter, where the subjects *Immunity* and *Susceptibility* can be taken up at length.

CHAPTER III.

IMMUNITY AND SUSCEPTIBILITY.

ONE of the most astonishing facts observed in physiology and pathology is the resistance which certain animals show to the invasion of their bodies by the germs of disease.

Thus, man suffers from typhoid fever, cholera, and other infectious diseases which are never observed in the domestic animals; cattle are subject to a pleuro-pneumonia which does not affect their attendants; man, the cow, and the guinea-pig are peculiarly susceptible to tuberculosis, which the cat, dog, and horse resist; yellow fever is a highly contagious, infectious disease which is almost certain to attack all new arrivals of the human species when epidemic, but which rarely, if ever, attacks animals.

The popular mind accepts the statement of such facts as these without any other explanation than that the animals are different, and so of course their diseases are different; but the more the scientific man contemplates them, the more complicated the matter becomes; for, while it might be admitted that a difference in the body-temperature and chemistry might explain why a frog will resist anthrax, which readily kills a white mouse, it will not explain why a house-mouse, whose chemistry must be almost identical with that of the white mouse, can successfully combat the disease. Nor is this all. That one attack of yellow fever, of typhoid fever, or of scarlet fever renders a second attack almost impossible is not the less interesting because of its every-day observation. The mouse that has recovered from tetanus will not take tetanus again, and most interesting and

extraordinary is the fact that a few drops of blood from the recovered mouse injected into another will protect it from tetanus.

Immunity is the condition in which the body of an animal resists the entrance of disease-producing germs, or, having been compelled to allow them to enter, resists their growth and pathogenesis. The resistance so manifested is a distinct, potential vital phenomenon.

Susceptibility is the opposite condition, in which, instead of resistance, there is a passive inertia which allows the disease-producing organisms to develop without opposition. Susceptibility is accordingly the absence of immunity.

Immunity is either natural or acquired.

Natural Immunity.—By this term is meant the natural and constant resistance which certain healthy animals exhibit toward certain diseases.

The white rat is peculiar in resisting anthrax. It is almost impossible to develop anthrax in a healthy white rat, but Roger found that such an animal would easily succumb to the disease if compelled to turn a revolving wheel until exhausted. Susceptibility which follows such an exhaustion of the vital powers cannot be regarded as other than accidental, and makes no exception to the statement that the white rat is immune to anthrax. Animals such as man, sheep, cows, rabbits, and white mice are susceptible to anthrax, while birds and reptiles are generally immune. The great difference in the morphology between mammals and birds and reptiles, together with the fact that their temperature, blood, and tissues all differ, makes this immunity reasonably intelligible. Morphological differences, however, will not suffice to explain all cases, for the Caucasian nearly always succumbs to yellow fever, while the negro is rarely affected; and scarlatina, which is one of our commonest and most dangerous diseases of childhood, is said to be unknown among the Japanese. Nor is this all, for, close as is their resemblance in all respects except color, the house-mouse,

field-mouse, and white mouse differ very much in their susceptibility to various diseases.

Acquired immunity is resistance which is the result of accidental circumstances. It may result—

A. By recovery from a mild attack of the disease. Most adults have suffered from rubeola, scarlatina, and varicella in childhood, and in consequence of the attacks are now immune to these diseases—*i. e.* will not become affected again. One attack of yellow fever is always a complete guard against another. Typhoid fever is rarely followed by a second attack.

B. By recovery from an attack of a slightly different disease. Sometimes the immunity is experimentally produced, as when by vaccination we produce the vaccine disease and afterward resist variola. Acquired immunity is a little less complete and not so permanent as natural immunity, for in the latter it is only when the functions of the individual are disturbed or his vitality depressed that the resistance is lost, while in the former time seems to lessen the power of resistance, so that rubeola and scarlatina may return in a few months or years, and for complete protection vaccination may need to be done as often as every seven years.

C. By the injection of antitoxic substances. At present there is much agitation over the newly-discovered antitoxin of diphtheria, the injection of about 2 c.cm. of which will give complete protection against the disease for a period lasting from a month to six weeks.

Immunity may be destroyed in numerous ways:

(*a*) *By variation from the normal temperature* of the animal under observation. Pasteur observed that chickens would not take anthrax, and suspected that this immunity might be due to their high body-temperature. After inoculation he plunged the birds into a cold bath, reduced their temperature, and succeeded in destroying their immunity. The experiment was a success, but the reasoning seems to have been faulty, as the sparrow,

with a temperature equally high, readily falls a victim to anthrax without a cold bath.

(b) *By altering the chemistry of the blood* by changing the diet or by hypodermic injection. Leo found that when white rats were injected with or fed upon phloridzin an artificial glycosuria resulted which destroyed their natural resistance to anthrax. Hankin found that rats, which possess considerable immunity to anthrax, could be made susceptible by a diet of bread. Platania succeeded in producing anthrax in dogs, frogs, and pigeons, naturally immune, by subjecting them to the influence of curare, chloral, and alcohol.

(c) *By diminishing the strength of the animal.* Roger by compelling white rats to turn a revolving wheel until exhausted destroyed their immunity to anthrax.

(d) *By removing the spleen.* Bardach has shown that the chances of recovery from specific diseases are greatly lessened by the removal of the spleen.

(e) *By combining two different species of bacteria*, either of which, when injected alone, would be harmless or of slight effect. Roger found that when animals immune to malignant edema were simultaneously injected with 1 to 2 c.cm. of a culture of *Bacillus prodigiosus* and the bacillus of malignant edema, they would contract the disease. Pawlowski found that when rabbits, which are very susceptible to anthrax, were simultaneously injected with anthrax and prodigiosus, they recovered from the anthrax, as if the harmless microbe possessed the power of neutralizing the products of the pathogenic form.

Sometimes an apparent immunity depends upon the attenuation of the culture used for inoculation, and the erroneous results to which such a mistake may lead are obvious. Should a culture become attenuated, its virulence may sometimes be increased by inoculating it into the most susceptible animal, then from this to a less susceptible, and then to an immune animal. The virulence of anthrax is increased by inoculation into pigeons,

and also by cultivation in an infusion of the tissues of an animal similar to the one to be inoculated.

It must be understood that the term "immunity" is a relative one, and that while "a white rat is immune against anthrax in amounts sufficiently large to kill a rabbit, it is perhaps not immune against a quantity sufficiently large to kill an elephant."

It is not to be expected that such intricate phenomena as these which have been mentioned could be observed and suffered to go unexplained. We have explanations, but, unfortunately, they are as intricate as the phenomena, and, though each may possess its grain of truth, not one will satisfy the demands of the thoughtful student. In brief review, the theories of immunity are the following:

1. THE EXHAUSTION THEORY.—This hypothesis was advanced by Pasteur in 1880, and suggests that by its growth in the body the micro-organism uses up some substance essential to its life, and that when this substance is exhausted the microbe can no longer thrive. The removal of the necessary material, if complete, will cause permanent immunity.

As Sternberg points out, were this theory true we must have within us a material of small-pox, a material of measles, a material of scarlet fever, etc., to be exhausted by its appropriate organism. It would necessitate an almost inconceivably complex body-chemistry and a rather stable condition of the same.

2. THE RETENTION THEORY.—In the same year Chauveau suggested that the growth of the bacteria in the body might originate some substance prejudicial to their further and future development. There seems to be a large kernel of truth in this, but were it always the case we would have added to our blood a material of small-pox, a material of measles, a material of scarlet fever, etc., so that we would become saturated with the excrementitious products of the bacteria, instead of having so many substances subtracted from our chemistry.

3. THE THEORY OF PHAGOCYTOSIS.—In 1881, Carl

Roser suggested a relation between immunity and the already familiar phenomenon of phagocytosis. Sternberg in the United States and Koch in Germany observed the same thing, but little real attention was paid to the subject until 1884, when Metchnikoff appeared, with his careful observations upon the daphnia, as the great champion of the theory which is now known as "Metchnikoff's theory of phagocytosis."

Phagocytosis is the swallowing or incorporating of particles by certain of the body-cells which are called *phagocytes*. This activity of the cells toward inert particles had been observed by Virchow as early as 1840, and toward living bacteria by Koch in 1878, but was not carefully studied until 1884. Metchnikoff divides the phagocytes into *fixed phagocytes*, comprising the fixed connective-tissue cells, endothelium, etc., and the *free phagocytes*, which are the leucocytes. The terms "phagocyte" and "leucocyte" are not to be regarded as synonymous in this connection; all leucocytes are not phagocytic, the *lymphocyte* having never been observed to take up bacteria.

It is obvious that only those cells can be phagocytic which are without a resisting cell-wall and possess ameboid movement. When an ameba, in a liquid containing numerous diatoms and bacteria, is watched through the microscope, an interesting phenomenon is observed. The ameba will approach one of the vegetable cells, even though it may be at a distance, will apprehend and surround it, and within the animal cell the vegetable cell will be digested and assimilated. The ameba has no eyes, no nose, no volition, and, so far as we can determine, no nervous apparatus which gives it tactile sense, yet it will approach the particle fitted for its use and swallow it. The attraction which draws the cells together has been called by Peffer *chemotaxis*, *chemiotaxis*, or *chemotropism*.

Chemotaxis is the exhibition of an attractive force between cells and their nutriment, ameboid cells and

food-particles, and sometimes between ameboid cells and inert particles. This attractive force, when operating so as to draw the ameba to the particle it will devour, is further named *positive chemotaxis* in order to distinguish it from a repulsive force sometimes exerted causing the ameboid cells to fly from an enemy, as it were, and which is called *negative chemotaxis*.

The force that operates and guides the ameba in its movements is exactly the same as that which governs the movement of the phagocytic cells of the human body, and observation of these phenomena is not difficult. If a small capillary tube be filled with sweet oil and placed beneath the skin, only a short time need pass before it will be found full of leucocytes—positive chemotaxis. If, instead of sweet oil, oil of turpentine be used, not a leucocyte will be found—negative chemotaxis.

Phagocytosis is almost universal in the micro-organismal diseases at some stage or another. If the blood of a patient suffering from relapsing fever be studied beneath the microscope, it will be found to contain numerous active mobile spirilla, all free in the liquid portion of the blood. As soon as the apyretic stage comes on not a single free spirillum can be found. Every one is seen to be enclosed in the leucocytes.

At the edge of an erysipelatous patch a most active warfare is waged between the streptococci and the cells. Near the centre of the patch there are many free streptococci and a few cells. At the margin there are free streptococci, and also a great many streptococci enclosed in cells (leucocytes) which are, for the most part, dead. In the newly-invaded tissue we find hosts of active living cells engaged in eating up the enemies as fast as they can. The phagocytologists tell us that at the centre the bacteria are fortified, actively growing, and virulent; in the next zone the leucocytes which have feasted upon the bacteria are poisoned by them; outside, the cells, which are more powerful and which are constantly being reinforced, are waging successful warfare

against the streptococci. In this manner the battle continues, the cells now being obliged to yield to the bacteria and the patch spreading, while the cells subsequently reinforce and destroy the bacteria, so that the disease comes to a termination.

Metchnikoff introduced fragments of tissue from animals dead of anthrax under the skin of the back of a frog, and found it surrounded and penetrated by leucocytes containing many of the bacilli.

It need scarcely be pointed out that a loophole of doubt exists in all these illustrations: the bacteria may have been dead before the cells ingested them, and the phenomena of digestion and destruction which have gone on in their interiors may have been exerted upon dead bacteria. To the relapsing-fever illustration we may take exceptions, and state that the apyrexia may have marked the death of the spirilla, which were taken up by the leucocytes only when dead. In the erysipelas illustration the streptococci remote from the centre of the lesion may have met from the body-juices or some other cause a more speedy death than that from the digestive juices of the leucocyte.

Metchnikoff, however, is prepared to show us that the leucocytes do take up living pathogenic organisms. He succeeded in isolating two leucocytes, each containing an anthrax spore, and conveying them to artificial culture-media, where he watched them. The new environment being better adapted to the growth of the spore than for the nourishment of the leucocyte, the latter died, and the spore developed under his eyes into a healthy bacillus. Seeing that the animal cells take up bacteria, and seeing that the ameba can ingest and digest "threads of leptothrix ten times as long as itself," we need only put two and two together to see that Metchnikoff's theory rests upon a very substantial foundation. The more virulent the bacteria, the less ready the leucocytes are to seize them. The more immune the animal, the greater is the affinity of the leucocyte for the bacteria.

The organisms which are seized upon by the leucocytes

do not remain in the blood, but are collected in the spleen and the lymphatic glands; and not the least important fact in favor of phagocytosis is that observed by Bardach, that excision of the spleen diminishes the resistance to infectious disease.

Quinin also furnishes a therapeutic support to the theory. It is known that quinin increases the destruction of leucocytes. Woodhead inoculated a number of rabbits with anthrax, giving quinin to some of them. Those which had received the drug died earliest—a result probably dependent upon the destruction of part of the phagocytic army.

Ruffer found that the "phagocytes evince a distinct selective tendency between various kinds of organisms. They will leave the bacillus of tetanus in order to seize upon the *Bacillus prodigiosus* if simultaneously introduced; also the streptococci in diphtheria for the Klebs-Löffler bacilli. This is illustrated in the diphtheritic membrane, where at the surface one can see leucocytes taking in numbers of the bacilli, but leaving the streptococci almost untouched, with the immediate result that streptococci are often found in the deeper parts of the membrane, and with the remote result that secondary abscesses occurring in the course of diphtheria are never due to the bacillus of diphtheria, but to some other organism."

Hankin and Hardy found that the three varieties of leucocytes in the frog's blood play important parts in the destruction of anthrax bacilli, this destructive process being accomplished thus:

1. The eosinophile cells are first to approach and swallow the bacteria. As this takes place the eosinophile granules are seen to dissolve and act upon the bacteria.
2. The hyaline cells take up the remains of the bacteria destroyed by the eosinophile leucocytes.
3. The basophile cells come to the field loaded with basophilic granules, supposed to be antidotal to the poisons of the bacteria, surround the combatants, neu-

tralize the bacterial poisons, and liberate the contesting cells.

Wyssokowitsch found that saprophytic micro-organisms are quickly eliminated from the blood when injected into the circulation. This elimination is not by excretion through organs nor by destruction in the streaming blood, but by collection in the small capillaries, where the blood-stream is slow and where the micro-organisms are taken up by the endothelial cells. Wyssokowitsch found them most numerous in the liver, spleen, and bone-marrow, and found that in these situations they were destroyed in a short time—saprophytic in a few hours, pathogenic in from twenty-four to forty-eight hours. Spores of *Bacillus subtilis* remained as living entities in the spleen for three months.

4. THE HUMORAL THEORY.—It was observed that if anthrax bacilli were introduced into a few drops of rabbit's blood, they were instantly killed. This observation was one of immense importance, and from it and similar observations Buchner deduced the principles of his theory, which teaches that the destruction of pathogenic bacteria in the body is due to the *bactericidal action of the blood-plasma*, not to phagocytosis; which phenomenon amounts to nothing more than the burial of the dead bacteria in "cellular charnel-houses." The experiments of Buchner and his followers have shown that freshly-drawn blood, blood-plasma, defibrinated blood, aqueous humor, tears, milk, urine, and saliva possess marked destructive influence upon the organisms brought in contact with them—an influence easily destroyed by heat.

The apparent paradox of rapid multiplication of anthrax bacilli in the rabbit's blood enclosed in the rabbit's body, and the reversed action in the test-tube, caused immediate and prolonged opposition to the theory. Each side of the question seemed well supported. The phagocytologists, however, showed that bacteria were often injured and their vegetative powers destroyed by sudden changes

from one culture-medium to another, this being proved by Haffkine, who in experimenting with aqueous humor has shown that its germicidal actions are largely imaginary, and due to the dispersion of the organisms in a large amount of watery liquid. When the micro-organisms are introduced into it in such a manner as to remain together, they grow well. If the tube be shaken, so as to distribute them, they die. Again, Adami has shown that when blood is shed there is almost always a pronounced destruction of corpuscles, and suggests that the antibiotic property of the shed blood may be due to solution of the nucleins formerly in the substance of the leucocytes. Jetter endeavored to prove the germicidal action of the serum to be due to certain salts which it contained. His experiments, which consisted in observing the action of solutions of various salts in mixtures of water, glycerin, and gelatin, were justly condemned by Buchner on the ground that such mixtures, though they might contain constituents of blood-serum, were far from approximating the normal serum in composition.

Wysokowitsch, however, surely argued against humoral germicide when he showed that the spores of *Bacillus subtilis* could reside in the spleen for three months uninjured.

In supporting their theory the humoralists experimented by placing beneath the skin micro-organisms enclosed in little bags of pith, collodium, etc. These bags allowed the fluids of the body free access to the bacteria, but would shut out the phagocytes. By these means Hüppe and Lubarsch have repeatedly seen the bacteria grow well, while the attempts of Baumgarten have failed. Such experiments are by no means conclusive, for we should remember that the operation necessary and the presence of the foreign body in which the bacteria are encased produce an inflammatory transudate which may have properties very different from those of the normal juices.

How much of the immunity which animals enjoy de-

depends upon the antibactericidal action of their body-juices must remain an open question. In some cases the germicidal action of the blood seems to be unquestionable. Buchner has shown that the blood-serum of animals only possesses this germicidal power when freshly drawn, and that exposure of the serum to sunlight, its mixture with the serum from another species of animal, its mixture with distilled water or with dissolved corpuscles, and heating it to 55° C., check the bactericidal power. Buchner also points out that the bactericidal and globulicidal actions of the blood are simultaneously extinguished.

Much discussion has arisen as to exactly what the protective substances are. Buchner has applied the term *alexin* to the protective proteid substances found in the blood of naturally immune animals. Hankin has given us, together with an extension of Buchner's idea, a considerable nomenclature of somewhat questionable utility. He divides the protective substances (alexins) into *sozins*, which occur in the blood of animals with natural immunity, and *phylaxins*, which occur in the blood of animals with acquired immunity. Both *sozins* and *phylaxins* are divisible into two groups—thus: a *sozin* which acts destructively upon bacteria is called a *myco-sozin*; one which neutralizes bacterial poisons, a *toxosozin*. A *phylaxin* which acts destructively upon bacteria is called a *myco-phylaxin*; one which neutralizes bacterial toxins, a *toxophylaxin*. A glance will show that this classification is based upon the somewhat doubtful existence of alexins.

5. THE THEORY OF ANTITOXINS.—It is a well-known fact that individuals can accustom themselves to the use of certain poisons, as tobacco, opium, and arsenic, so as to experience no inconvenience from what would be poisonous doses for other individuals. This is purely a matter of tolerance, but is of interest in connection with the observations which are to follow.

Ehrlich has shown that animals can tolerate gradually-

increasing doses of ricin and abrin, provided that up to a certain point the increase of dosage is very small. When this point is, however, safely passed, the increase in dosage can be very rapid, yet without signs of poisoning, seemingly because the drug is no longer simply tolerated, but tolerated and simultaneously neutralized. By experimentation Ehrlich has shown that during the period of simple tolerance the blood of the animal is unaltered, but that as soon as the tolerance becomes unlimited the blood contains a new substance, capable not only of protecting the animal by which it is produced, but also other animals into whose blood it is introduced. In the ricin experiments this substance was described as *antiricin*; in the experiments with abrin, as *antiabrin*.

These investigations of Ehrlich with the poisons of higher plants succeeded, but threw much light upon, the extraordinary work of Behring, Wernicke, and Kitasato, who experimented with the toxins of diphtheria and tetanus, and showed that in the blood of animals accustomed to these poisons, new substances—*antitoxins*, found by Brieger to be proteid in nature—were produced.

The antitoxic theory of immunity, being, in the cases cited at least, a fact capable of demonstration, has established itself at present as the most important hypothesis. According to it, acquired immunity, at least, depends upon the development in the blood of a neutralizing substance probably related to the nucleins.

It is of prime importance to remember that the antitoxin is an entirely new substance which does not occur in the blood of normal animals.

The difference between this theory of neutralization by antitoxins and Chaveau's retention hypothesis is quite marked. The retention theory teaches that a bacterium leaves behind it a substance prejudicial to its future growth in the economy—a distinct metabolic product. The antitoxic theory shows the protective substance to be a product not of bacterial growth, but of tissue-energy,

not depending upon the presence of the bacteria, but upon the presence of a poison.

The antitoxins do not act harmfully upon the bacteria, do not preclude their growth in the animal body, but prevent their pathogenesis by annulling their toxicity—*i. e.* enabling the body-cells to endure the injury—and placing them in a position exactly parallel with non-pathogenic bacteria.

The diseases which are at present controllable by antitoxins are *toxic* diseases, caused by the entrance of toxin-producing bacteria into the body. The growth of these toxin-producers probably depends upon the inability of the body-cells or bactericidal body-juices to properly cope with them, so that they develop and engender the poisonous substances which are the essential factors of disease-production. The more the body and its component elements are injured, the more successful the inroads of the bacteria, the more prolific the toxin-production, and the more severe the affection.

The presence of the antitoxin annuls the poison, maintains the vitality of the organism as a whole, sustains the integrity of its tissues, and so places the pathogenic bacterium on a very different footing in relation to the organism.

An antitoxin is a neutralizing or annulling agent, not a regenerating one, and therefore in therapeutics finds its proper sphere only in the beginning of the disease combated. Up to a certain point the symptoms of diphtheria and tetanus are due to the circulation of toxins in the blood, and can be successfully combated by antitoxic neutralization. Later in both diseases we have symptoms resulting from disorganization of the nervous system, degeneration of the heart-muscle, destruction of the kidneys, etc., and the neutralization of the poison can be of no avail because the lesions are irreparable, and the patient must succumb.

I have used the term "neutralization," in speaking of the antitoxins, in a rather free and scarcely warranted

manner, and must call attention to the fact that their operation is in no way analogous to chemical neutralization. From mixtures of toxin and antitoxin the unchanged poison has been recovered. The effect of an antitoxin, unlike that of a toxo-phylaxin, seems to be a biologic one, by which the tissues are so stimulated as to endure the action of a substance ordinarily disorganizing in effect.

Buchner and Roux have both pointed out that when the toxins and antitoxins are mixed and introduced into animals of greater susceptibility than are ordinarily used, the presence of an unaltered toxin can easily be demonstrated.

According to Buchner, the antitoxins differ from the alexins in being new substances in the blood, in being without germicidal or chemical neutralizing power against the toxins, and in being stable compounds which can resist heat to 75° C., can resist a reasonable amount of exposure to light, and which are not altered by decomposition of the substances containing them.

The antitoxins are specific for one poison only. Ehrlich found that antiricin was powerless against abrin, and *vice versa*. Diphtheria antitoxin is of no avail against tetanus, and *vice versa*.

The immunity which the antitoxins produce is fugacious, varying considerably according to the particular substance employed. As a rule, it is limited to a few months—at least in the case of such antitoxins as we can produce experimentally.

From all that has gone before it must be clear to the reader that no single theory thus far advanced can explain immunity. Acquired immunity may depend in the great majority of cases upon antitoxins, but as yet we have no satisfactory explanation of natural immunity. The humoral theory may be applicable in some cases; in others one cannot deny the importance of the rôle played by the phagocytes.

CHAPTER IV.

METHODS OF OBSERVING BACTERIA.

WHOEVER would study bacteria must be equipped with a good microscope. The instruments generally provided for the use of medical students in college laboratories, as well as those seldom-employed "show microscopes" seen in physicians' offices, are ill adapted for the purpose. The essential features of a bacteriological instrument are lenses giving a *clear* magnification extending as high as one thousand diameters, and a good condenser for intensifying the lights thrown upon the objects. It naturally follows that the best work requires the best lenses. The cheapest good microscope which is at present offered to the public is the BB. Continental stand, made by Bausch and Lomb. This stand is provided with everything necessary, is fitted with very creditable objectives, including an excellent $\frac{1}{2}$ " oil-immersion lens, and seems capable of doing very good work. I do not recommend this as the best instrument obtainable, but as one that is both good and cheap. For those who desire the very best the rather costly outfits made by Carl Zeiss of Jena are unexcelled.

For those who may begin the use of the Abbe condenser and oil-immersion lenses without the advantage of personal instruction a few hints will not be out of place:

Always employ good slides without bubbles, and thin cover-glasses; No. 1 are best.

Place a drop of oil of cedar upon the cover-glass of the specimen to be examined; rack the body of the instrument down until the oil-immersion lens touches the oil;

keep on until it *almost* touches the glass, then look into the microscope and find the object by slowly and firmly racking *up*. As soon as the object comes into view leave the rack and pinion and focus with the fine adjustment.

Always select the light from a white cloud if possible ; if there are no white clouds, choose the clearest whitest light possible. *Never under any circumstances employ sunlight*, which is ruinous to the eyes and useful only for photomicrography.

In using low-power lenses the Abbe condenser must be moved away from the object and the light modified by the iris-diaphragm. The distance between condenser and object should correspond more or less closely with the distance between objective and object.

In using high powers the Abbe condenser must be brought near the object and the light modified by the iris-diaphragm.

If the oil-immersion lens is used, it is perhaps best to employ the plane side of the mirror. When with this lens a section of tissue is examined for details, the light must be modified by the iris-diaphragm, opening and closing it alternately until the best effect of illumination is achieved. If tissue be searched for stained bacteria, and no cellular detail is required, the diaphragm should be wide open to admit a great flood of light and extinguish everything except the deeply-colored bacteria.

When unstained bacteria are to be examined with the oil-immersion lens, the diaphragm should be closed so as to leave only a small opening through which the light can pass.

Bacteria may be examined either stained or unstained. The former condition would always be preferable if the process of coloring the organisms did not injure them. Unfortunately, it is generally the case that the drying, heating, boiling, macerating, and acidulating to which we expose the organisms in the process of staining alter

their shape, make their outlines less distinct, break up their arrangement, and disturb them in a variety of other ways. Because of the possible errors of appearance resulting from these causes, as well as because it must be determined whether or not the individual is motile, in making a careful study of a bacterium it must always be examined in the living, unstained condition.

The simplest method of making such an examination would be to take a drop of the liquid, place it upon a slide, put on a cover, and examine.

While this method is simple, it cannot be recommended, for if the specimen should need to be kept for a time much evaporation takes place at the edges of the cover-glass, and in the course of an hour or two has changed it too much for further use. The immediate occurrence of evaporation at the edges also 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.

The best way to examine living micro-organisms is in what is called the *hanging drop* (Fig. 5). A hollow-

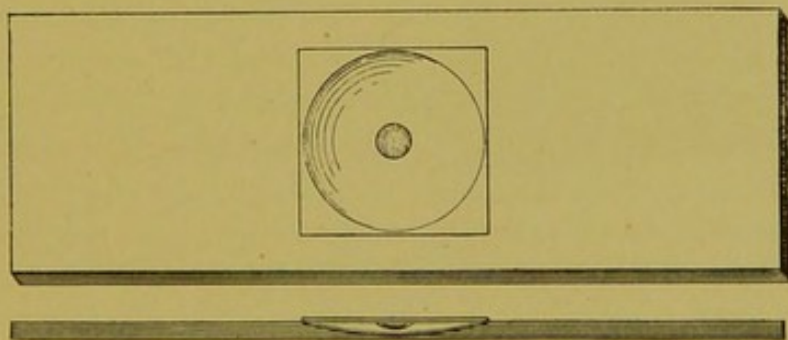


FIG. 5.—The "hanging drop" seen from above and in profile.

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 at its centre. A drop of the material to be examined is placed in the centre of a large clean cover-glass, and then placed upon the slide so

that the drop hangs in, but does not touch, the concavity. The micro-organisms are now hermetically sealed in an air-chamber, and appear under almost the same con-

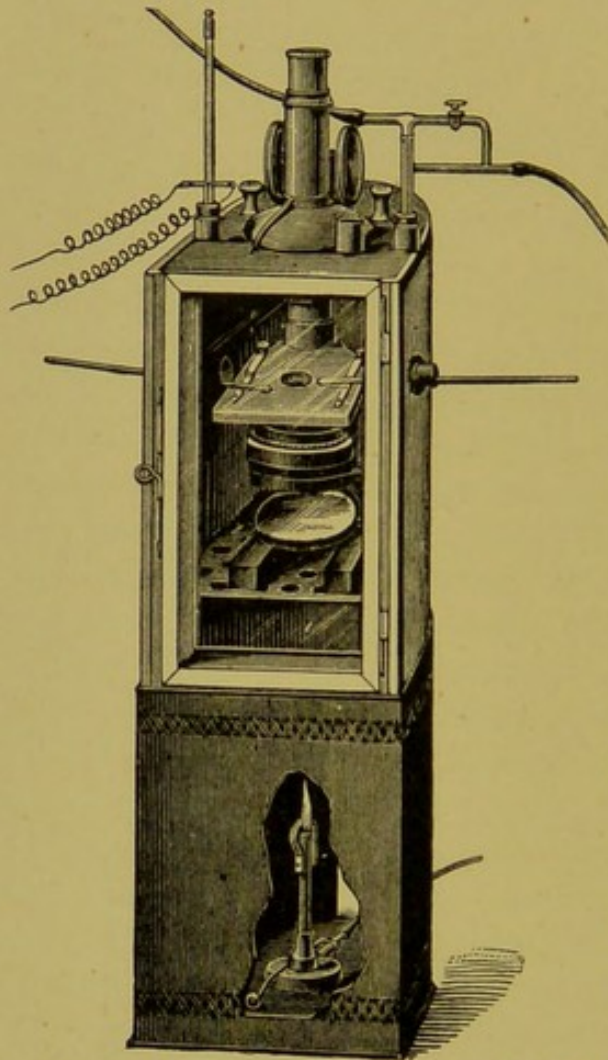


FIG. 6.—Apparatus for keeping objects under microscopic examination at constant temperatures.

ion, 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 very readily 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

conditions as in the culture. Such a specimen may be kept from day to day and examined, the bacteria continuing to live until the oxygen or nutriment is exhausted. By means of a special apparatus (Fig. 6), in which the microscope is stood, the growing bacteria may be watched at any temperature, and very exact observations made.

The hanging drop should always be examined at the edge, as the centre is too thick.

In such a specimen it is possible to determine the shape, size, grouping, division,

mixed up in a drop of sterile bouillon or water and examined.

In the early days of study efforts were made to facilitate the observation of bacteria by the use of carmin and hematoxylon. Both of these reagents tinge the protoplasm of the organisms a little, but so unsatisfactorily that since Weigert introduced the anilin dyes for the purpose both of these tissue-stains have been rejected. The affinity between the bacteria and the anilin dyes is peculiar, and many times is so certain a reaction as to become an essential factor in the differentiation of species.

For the study of bacteria in the stained condition we now employ the anilin dyes only. These wonderful colors, as numerous as the rainbow hues, are coal-tar products. Hüppe classifies them as follows:

A. Dyes prepared from anilin oil.

1. Oxidation-products of pure anilin :
Methylene blue,
Chlorhydrin blue (basic indulin).
2. Oxidation-products of pure toluol :
Safranin.
3. Oxidation-products of mixed anilin and toluol :
(*a*) Rosanilin. When pure this is triamido-diphenyl-toluyl-karbinol.
Fuchsin—rosanilin hydrochlorate. It is often mixed with the acetate and the pararosanilin acetate and hydrochlorate. The pure rosanilin hydrochlorate should always be chosen for purposes of staining.
Azalein is rosanilin nitrite.
Methylized and ethylized rosanilin :
Iodin violet,
Dahlia,
Iodin green.
- (*b*) Pararosanilin. The colorless pure pararosanilin is triamido-triphenyl-karbinol.

Rubin-pararosanilin hydrochlorate.
 Methylized, ethylized, and benzylicized
 pararosanilid :
 Crystal violet,
 Gentian violet,
 Victoria blue,
 Methyl green,
 Auramin.

The rosanilins are more difficult to prepare than the pararosanilins, and are generally mixed with them. The pararosanilins color more sharply than the rosanilins.

4. Amido-azo combinations :
 Bismarck brown,
 Phenylene brown,
 Vesuvin.
5. Chinolin derivatives :
 Cyanin.

B. Naphthalin group.—Magdala red.

The best anilin dyes made at the present time, and those which have become the standard for all bacteriological work, are made in Germany by Dr. Grüber. In ordering the stain the name of this manufacturer should always be specified.

A whole volume could easily be devoted to scientific staining. Indeed, the technical difficulties encountered are so great that no explanations can be too thorough to be useful. The special methods essential for such bacteria as have peculiar staining reactions will be given with the description of the organism. General methods only will be discussed in this chapter.

Cover-glass Preparations for General Examination.
 —The material to be examined must be spread in the thinnest possible layer upon the surface of a perfectly clean cover-glass, and dried. Here it may be remarked that for bacteriological purposes thin covers (No. 1) are generally required, because thick glasses interfere with the focussing of the oil-immersion lenses, and that cover-

glasses can never be too clean. It is best to immerse them first in a strong mineral acid, then to wash them in water, then in alcohol, then in ether, and keep them in ether until they are to be used. Except that it sometimes cracks, bends, or fuses the edges of the glasses, a better and more convenient method of cleaning them is to wipe them as clean as possible, seize them in fine-pointed forceps, pass them repeatedly through a small Bunsen flame until it becomes greenish yellow, then slowly elevate the glasses above the flame, so as to allow them to anneal. This manœuvre removes the organic matter by combustion. It is not expedient to use covers twice for bacteriological work, though if well cleaned they may subsequently be employed for ordinary microscopic objects.

To return: After the material spread upon the cover has dried, it must be fixed to the glass by immersion for twenty-four hours in equal parts of absolute alcohol and ether, or, as is much easier and more rapid, be passed *three times through a flame*. Three is not a magic number, but experience has shown that when drawn through the flame three times the desired effect seems best accomplished. The Germans recommend that a Bunsen burner or a large alcohol lamp be used, that the arm holding the forceps containing the cover-glass inscribe a circle a foot in diameter, and that, as each revolution occupies a second of time, the glass be 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.

After fixing, the material is ready for the stain. Every laboratory should be provided with several *stock-solutions* of the more ordinary dyes. These stock-solutions are *saturated alcoholic* solutions made by adding 25 grams of the dye to 100 c.cm. of alcohol. Of these it is well to have fuchsin, gentian violet, and methylene blue always made up. The stock-solutions will not stain, but are the standards for the manufacture of the working stains.

For ordinary staining an aqueous solution made in a simple manner is employed. A small bottle is nearly filled with distilled water, and the stock-solution is added, drop by drop, until the color becomes just sufficiently intense to prevent the ready recognition of objects through it. Such a watery solution possesses the power of readily penetrating the dried protoplasm of the bacterium, taking the stain with it. Alcohol does not have this power.

As in the process of staining the cover is apt to slip from the fingers and spill the stain, it is well to be provided with cover-glass forceps (Fig. 7), which hold the

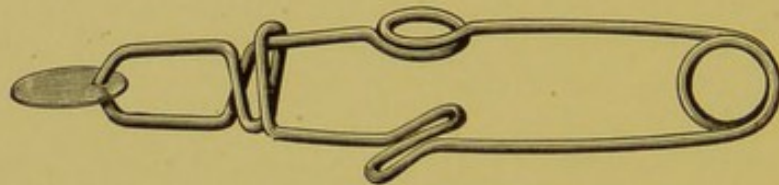


FIG. 7.—Stewart's cover-glass forceps.

glass in a firm grip and allow of all manipulations without danger to the fingers or clothes. The ordinary instruments are entirely unfitted for the purpose, as capillary attraction draws the stain between the blades and makes certain the soiling of the fingers. Sufficient stain is allowed to run from a pipette upon the smeared side of the cover-glass 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. If the specimen is one prepared for temporary use, it can be examined at once, mounted in a drop of water, but under these conditions will not appear as advantageously as if dried and then mounted in Canada balsam.

Sometimes the material to be examined is too solid to spread upon the glass conveniently. Under such circumstances a drop of distilled water can be added and a minute portion of the material be mixed in it upon the glass.

The entire process is, in brief :

1. Spread the material upon the cover ;
2. Dry—do not heat ;
3. Pass three times through the flame ;
4. Stain

two to three minutes; 5. Wash thoroughly in water; 6. Dry; 7. Mount in Canada balsam.

This simple process suffices to stain most bacteria.

Staining Bacteria in Sections of Tissue.—It not infrequently happens that the bacteria to be examined are scattered among or enclosed in the cells of tissues. Their demonstration is then a matter of some difficulty, and the method employed is one which must be modified according to the kind of organism to be stained. Very much, too, depends upon the 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 the cells and bacteria. Afterward they are removed from the absolute alcohol and kept in 80–90 per cent., which does not shrink the tissue. Bichlorid of mercury may also be used, but absolute alcohol seems to answer every purpose.

For ordinary work the following simple method is recommended: After the sections are cut, the paraffin must be, and the celloidin would better be, removed. From water the sections are placed in the same watery stain used for cover-glasses and allowed to remain five to eight minutes. They are next washed in water for several minutes, then decolorized in 0.5–1 per cent. acetic-acid solution. The acid removes the stain from the tissues, and ultimately from the bacteria as well, so that one must watch carefully, and as soon as the color almost disappears from the sections remove them to absolute alcohol. At this point the process may be interrupted to allow the tissue-elements to be counter-stained 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.

As will be mentioned hereafter, certain of the bacteria which occur in tissue do not allow of the ready penetra-

tion of the color. For such forms a more intense stain must be employed. One of the best of these stains, which can be employed by the given method both for cover-glasses and tissues, is Löffler's alkaline methylene blue :

Saturated alcoholic solution of methylene blue, 30 ;
1 : 10,000 aqueous solution of caustic potash, 100.

Some bacteria, as the typhoid-fever bacillus, decolorize so rapidly as to contraindicate the use of acid for the differentiation, washing in water or alcohol being sufficient.

Gram's Method of Staining Bacteria in Tissue.—Gram was the fortunate discoverer of a method of staining bacteria in such a manner as to saturate them with an insoluble color. It will be seen at a glance what a marked improvement this is on the method given above, for now 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. Its prosecution is 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, fuchsin, or any basic anilin color may be used. The mixture does 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 it in very small quantity by pouring about 1 c.cm. of pure anilin into a test-tube, filling the tube about one-half with distilled water, shaking the mixture well, then filtering as much as is desired into a small dish. To this the saturated alcoholic solution of the basic 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.

While the specimen is in the Gram's solution it appears to turn a dark blackish-brown color. When removed from the solution it is carefully washed in 95 per cent. alcohol until no more color is given off and the tissue assumes a grayish color. If it is simply desired to find the bacteria, the section is dehydrated in absolute alcohol for a moment, cleared up in xylol, and mounted in Canada balsam. If it is necessary to study the relation between the bacteria and the tissue-elements, a nuclear stain, such as alum carmin or Bismarck brown, may be subsequently used. Should a nuclear stain requiring acid for its differentiation be desirable, the process of staining must precede the Gram method altogether, so that the acid shall not act upon the stained bacteria.

The success of Gram's method rests upon the fact that *the combination of mycoprotein, basic anilin, 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 momentarily 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 up in xylol ;

Mount in Canada balsam.

This method stains a large variety of bacteria very beautifully, but, unfortunately, does not stain them all, and as some of those which do not stain are important, it seems well to mention the—

Spirillum of cholera and of chicken-cholera ;
Bacillus mallei (of glanders) ;
Bacillus of malignant edema ;
Bacillus pneumoniæ of Friedländer ;
Micrococcus gonorrhœæ of Neisser ;
Spirochæte Obermeieri of relapsing fever ;
Bacillus of typhoid fever ;
Bacillus of rabbit-septicemia.

Gram's method is a method of staining bacteria in tissues, but the fact that the method colors some but not all bacteria is one of considerable importance from a differential point of view ; and as the difficulty of separating the species of bacteria is so great that every such point must be eagerly seized for assistance, this method becomes one much employed for cover-glass preparations, where it is more easily performed than for sections.

Gram's Method for Cover-glass Preparations.—A thin layer of the bacteria to be examined is spread upon the cover-glass, dried, and fixed. The cover, held in the grip of a cover-glass forceps, is flooded with Ehrlich's solution. By holding the cover flooded with stain over a small flame for a moment or two the solution is kept warm, and the process of staining is continued from two to five minutes. If the heating causes the stain to evaporate, more of it must be dropped upon the glass, so that it does not dry up and incrust.

The stain is poured off, and the cover placed in a small dish of Gram's solution and allowed to remain one-half to two minutes, the solution being agitated. It is possible to apply the Gram solution in the same manner in which the stain is used, but as a relatively larger quantity should be employed, the dish seems preferable.

The cover is next washed in 95 per cent. alcohol until

the blue color is wholly or almost lost, after which it can be counter-stained with 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 ;
- Counter-stain if desired ; wash the counter-stain off with water ;
- Dry ;
- Mount in Canada balsam.

Method of Staining Spores.—It has already been remarked that the peculiar quality of the spore-capsules protects them from the influence of stains and disinfectants to a certain extent. 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 five to fifteen minutes. The cover is now 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, then removed, washed in water, and counter-stained with an aqueous solution of methyl or methylene blue.

In such a specimen the spores should appear red, the bacilli blue.

I have not generally found that spores color so easily, and for many species the best method seems to be to place the prepared cover-glass in a test-tube half full of carbol-fuchsin:

Fuchsin,	1 ;
Alcohol,	10 ;
5 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 counter-stained blue.

Fiocca suggests the following rapid method: "About 20 c.cm. of a 10 per cent. solution of ammonium are poured into a watch-glass, and 10-20 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 counter-stained with a watery solution of vesuvin, chrysoïdin, 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."

Method of Staining Flagella.—This is much more difficult than the staining of either the bacteria or their spores, because each species seems to behave differently in its relation to the stain, so that the chemistry of the micro-organismal products must be taken into consideration.

The best method introduced is that of Löffler. In it three solutions are used :

- A. A 20 per cent. solution of tannic acid, 10 ;
Cold saturated aqueous solution of ferrous sulphate, 5 ;
Alcoholic solution of fuchsin or methyl violet, 1.
- B. A 1 per cent. solution of caustic soda.
- C. An aqueous solution of sulphuric acid of such strength that 1 c.cm. will exactly neutralize an equal quantity of Solution B.

Some of the bacteria to be stained are mixed upon a cover-glass with a drop of distilled water. This is the first dilution, but is too rich in bacteria to allow the flagella to show well, so that it is recommended to prepare a second dilution by placing a small drop of distilled water upon a cover and taking a small portion from the first cover to the second, spreading it over the entire surface. The material 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, is dropped upon it until it is well covered. The cover is warmed until it begins to steam, and the stain 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 in distilled water, then in 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 now dropped on so as to cover the specimen, and heated for a minute until vapor begins to arise; it is then washed off carefully, dried, and mounted in Canada balsam. To obtain this neutral reaction 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. Such a specimen may or may not show the flagella. If not, before proceeding farther it is necessary to study the products of the bacterium in culture-media. If by its growth the organism elaborates alkalies, Solution C, in proportion from 1 drop to 1 c.cm. in 16 c.cm. of the mordant A, must be added, and the process repeated again and again until the proper amount is determined. On the other hand, if the organism by

its growth produces acid, Solution B must be added, drop by drop, until 1 in 16 cm. have been attained, and numerous experiments made to see when the flagella will appear. Löffler has fortunately worked out the amounts required for some of the species, and of the more important ones the following amounts of Solutions B and C must be added to 16 c.cm. of Solution A to attain the desired effect:

Cholera spirillum,	$\frac{1}{2}$ -1 drop of Solution C;
Typhoid fever,	1 c.cm. of Solution B;
Bacillus subtilis,	28-30 drops of Solution B;
Bacillus of malignant edema,	36-37 drops of Solution B.

Part of the success of the staining depends upon having the bacteria thinly spread upon the glass, and 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.

Bunge suggests a mordant consisting of a concentrated aqueous tannin solution and a 1:20 solution of liq. ferri sesquichloridi in water. The best mixture seems to be 3 parts of the tannin solution to 1 part of the diluted iron solution. To 10 c.cm. of this mixture 1 c.cm. of a concentrated aqueous fuchsin solution is added. It is not necessary to prepare this mordant fresh for each staining, as it seems to improve with age. The use of acid and alkaline solutions added to the mordant is dispensed with.

The bacteria are carefully fixed to the glass, stained with the mordant for five minutes, warming a little toward the end, washed, dried, and subsequently colored with carbol-fuchsin warmed a little.

Bacteria can best be measured by an eye-piece micrometer. As these instruments vary somewhat in con-

struction, the unit of measurement for each objective magnification or the method of manipulating the adjustable instruments must be learned from dealers' catalogues.

Photographing bacteria requires special apparatus and methods, which are fully described in text-books upon the subject.

CHAPTER V.

STERILIZATION AND DISINFECTION.

BEFORE considering the cultivation of bacteria and the preparation of media for that purpose it is necessary to discuss methods of destroying bacteria whose accidental presence might ruin our experiments.

The dust of the atmosphere, as has already been shown, is almost constant in its micro-organismal contamination, so that the spores of moulds and bacilli, together with yeasts and micrococci, constantly settle from it upon our glassware, enter our pots, kettles, funnels, etc., and would ruin every culture-medium with which we operate did we not take measures for their destruction.

Micro-organisms may be killed by heat or by the action of chemicals, the processes being generically termed *disinfection*. The destruction of the germs by heat is generally called *sterilization*. A chemical agent causing the death of bacteria is called a *germicide*. An object which is entirely free from bacteria and their spores is described as *sterile*. Certain substances whose action is detrimental to the vitality of bacteria and prevents their growth amid otherwise suitable surroundings are termed *antiseptics*.

The study of sterilization, disinfection, and antisepsis will naturally lead us through the following subdivisions :

I. The sterilization and protection of instruments and glassware used in experimentation.

II. The sterilization and protection of culture-media.

III. The disinfection of the instruments, ligatures, etc. and the hands of the surgeon, and the use of antiseptics.

IV. The disinfection of sick-chambers and their contents, as well as the dejecta and discharges of patients suffering from contagious and infectious diseases.

The Sterilization and Protection of Instruments and Glassware Used in Experimentation.—Sterilization may be accomplished by either moist or dry heat. For the perfect sterilization of objects capable of withstanding it dry heat is preferable, because more certain in its 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 while some simple non-spore-producing forms are killed at a temperature of 60° C., others can withstand boiling for an hour; it is therefore best to employ a temperature high enough to kill all with certainty. Platinum wires used for inoculation are held in the direct flame until they become incandescent. In sterilizing such wires attention must be bestowed upon the glass handle, which should be held in the flame for at least half its length for a few moments when used for the first time each day. Carelessness in this respect may cause the loss of much time by contaminating cultures.

Knives, scissors, and forceps may be exposed for a very brief time to the direct flame, but this affects the temper of the steel when continued too long. They may also be boiled, steamed, or carbolized.

All glassware is sterilized by exposure to a sufficiently high temperature, 150° C. or 302° F., for one hour in the well-known hot-air closet (Fig. 8). A temperature of 150° C. is sufficient to kill all known bacteria and their spores if continued for an hour.

Rubber stoppers, corks, wooden apparatus, and other objects which are warped, cracked, charred, or melted by so high a temperature must be sterilized by moist heat in the steam apparatus for at least an hour before they can be pronounced sterile.

It must always be borne in mind that after sterilization has been accomplished the same sources of contamination that originally existed are still present, and begin to operate as soon as the objects are removed from the sterilizing apparatus.

To Schröder and Van Dusch belong 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 observation has been of inestimable value to every bacteriologist. Before sterilizing

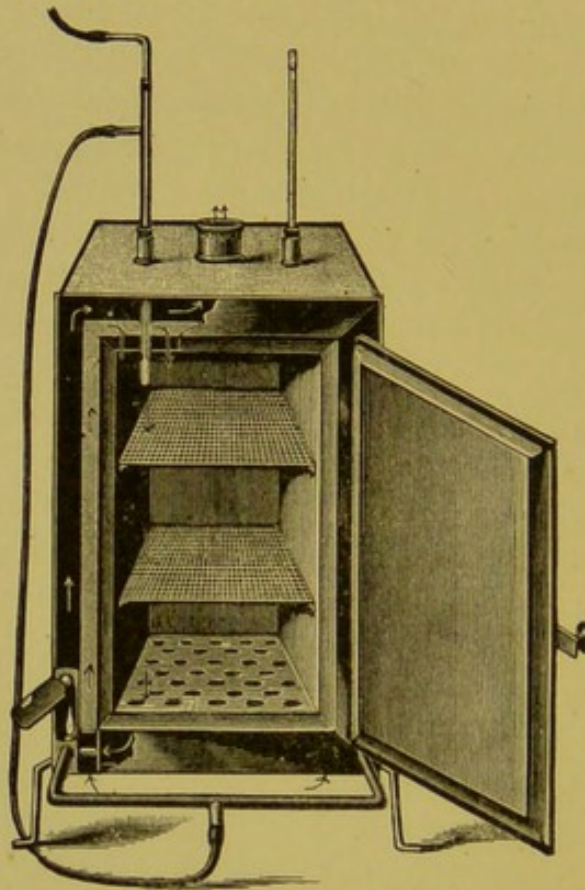


FIG. 8.—Hot-air sterilizer.

flasks and tubes we plug them with ordinary raw cotton, and are sure that afterward their interiors 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, and to allow as little dust to collect upon it as possible, in order that the object of the sterilization be not defeated. As the spores of moulds falling upon cotton sometimes grow and allow their mycelia to work their way through and drop into a culture-medium, Roux

has introduced little paper caps with which the cotton stoppers are protected from the dust. These are easily made by curling a small square of paper into a "cornucopia," 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.

Sterilization of Culture-media.—As almost all of the culture-media contain about 80 per cent. of water, which would be evaporated in the hot-air closet, so that the material would be destroyed, hot-air sterilization is not appropriate for them. Sterilization by streaming steam is the best and surest method. The prepared media are placed in flasks or tubes carefully plugged with cotton and previously sterilized with dry heat, and then sterilized in what is known as Koch's steam apparatus (Fig. 9) or in Arnold's



FIG. 9.—Koch's steam sterilizer.

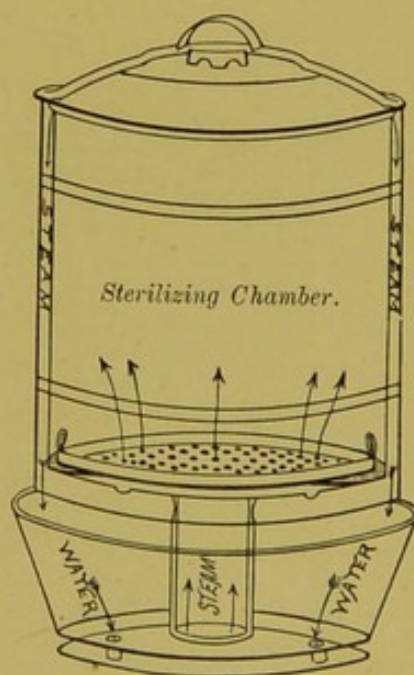


FIG. 10.—Arnold's steam sterilizer.

steam sterilizer (Fig. 10), which is more convenient and more generally useful.

The temperature of boiling water, 100° C., does not

kill many spores, so that the exposure of culture-media to streaming steam is of little use unless applied in a systematic manner—*intermittent sterilization*—based upon a knowledge of sporulation.

In carrying out the intermittent sterilization the culture-medium is exposed for fifteen minutes to the passage of streaming steam in the apparatus or to some temperature judged to be sufficiently high, so that the bacteria 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 to develop into perfect bacteria.

When the twenty-four hours have passed the culture-medium is again placed in the apparatus and 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 and capable of spoiling the material. When properly sterilized in this way, culture-media will remain free from contamination until time and evaporation cause them to dry up. If hermetically sealed, a sterile medium will keep indefinitely.

If it should be necessary to sterilize culture-media at once, not waiting the three days re-

quired by the intermittent method, it may be done by superheated steam in an autoclave (Fig. 11). Here under

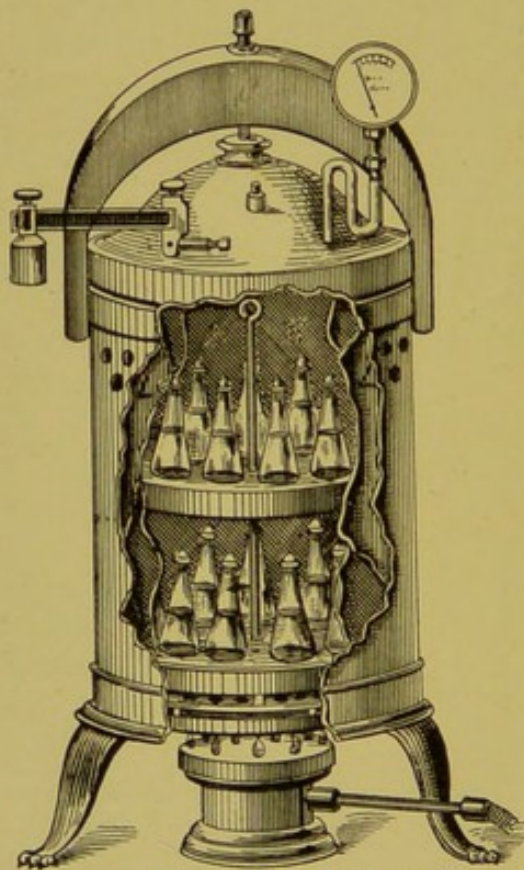


FIG. 11.—Autoclave for rapid sterilization by superheated steam under pressure.

a pressure of two or three atmospheres sufficient heat is generated to destroy the spores. The objections to this method are that it sometimes turns the agar-agar dark, and that it is likely to destroy the gelatinizing power of the gelatin, which after sterilization remains liquid.

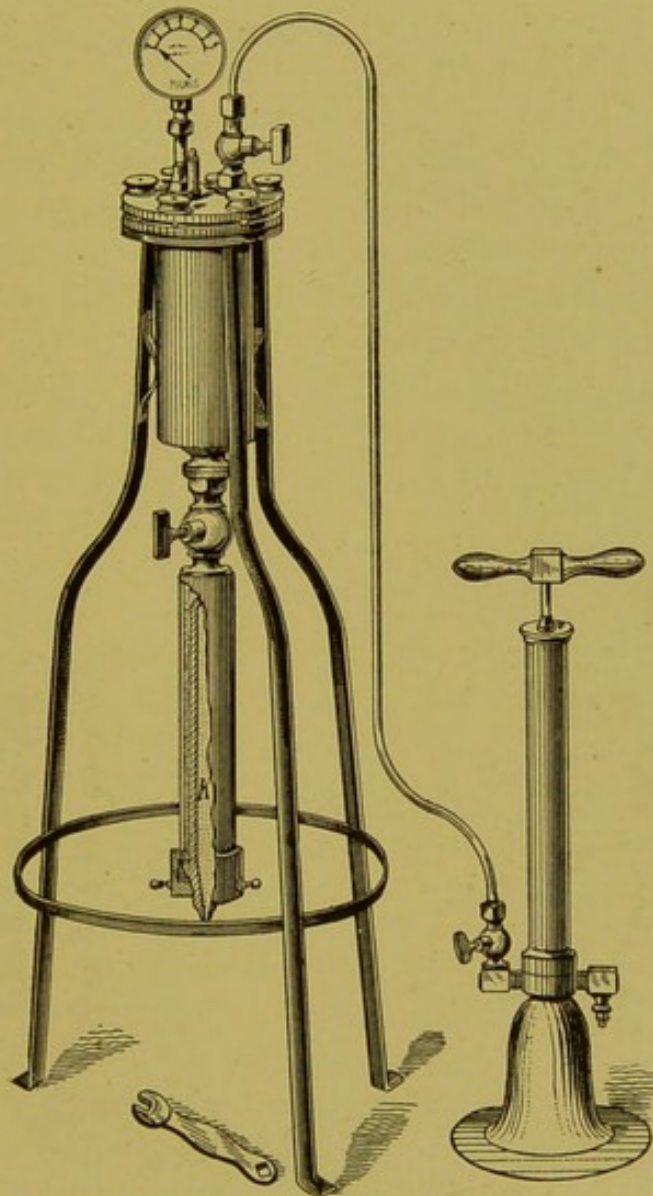


FIG. 12.—Pasteur-Chamberland filter arranged to filter under pressure.

Liquids may also 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 the bacteria. This method is largely employed

for the sterilization of the unstable toxins and anti-toxins, which are destroyed by heat. Various substances have been used for filtration, as stone, sand, powdered glass, etc., but experimentation has shown porcelain to be the only reliable filter against 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 used considerably the bacteria seem able to work their way through. To be certain of the efficacy of such a filter the fluid first passed through must be tested by cultivation methods. The complicated Pasteur-Chamberland and the simple Kitasato and Reichel filters are shown in Figures 12, 13, and 14.

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



FIG. 13.—Kitasato's filter: *a*, porcelain bougie; *b*, attachment for suction-pump; *c*, reservoir; *d*, sterile receiver.

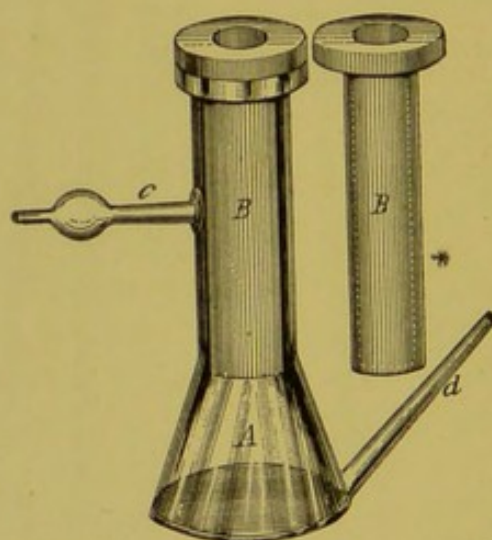


FIG. 14.—Reichel's bacteriologic filter of unglazed porcelain: *A*, sterile receiver; *B*, porcelain filter; *c*, *d*, attachments for pump.

matter is consumed. In this firing process the filter first turns black as the organic matter chars, then becomes

white as it is consumed. The greatest care must be exercised in cleansing, and especially must care be taken that the porcelain is dry before entering the fire, as it will certainly crack if moist.

Before using a new filter it should be sterilized by dry heat, then connected with receivers and tubes, also carefully sterilized. It should not be forgotten that the filtered material is still a good culture-medium and must be handled with the greatest care.

While the filtration of water, peptone solution, and bouillon is comparatively easy, gelatin and blood-serum pass through with great difficulty, and speedily gum the filter, so that it is useless until fired.

A convenient apparatus used by the author for the rapid filtration of large quantities is shown in the accompanying illustration (Fig. 15).

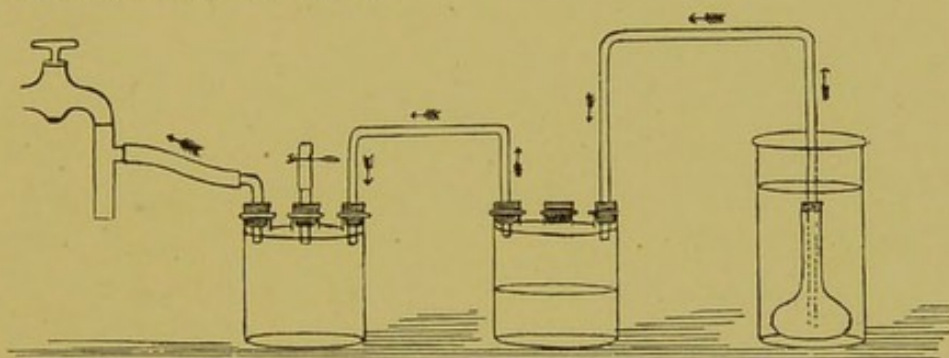


FIG. 15.—Apparatus for the rapid filtration of toxins, etc.

The Disinfection of Instruments, Ligatures, Sutures, the Hands, etc.—There are certain objects used by the surgeon which cannot well be rendered incandescent, exposed to dry heat at 150° C., steamed, or intermittently heated without injury. For these objects disinfection must be practised. Ever since Sir Joseph Lister introduced antiseptics, 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; hence the origin of the

successful *aseptic* surgery of the present day, which strives to prevent the entrance of germs into, rather than their destruction after admission to, the wound.

For a complete discussion of the subject of antiseptics in relation to surgery the reader must be referred to the large text-books of surgery, where much space is thus occupied. A short list of useful germicides of which the respective values are given, and a brief discussion of some of the more important measures, can alone find space in these pages. The antiseptic value of some of the principal substances used may be expressed as follows, the figures indicating the strength of the solution necessary to prevent the development of bacteria :

Pyoktanin (methyl violet)	1 : 2,000,000--1 : 5000.
Bichlorid of mercury	1 : 14,300.
Hydrogen peroxid	1 : 20,000.
Formalin	1 : 20,000.
Nitrate of silver	1 : 12,500.
Creolin	1 : 5000 to 1 : 200 (does not kill anthrax).
Chromic acid	1 : 5000.
Thymol	1 : 1340.
Salicylic acid	1 : 1000.
Potassium bichromate	1 : 909.
Zinc chlorid	1 : 526.
Potassium permanganate	1 : 285 ; not prompt in action.
Nitrate of lead	1 : 277.
Boracic acid	1 : 143.
Chloral hydrate	1 : 107.
Ferrous sulphate	1 : 90—1 : 200, Sternberg.
Calcium chlorid	1 : 25.
Creosote	1 : 20.
Carbolic acid	1 : 20 :: 1 : 50.
Alcohol	1 : 10.
Ether. Pure ether will not kill anthrax spores immersed in it for eight days.	

The value of antiseptics, like that of disinfectants, is always relative, the destructive as well as the inhibitory power of the solution varying with the micro-organism upon which it acts. The following table, from Boer, will illustrate this :

Methyl Violet (Pyoktanin).

	Restrains.	Kills.
Anthrax bacillus	1 : 70,000	1 : 5000
Diphtheria	1 : 10,000	1 : 2000
Glanders	1 : 2500	1 : 150
Typhoid	1 : 2500	1 : 150
Cholera spirillum	1 : 30,000	1 : 1000

Large numbers of both strongly and feebly antiseptic substances have purposely been omitted from the above lists, compiled from Sternberg and Micquel, as either inappropriate for ordinary use or as having been replaced by better agents.

The disinfection of the skin, both the hands of the surgeon and the part about to be incised, is a matter of importance. It is almost impossible to secure absolute sterility of the hands, so deeply do the skin-cocci penetrate between the layers of the scarf-skin. 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 brush previously sterilized 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, after which they are ready for use.

Surgical dressings are generally sterilized by superheated steam, which, as has been shown, destroys all germs. Ligatures and sutures of silk, gut, chromicized gut, silkworm gut, etc., having been boiled, are kept either in alcohol or in an alcoholic solution of bichlorid

of mercury, or, if this causes them to become too brittle, in a watery solution of bichlorid.

At present, in most hospitals, instruments are boiled before using, and during the operation are either kept in the boiled water or in carbolic solution.

During the operation the wound is frequently to be washed with carbolic solution or bichlorid of mercury, 1 : 2000, applied by sterile sponges. To La Place belongs the credit of observing that the efficacy of these germicides is greatly increased by the addition of a small amount of acid, by which their penetration is increased and the formation of insoluble albuminates lessened.

The knowledge that the action of germicides is chemical, and that the destruction of the bacteria is due to the combination of the germicide with the mycoprotein, is apt to lessen our confidence in the permanence of their action. Geppert has shown of bichlorid of mercury that in the reaction between it and anthrax spores the vitality of the latter seems lost, but that the precipitation of the bichlorid from this combination by the action of ammonium sulphid restores the vitality of the spore.

Again, the fact that some of the antiseptics, as nitrate of silver and bichlorid of mercury, are at once precipitated by albumins, and thus lose their germicidal and antiseptic powers, limits the scope of their employment. I think it may be safely said that carbolic acid is the most reliable and most generally useful of all the germicides and antiseptics.

The Disinfection of Sick-chambers, Dejecta, etc.—What has just been remarked concerning the unreliability of many of the germicidal substances is eminently *a propos* of the disinfection of dejecta. It is useless to mix bichlorid of mercury with typhoid stools or tubercular sputum rich in albumin, and imagine these substances rendered harmless in consequence. It should not be forgotten that the sick patient is less the means of conveying the contagium than the objects with which he is in contact, which can be carried to other rooms or houses

during or after the progress of the disease. A careful consideration of the condition of the sick-room will lead us to a clear understanding of its bacteriological condition.

The Air of the Sick-room.—It is impossible to sterilize or disinfect the atmosphere of a room during its occupancy by the patient, or in all probability after his exitus from it. The concentration of the disinfecting solutions given above must make obvious the foolishness of placing beneath the bed or in the corners of a room small receptacles filled with carbolic acid or chlorinated lime. These can serve no purpose for good, and may be potent for harm by obscuring the disagreeable odors emanating from materials which should be removed from the room by the still more disagreeable odors of the disinfectants. The practice of such a custom is only comparable to the old faith in the virtue of asafetida tied up in a corner of the handkerchief as a preventive of cholera and small-pox.

During the period of illness a chamber in which the patient is confined should be freely ventilated, so that its atmosphere is constantly changing and replacing the closeness so universally an accompaniment of fever by fresh, pure air—a comfort to the patient and a protection to the doctors and nurses.

After recovery or death one should rely less upon fumigation than upon the 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 may do some good—when combined with steam, much good—but are greatly overestimated, and disinfection combined with fresh air and sunlight is much better.

Indeed, I would recommend that after opening the doors and windows of the sick-chamber the atmosphere of the whole house be forgotten and attention be devoted to other things.

The Dejecta.—A little thought will direct attention to

those of the dejections which are dangerous to the community and promote efforts for their complete sterilization. In cases of diphtheria the vomit, expectorations, and nasal discharges are most important. They should be received in old rags or in Japanese paper napkins—not handkerchiefs or towels—and 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 as good as the small pasteboard boxes (Fig. 16) recommended by

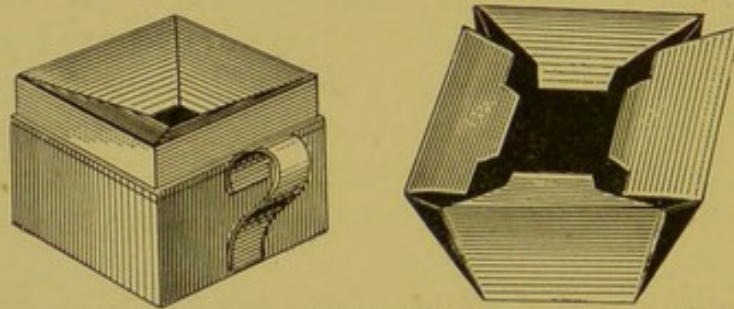


FIG. 16.—Pasteboard cup for receiving infectious sputum. When used the pasteboard can be removed from the iron frame and burned.

some city boards of health, because, being highly absorbent, the sputum is apt to soak through and soil the fingers, etc. Tuberculous patients should be provided with rice-paper instead of handkerchiefs, and should have their towels, knives, forks, spoons, plates, etc. kept strictly apart from the others of the household (though the patients, whose mental acuity makes their sensibilities very pronounced, need never be told of their isolation), and frequently boiled for considerable lengths of time.

The excreta from typhoid-fever and cholera cases require particular attention. These, and indeed all alvine matter possibly the source of infection or contagion, should be received in glazed earthen vessels and immediately 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.

The Clothing, etc.—All bed-clothing which has been used in the sick-room, all towels, napkins, handkerchiefs, night-ropes, underclothes, etc. which have been used by the sick, and all towels, napkins, handkerchiefs, caps, aprons, and outside dresses worn by the nurse, should be regarded as infected and subjected to sterilization. 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. When possible, the clothes should be soaked in 1:2000 bichlorid solution before or after boiling, and in drying should hang in the sun and wind. Woollen underwear can be treated exactly as if of cotton. The woollen clothing of the patient, if infected, requires special treatment. Fortunately, the infection of the outer woollen garments is unusual. The only reliable method for their purification is prolonged exposure to hot air at 110° C. In private practice it becomes a grave question what shall be done with these articles. Prolonged exposure to fresh air and sunlight will aid in rendering them harmless; when it is certain that articles of wool are infected, they may be sent to the city hospital or to certain of the moth-destroying and fumigating establishments which can be found in all large cities, and be baked.

The Furniture, etc.—The wholesale destruction of furniture practised in earlier times has at present become unnecessary. The doctor, if he properly performs his functions, will save much trouble and money for his patient by ordering the immediate isolation of his charge in an uncarpeted, scantily- and cheaply-furnished room the moment an infectious disease is *suspected*, before much infection can have occurred. However, if before his removal the patient has occupied another bed, its clothing should be promptly handled in the above-described manner.

After the illness the walls of the rooms, including the ceiling, may be rubbed with fresh bread, which Löffler has shown to be efficacious in collecting the bacteria, or, if possible, should be whitewashed. If the walls are hung with paper, it should be dampened with 1 : 1000 bichlorid-of-mercury solution before new paper is hung. The floor should be scoured with 5 per cent. carbolic-acid solution or 1 : 1000 bichlorid of mercury, and all the wooden articles wiped off two or three times with the same solution employed for the floor. In this scouring no soap can be used, as it destroys the virtue of the germicide. 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 generally have ovens for the purpose. Curtains, shades, etc. should receive proper attention, but of course the greater the precautions exercised in the beginning, the fewer the articles which will need attention in the end. They should be removed before the case has developed.

The patient, whether he lives or dies, may also be a means of spreading the disease unless specially cared for. After convalescence the body should be 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 above solution. In desquamative diseases it seems best to have the entire body anointed with cosmolin once daily, the unguent being well rubbed in, in order to prevent the particles of epidermis being distributed through the atmosphere. Carbolated cosmolin may be better than the plain, not because of the very slight antiseptic value it possesses, but because it helps to allay the itching which may be part of the desquamative process.

After the patient is about the room again, common sense will prevent the admission of strangers until all

the disinfective measures have been adopted, and after this, touching, and especially kissing him, should be omitted for some time.

The dead who die of infectious diseases should be washed in a strong disinfectant solution, and should be given a private funeral in which the body, if exposed, should not be touched. In my judgment, the body is best disposed of by cremation.

CHAPTER VI.

CULTIVATION OF BACTERIA; CULTURE-MEDIA.

ACCURACY of observation requires that the bacteria be separated from their natural surroundings and artificially grown upon certain prepared media of standard composition, in such a manner that only organisms of the same kind are together.

One after another various organic and inorganic mixtures have been suggested, but, although almost any compound containing organic matter, even in small amounts, will suffice for the nourishment of bacteria, a certain few have met with particular favor as being most valuable.

Rather than give a complete review of the work which has already been done, in the following pages the most useful preparations only will be considered.

Our knowledge of the biology of the bacteria has shown that they grow best in a mixture containing at least 80 per cent. of water, of a 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 readily liquefied and solidified.

Bouillon is one of the most useful and most simple of the media. Its preparation is as follows: To 500 grams of finely-chopped lean, boneless beef, 1000 c.cm. 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 c.cm. This liquid is called the *meat-infusion*. To it 10 grams of Whitte's dried beef-peptone and 5 grams of sodium chlorid are added, and the whole boiled until the albumins coagulate. The reaction is then carefully tested, in order that whatever sarcolactic acid may have been present in the meat may be neutralized by the addition of a few drops of a saturated aqueous solution of sodium carbonate. The solution is added drop by drop, and the reaction frequently tested with litmus-paper. When a neutral reaction, or, better, a faint alkaline reaction, is attained, the mixture is well stirred, boiled again for about half an hour, to precipitate the alkaline albumins formed, and filtered. The bouillon thus prepared is a clear fluid of a straw color, much resembling normal urine in appearance. It is dispensed in tubes—about 10 c.cm. to each—and is then sterilized by steam three successive days for fifteen to twenty minutes each, according to the directions already given for fractional sterilization. (See p. 94.)

For the preparation of bouillon, as well as gelatin, agar-agar, and glycerin agar still to be described, beef-extract (Liebig's) may be employed, but for the most delicate work this is rather undesirable, because of its unstable composition and because of the precipitation of meat-salts, which can scarcely be filtered out of the agar-agar, owing to the fact that they only crystallize when the solution cools. When it is desirable to prepare the bouillon from beef-extract, the method is very simple. To 1000 c.cm. of clean water 10 grams of Whitte'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, neutralized, if necessary, and filtered *when cold*. If it is 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 bulk. A very convenient simple apparatus used by bacteriologists for filling tubes with liquid media is shown in Figure 17. It



FIG. 17.—Funnel for filling tubes with culture-media (Warren).

need not be sterilized before using, as the culture-medium will be sterilized by the intermittent method 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. The dry-heat sterilization is done, of course, after the cotton plugs are in place.

Bouillon 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." The preparation of these media, however, varies somewhat from that of the plain bouillon.

Gelatin.—The culture-medium known as gelatin has decided advantages over the bouillon, not only because it is an excellent food for bacteria, and, like the bouillon, transparent, but because it is also *solid*. Nor is this all: it is a transparent solid which can be made liquid or solid at will. It is prepared as follows: To 1000 c.cm. of meat-infusion or to 1000 c.cm. of water containing 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 boiled for about an hour over a moderately hot flame. Double boilers are very slow, and if proper care is exercised there is little danger of the gelatin burning. It must be stirred occasionally, and the flame should be so distributed by wire gauze as not to act upon a single point of the bottom of the kettle. 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. The liquid is now cooled to 60° C. and neutralized—*i. e.* alkalinized. As the gelatin is itself acid, a relatively larger amount of the sodium-carbonate solution will be needed than was required for the bouillon. When the proper reaction is attained, as much water as has been lost by vaporization during the process of boiling, intimately mixed with the white of an egg, is added, well stirred in, and the whole boiled for half an hour, then filtered.

If the filter-paper be of good quality and properly folded (pharmaceutical filter), 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 generally filters readily. A wise precaution is to catch the first few centimeters in a test-tube and boil them, so that if a cloudiness shows the presence of uncoagulated albumin,

the whole mass can be boiled again. The finished gelatin is at once distributed into sterilized tubes, and then sterilized like the bouillon by the fractional method.

Of course, the gelatin or any other culture-medium can be kept *en masse* indefinitely, but should a contaminating micro-organism accidentally enter, the whole quantity will be spoiled; if, on the other hand, it is kept in tubes, several of them may be lost without much inconvenience. Under proper precautions of sterilization and protection it should all keep well.

Agar-agar.—Agar-agar is the commercial name of a Japanese sea-weed which dissolves in boiling water with resulting thick jelly when cold. The jelly, which solidifies between 30° and 40° C., cannot again be melted except by the elevation of its temperature to the boiling-point, so that this culture-medium, which is nearly transparent, is almost as useful as gelatin. In addition to its readiness to liquefy and solidify, it is sufficiently firm to allow of the incubation-temperature—*i. e.* 37° C.—at which gelatin is always liquid, and no better than bouillon.

The preparation of this medium is generally 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 be made to filter the solution until the agar-agar is perfectly dissolved. It is prepared as follows: To 1000 c.cm. of bouillon made as described above, preferably of meat instead of beef-extract, 10 grams of agar-agar are added. The mixture is boiled for an hour, or, if possible, two. At the end of the first hour it is cooled to about 60° C., and after neutralization, which may not be necessary if the bouillon was neutral, an egg beaten up in water is added, and the liquid is boiled again until the egg is entirely coagulated. The reaction of the agar-agar should be *neutral* rather than alkaline, as, for an unknown reason, alkalinity seems to interfere slightly with filtration.

After the boiling, which should be brisk, has caused

the thorough solution of the agar-agar, it is filtered, just as the gelatin was, through a carefully-folded pharmaceutical 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 when necessary. Experience shows that 1000 c.cm. of agar-agar rarely go through one paper, and I always expect when beginning the filtration to be compelled to boil the material which remains on the paper again, and pour it through a new filter.

The formerly much-employed hot-water and gas-jet filters seem unnecessary. If properly prepared, the whole quantity will filter in from fifteen to thirty minutes.

If made from beef-extract, the agar-agar almost always precipitates a considerable amount of meat-salts as it cools. This should be anticipated, but, so far as I can determine, cannot always be prevented. The amount is certainly lessened by making the bouillon first, filtering it *cold*, then adding the agar-agar, and dissolving and filtering it.

The difficulty of filtering the agar-agar has led Flügge and others to adopt a method of sedimentation. An ingenious apparatus for this purpose has lately been devised by Bleisch. The method can be simplified by using a small pharmaceutical percolator, the bottom of which is closed by a rubber cork containing a tube which extends nearly to the top of the percolator and is attached to a rubber tube with a pinchcock below. The melted agar-agar is poured into this, and kept in the steam apparatus until the sedimentation is sufficient to allow clear fluid to be drawn from the top. As the clear agar-agar is drawn off the tube is pulled down through the rubber cork, and more drawn off until only the sediment is left.

Agar-agar is dispensed in tubes like the gelatin and bouillon, sterilized by steam by the intermittent process, and after the last sterilization, before cooling, each tube is inclined against a slight elevation, so as to offer an extensive flat surface for the culture.

After the agar-agar jelly solidifies its contraction causes some water to collect at the lower part of the tube. This should not be removed, as it keeps the material moist, and also because it has a distinct influence upon the character of the growth of the bacteria.

Glycerin Agar-agar.—For an unknown reason certain of the bacteria which will not grow upon the agar-agar as prepared above will do so if 3-7 per cent. of glycerin be added. Among these is the tubercle bacillus, which, not growing at all upon plain agar-agar, will grow well when glycerin is added—a fact discovered by Roux and Nocard. The glycerin may also be added to gelatin or any other medium.

Blood-serum.—The great advantage possessed by this medium is that it is itself a constituent of the body, and hence offers opportunities for the development of the parasitic forms of bacteria under the most natural conditions possible. It is the most difficult of all the media to prepare. The blood must be obtained from a slaughter-house in an appropriate receptacle, the best things for the purpose being tall narrow jars of about 1 liter capacity, with a tightly-fitting lid. The jars are sterilized by heat or by washing with alcohol and ether, are carefully dried, closed, and carried to the slaughter-house where the blood is to be obtained. As the blood flows from the severed vessels of the animal the jars are filled one by one. 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 obviated. The jars when full are allowed to stand undisturbed until quite firm coagula form within them. If these have any tendency to cling to the glass, each one should be given a few violent twists, so as to break away the fibrinous attachments. After this the jars 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 pipettes and placed in

sterile tubes. If the serum obtained is 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. It is evident that such complicated manœuvring will offer many possible chances of infection; hence the sterilization of the serum is of the greatest importance.

If it is desirable to use the serum as a liquid medium, it is exposed to a temperature of 60° to 65° C. for one hour upon each of five consecutive days. If it is thought best 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 is distinct reason to think it contaminated—to a temperature just short of the boiling-point. During the process of coagulation the tubes should be inclined, so as to offer a large surface for the growth of the culture. The serum thus prepared may be white, or have a reddish-gray color if many corpuscles are present, and is opaque. It cannot be melted, but once solid remains so.

Koch devised a very good apparatus (Fig. 18) for coag-

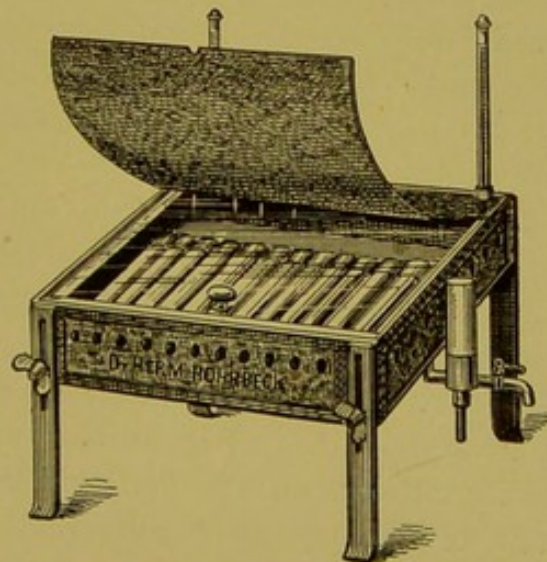


FIG. 18.—Koch's apparatus for coagulating and sterilizing blood-serum.

ulating blood-serum. The bottom should be covered with cotton, a single layer of tubes placed upon it, and

the temperature elevated until coagulation occurs. The repeated sterilizations may be conducted in this apparatus, or may be done equally well in the steam apparatus, the cover of which is not completely closed, for if the temperature of the serum is raised too high it is certain to bubble.

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 this is distributed in tubes, and sterilized and coagulated like the blood-serum itself. Most organisms grow more luxuriantly upon it than upon either plain blood-serum or other culture-media. Its special usefulness is for the *Bacillus diphtheriæ*, which grows upon it with rapidity and with quite a characteristic appearance.

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 of using this valuable medium—that of Bolton and Globig:

With the aid of a cork-borer a little smaller in diameter than the test-tube ordinarily used a number of cylinders are cut from potatoes. Rather large potatoes should 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 then are exposed three times, for half an hour each, to the passing steam of the sterilizer. This steaming cooks the

potato and also sterilizes it. Such cultures 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 do not turn dark. Drying may be prevented by adding a few drops of clean water to each tube before sterilizing. It is not necessary to have a special small chamber blown in the tube to contain this water; only a small quantity need be added, and this will not touch the potato, which does not reach the bottom of the rounded tube.

A potato-juice has also been suggested, and is of some value. It is made thus: To 300 c.cm. of water 100 grams of grated potato are added, and allowed to stand on ice over night. Of the pulp 300 c.cm. are expressed through a cloth and cooked for an hour on a water-bath. After cooking, the liquid is filtered and receives 4 per cent. of glycerin. It may or may not need neutralization. Upon this medium the tubercle bacillus grows well, especially when the reaction of the medium is acid, but loses its virulence.

Milk.—Milk is useful as a culture-medium. As when the milk stands the cream which rises to the top is a source of inconvenience, it is best to secure from a dairy fresh milk from which the cream has been removed by a centrifugal machine. It is placed in sterile tubes and sterilized by steam by the intermittent method. The opaque nature of this culture-medium often permits the undetected development of contaminating organisms. A careful watch should therefore be kept upon it 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. Cow's milk is inclined to be acid in reaction, and a small amount of sodium carbonate may be necessary to give it a distinct blue. The use of litmus is probably the best method of determining

whether bacteria by their growth produce acids or alkalis.

The watery solution of litmus, being a vegetable infusion, is likely to spoil, hence should always be treated like the culture-media and sterilized by steam every time the receptacle in which it is kept is opened.

Peptone solution, or Dunham's solution, is very useful for the detection of certain faint colors. It is a perfectly clear, colorless solution, made as follows:

Sodium chlorid,	0.5	} Boil until the ingredients dissolve; then filter, fill into tubes, and sterilize.
Whitte's dried peptone,	1.	
Water,	100.	

It is the best medium for the detection of indol. In it the *Bacillus pyocyaneus* produces its blue color. The addition of 4 c.cm. of the following solution—

Rosalic acid,	0.5,
80 per cent. alcohol,	100.

makes it become an excellent reagent for the detection of acids and alkalies. The solution is pale rose in color. If the bacterium produces acids, the color fades; if alkalis, it intensifies.

It is not intended that the student shall infer that there are no culture-media other than these, which have been selected because of their usefulness and popularity. Many other compounds and as many simple substances are employed; for example, eggs, white of egg, urine, bread, sputum, sugar solutions, hydrocele fluid, and aqueous humor.

CHAPTER VII.

CULTURES, AND THEIR STUDY.

THE objects which we have had before us in the preparation of the culture-media were numerous. We have prepared them so as to allow us to separate—or, rather, to *isolate*—bacteria, to keep them in healthy growth for considerable lengths of time, to enable us to observe their biologic peculiarities, and to introduce them without difficulty into the bodies of animals.

The isolation of bacteria was impossible until the fluid culture-media of the early observers were replaced by the solid media, and was exceedingly crude until Koch gave us the solid, transparent media and the well-known “plate-cultures.”

A growth of artificially-planted micro-organisms in which an immense number are massed together is called a *culture*. If such a growth contains but one kind of organism, it is known as a *pure culture*.

It has become the habit at present to use the term “culture” rather loosely, so that it does not always signify a growth of micro-organisms artificially planted, but may signify a growth taking place under natural conditions; thus, typhoid bacilli are said to exist in the spleens of patients dead of that disease “in pure culture,” because no other bacteria are there; and sometimes, when in expectorated fragments of cheesy matter from tuberculosis pulmonalis the tubercle bacilli are very numerous and unmixed with other bacteria, the term “pure culture” is again used to describe the condition.

Three principal methods are at present employed to enable us to secure pure cultures of bacteria, but before beginning a description of them it is well to observe that

the peculiarities of certain pathogenic forms enable us to use special means, taking advantage of their eccentricities, for their isolation, and that the general methods are in reality more useful for the non-pathogenic than for the pathogenic forms.

All three methods depend upon the observation of Koch, that when germs are equally distributed throughout some liquefied nutrient medium which can be solidified in a thin layer, the growth of the germs takes place in little scattered groups or families, called *colonies*, distinctly separated from each other and capable of transplantation to tubes of culture-media.

Plate-cultures.—The plate-cultures, originally made by Koch, require considerable apparatus, and of late years have given place to the more ready methods of Petri and Von Esmarch. So great, however, is the historic interest attached to the plates that it would be a great omission not to describe Koch's method in full.

Apparatus.—Half a dozen glass plates, about 6 by 4 inches in size, free from bubbles and scratches and ground at the edges, are carefully cleaned, placed in a sheet-iron box made to receive them, and then put in

the hot-air closet, where they are sterilized. The box, which is tightly closed, allows the sterilized plates to be kept on hand indefinitely before using.

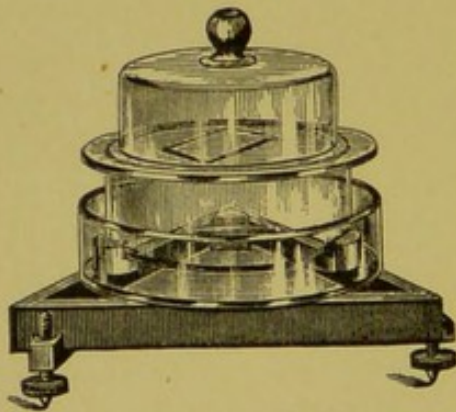


FIG. 19.—Complete levelling apparatus for pouring plate-cultures, as taught by Koch.

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 solution is 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 always kept closed, is ready for use.

A levelling apparatus is required (Fig. 19). This 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 levelled by adjusting the screws under the legs of the tripod. When level the cover is placed upon it, and it is ready for use.

Method (Fig. 20).—A sterile platinum loop is dipped into the material to be examined, a small quantity se-

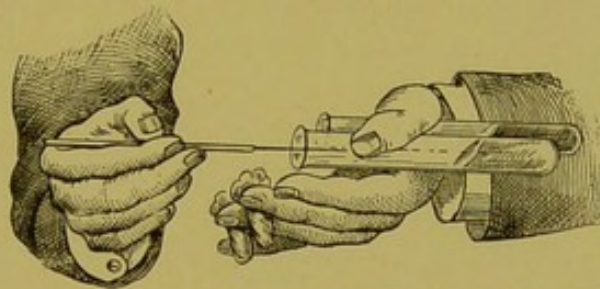


FIG. 20.—Method of holding tubes during inoculation.

cured, and stirred about so as to distribute it evenly through a tube of the melted gelatin. If the material under examination is 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 the necessity for a dilution. From the first tube a loopful of gelatin is carried to a second tube of melted gelatin and stirred well, so as to distribute the organisms evenly through it. 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 hundreds, and a fourth only a few dozen colonies.

After the tubes are prepared, 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 levelling 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 cold from the ice



FIG. 21.—Glass bench.

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 (Fig. 21) made for the purpose and sterilized. After the contents of all the tubes are thus distributed, the moist chamber and its contents are allowed to stand for some hours, 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 culture-medium where it can grow unmixed and undisturbed.

The description must have made evident the fact that only such culture-media can be used for plate-cultures as can be melted and solidified at will—viz. gelatin, agar-agar, and glycerin agar-agar. Blood-serum and Löffler's mixture are entirely inappropriate.

The great drawback to this excellent method is 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

Petri's Dishes.—These small dishes (Fig. 22), about 4 inches in diameter and $\frac{1}{2}$ inch deep, with accurately fitting lids, are about as convenient as anything that has been devised in bacteriological technique. They dis-



FIG. 22.—Petri dish for making plate-cultures.

pense with plates and plate-boxes, with moist chambers and benches, and usually with the levelling apparatus, though this is still employed in connection with the Petri dishes in some laboratories.

The method of the employment of Petri dishes is very simple. The dishes are carefully cleaned, polished, and sterilized by hot air, care being taken that they are placed in the hot-air closet right side up, and after sterilization are kept covered and in that position. The dilution of the material under examination is made with gelatin or agar-agar tubes in the manner described above, the plugs are removed, the mouth of the tube is cautiously held for a moment in the flame, then the contents of each tube are poured into one of the sterile dishes, whose top is elevated just sufficiently to allow the mouth of the tube to enter. The gelatin is spread over the bottom of the dish in an even layer, is allowed to solidify, labelled, and then stood away for the colonies to develop.

Esmarch Tubes.—This method, devised by Esmarch, converts the walls 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 solid medium (Fig. 23). Thus the tube becomes the plate upon which the colonies develop.

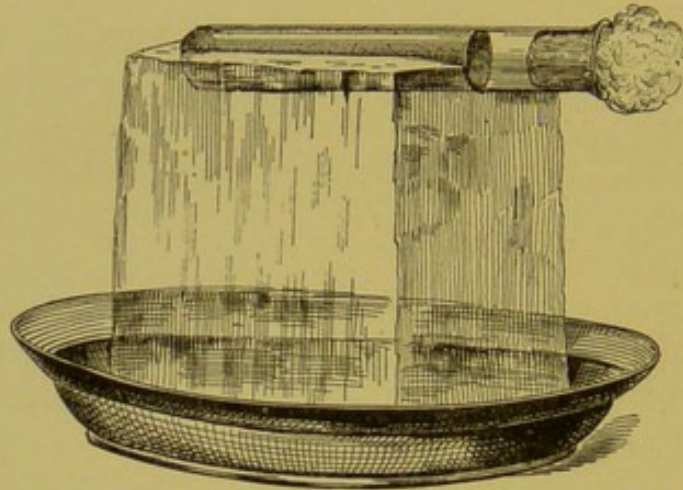


FIG. 23.—Esmarch tube on block of ice (redrawn after Abbott).

Several little points need to be observed in carrying out Esmarch's method. The tube must not contain too much culture-medium, or it cannot be rolled into an even layer. In rolling the contents should not touch the cotton plug, lest it be glued to the glass and its subsequent usefulness be injured. No water must be admitted from the melted ice.

The offspring of each bacterium growing upon the film of gelatin constituting a plate-culture form a mass which has already been pointed out as a *colony*. These small bacterial families may be seen through a microscope when still much too small for detection by the naked eye, and because of their minuteness should always be studied with the microscope.

The original plates of Koch are very inconvenient for such examination, because it is impossible to remove them from the moist chamber and lay them upon the stage of the microscope without exposing them to the danger of contamination by the atmosphere, so that the advantages of Petri dishes and Esmarch tubes, where the examination may be made through the glass tube or

through the bottom of the inverted dish, will be more than ever apparent.

The colonies should be viewed from time to time in their growth, drawings being made of the appearances, so as to form a series showing the developmental cycle. Most colonies will be found to originate as spherical, circumscribed, slightly granular, yellowish, greenish, or brownish dots, and later to send out offshoots or filaments or to develop concentric rings or characteristic liquefactions. A few appear from the very first as woolly clumps of entangled threads.

Some of the most diverse forms of colonies are represented in the accompanying illustrations (Figs. 24-28).



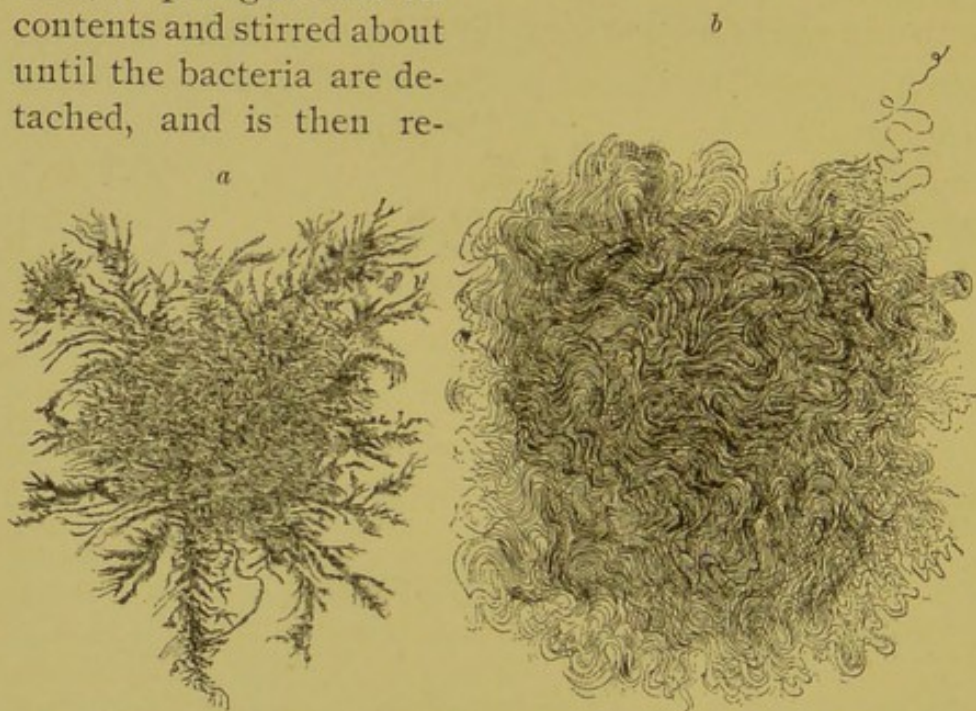
FIGS. 24, 25, 26.—The various appearances of colonies of bacteria under the microscope: *a*, colony of *Bacillus liquefaciens parvus* (Lüderitz); *b*, colony of *Bacillus polypiformis* (Liborius); *c*, colony of *Bacillus radiatus* (Lüderitz).

A pure culture, when obtained from colonies growing upon a plate, must always be made from a *single colony*, the transplantation being accomplished under a low power of the microscope. The naked eye can rarely be depended upon to recognize the purity of a colony or its isolation.

Selecting as isolated, large, and characteristic a colony as possible, it is brought to the centre of the field. A platinum wire, securely fused into a glass handle about 8 inches long, is sterilized by being made incandescent in a Bunsen flame, cooled, and then cautiously manipulated until, while it is watched through the microscope,

it is seen to touch the colony and take part of its contents away. *In this manœuvre the wire must not touch the objective, the glass, or anything except the colony.* Having secured the adhesion of a few bacteria to the sterile wire, the pure culture is made by introducing them into a sterile culture-medium.

If the pure culture is to be made in bouillon, the tube is held obliquely, so that when the cotton plug is cautiously removed no germs can fall in from the air. The plug is removed by a twisting movement. The wire, without being allowed to touch the mouth or sides of the tube, is plunged into its contents and stirred about until the bacteria are detached, and is then re-



FIGS. 27, 28.—The various appearances of colonies of bacteria under the microscope: *a*, colony of *Bacillus muscoides* (Liborius); *b*, colony of *Bacillus anthracis* (Flügge).

moved and the plug replaced. The wire should be immediately sterilized by heating to incandescence, lest the bacteria be pathogenic and capable of doing subsequent harm.

If the culture is to be made in gelatin, a different method is employed. The tube is either held horizontally, or, as is perhaps better, inverted; the cotton plug

is removed cautiously ; the wire bearing the bacteria from the colony is introduced until its point enters the centre of the gelatin, and is then carefully pushed on until a vertical puncture from the surface to the bottom of the gelatin is made. This is the *puncture-culture*—“*stichcultur*” of the Germans.

If the bacteria are only to be planted upon the surface of the culture-medium, the wire is drawn over the surface of a tube of obliquely solidified gelatin, agar-agar, blood-serum, etc. with a steady, slow movement, so as to scatter the germs along its path and cause the development of the bacteria in an enormous colony or mass of colonies in a line following the longest diameter of the exposed surface from end to end. This is the *stroke-culture*—“*strichcultur*.”

The method of holding the tubes, cotton plugs, and platinum wire during the process of inoculation is shown in Figure 20.

Sometimes it is desirable to preserve an entire colored colony as a 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 underneath, but the pressure must not be too great, as it may 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, is dried, fixed, stained, and mounted, and kept as a permanent specimen. It is called an *adhesion preparation*—“*klatsch präparat*.”

The development of bacteria in liquids is of less interest than that upon solid media. The growth generally manifests itself by a diffuse turbidity. Sometimes flocculi float in the otherwise clear medium. Some forms grow most rapidly at the surface of the liquid, and produce a

distinct membranous pellicle called a *mycoderma*. In such a growth multitudes of degenerated bacteria and large numbers of spores are to be observed. On the other hand, it occasionally happens that the growth occurs chiefly below the surface, and may produce gelatinous masses which are known as *zoöglea*.

In gelatin the bacteria exhibit a great variety of appearances, many of which are beautiful and interesting. Certain bacteria, as the tubercle bacillus, will not grow at all upon gelatin. Some forms which are rigidly aërobic will only grow upon or near the surface; others, anaërobic, only in the deeper parts. The majority, however, grow both upon the surface and in the puncture made by the wire. Sometimes the consistence of the gelatin is unaltered; sometimes it is liquefied throughout, sometimes only at the surface. Sometimes offshoots extend from the colonies into the gelatin, giving the culture

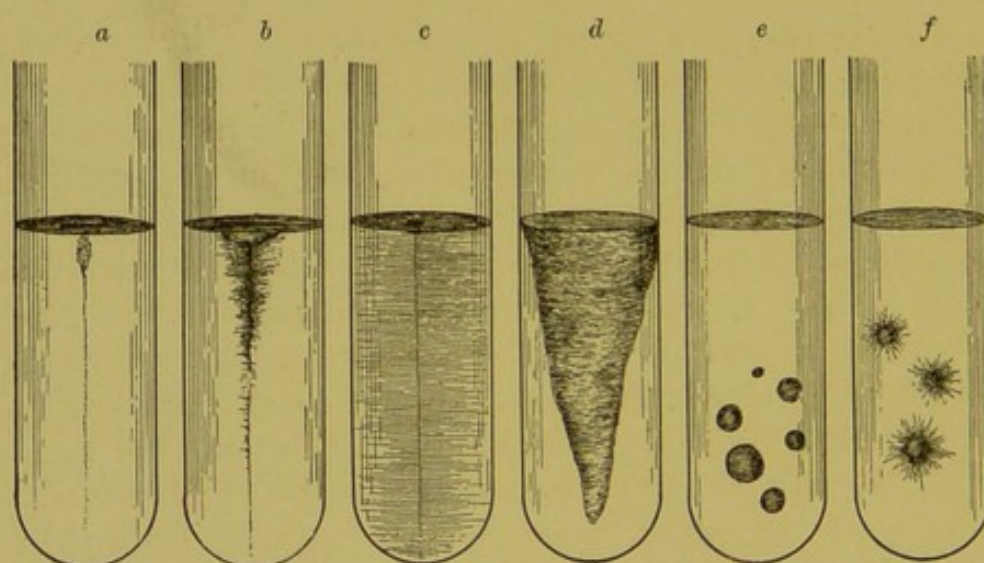


FIG. 29.—Various forms of gelatin puncture-cultures: *a*, *Bacillus typhi abdominalis*; *b*, *B. anthracis*; *c*, *B. mycoides*; *d*, *B. mesentericus vulgatus*; *e*, *B. of malignant edema*; *f*, *B. radiatis*.

a bristling appearance. Figure 29 will serve to illustrate different varieties of gelatin growth.

The growth in gelatin is generally so far removed from the walls of the tube (a central puncture nearly always

being made in the culture-medium, in order that the growth be symmetrical) that it is next to impossible to make a microscopical examination of it with any power beyond that given by a hand-lens.

Much attention has been given of late to the preparation of microtome sections of the gelatin growth. To accomplish this the glass is warmed sufficiently to allow the gelatin to be removed 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., imbedded in celloidin, cut wet, and stained like a section of tissue.

A ready method of doing this has been suggested by Winkler, who bores 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.

Very pretty museum specimens of plate- and puncture-cultures in gelatin can be made by simultaneously killing the micro-organisms and permanently fixing the gelatin with formalin, which can either be sprayed upon the gelatin or applied in dilute solution. As gelatin fixed in formalin cannot subsequently be liquefied, such preparations will last indefinitely.

The growths which occur upon agar-agar are in many ways less characteristic than those in gelatin, but as this medium does not liquefy except at a high temperature (100° C.), it has that great advantage over gelatin. The colorless or almost colorless condition of the preparation also aids in the detection of such chromogenesis as may be the result of the micro-organismal growth.

Sometimes the growth is colored, sometimes not; sometimes the production of a soluble pigment colors the agar-agar as well as the growth; sometimes the growth

is one color and the agar-agar another. Sometimes the growth is filamentous, sometimes 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).

Still less characteristic are the growths upon potato. Most bacteria produce rather smooth, shining, irregularly-extending growths, which often show very beautiful colors.

In milk and litmus milk one must observe the presence or absence of acid-production, the coagulation which may or may not accompany it, and the subsequent gelatinization or digestion of the coagulum.

Blood-serum is liquefied by some bacteria. The majority of organisms are not very characteristic in their development upon it. Others, as the bacillus of diphtheria, are, however, characterized by their shape, color, and rapidity of development at given temperatures.

While most of the saprophytic bacteria will grow well at the ordinary temperature of a well-warmed room, the important pathogenic forms require to be kept at the temperature of the body. To do this accurately an incubating oven becomes a necessity. Various forms, of wood and metal, are in the market, the one shown in the illustration (Fig. 30) being one of the newest and best.

It scarcely need be pointed out that gelatin cultures cannot be grown in the incubating oven, as the medium will not remain solid at temperatures above 20-22° C.

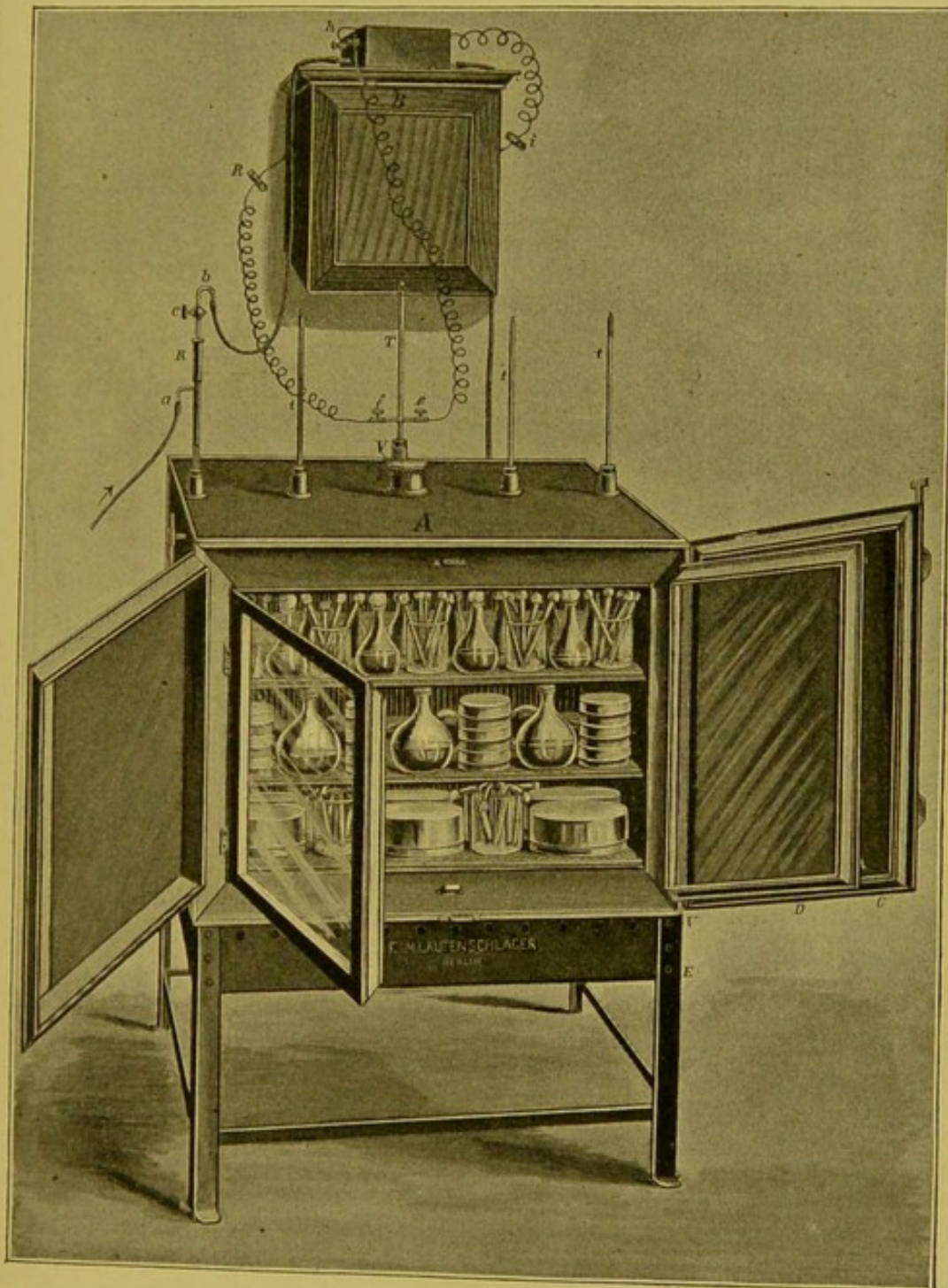


FIG. 30.—New model incubating oven with electro-regulator.

CHAPTER VIII.

THE CULTIVATION OF ANAËROBIC BACTERIA.

THE cultivation of micro-organisms which will not grow where the least amount of oxygen is present is always attended with much difficulty, and can seldom be accomplished with certainty. Many methods have been suggested, but not one can be described as satisfactory.

Koch originally cultivated anaërobic bacteria upon plates by covering the surface of the soft gelatin with a thin film of mica previously sterilized by incandescence. Some anaërobic forms will grow quite well by such a simple exclusion of the air, but the strictly anaërobic forms will not develop at all.

Hesse originated the plan, still sometimes followed, of making a deep puncture in recently boiled and rapidly sterilized gelatin or agar-agar, then covering the surface with sterilized oil, through which no oxygen was supposed to penetrate (Fig. 31).

Liborius suggested the plan of having a tube nearly full of gelatin or agar-agar, boiling it just before inoculation, so as to expand and drive out whatever air it might contain, making the inoculation while the culture-medium was still fluid, cooling rapidly in ice-water, and sealing up the tube in a blowpipe as near the surface of the gelatin as possible.

Esmarch used a regular "Esmarch tube," into the central cavity of which melted sterile gelatin was poured to exclude the air.

Buchner invented a method by which, by the use of pyrogallic acid, the oxygen was absorbed from the atmosphere in which the culture was kept, and the growth allowed to continue in the nitrogen and carbonic acid

which remained (Fig. 32). His method was to place the tube which had been inoculated in a much larger outer test-tube containing alkaline pyrogallic acid. The large



FIG. 31. — Hesse's method of making anaerobic cultures.



FIG. 32.—Buchner's method of making anaerobic cultures.

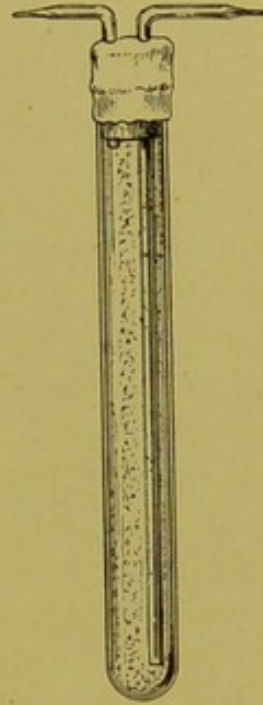


FIG. 33.—Fränkel's method of making anaerobic cultures.

tube was closed with a rubber cap, and the absorption of the oxygen allowed to progress.

Gruber, instead of absorbing the oxygen as Buchner does, prefers to use an air-pump and exhaust the contents of the tube. He uses a tube having a slender neck and a perforated rubber stopper. After the inoculation is made the air is pumped out and the slender neck sealed in the blowpipe. After this the tube can be warmed and the melted gelatin or agar-agar rolled on its sides, as suggested by Esmarch, if desired.

Better than any of the preceding is the method of Fränkel, which removes the air and replaces it by hydrogen. Fränkel prepares an ordinary Esmarch tube, removes the cotton stopper, and replaces it by a carefully sterilized rubber cork containing two tubes (Fig. 33). The

tubes are connected with a hydrogen generator, and the gas is allowed to pass through until all the oxygen is forced out and replaced by the hydrogen, after which the ends of the tubes are sealed in the flame (Fig. 32).

Liborius has designed a special tube for accomplishing the same thing.

Kitasato and Weil found the addition of 0.3–0.5 per cent. of sodium formate to be of use in aiding the rapidity of the development of anaërobic cultures. Liborius found that 2 per cent. of glucose added to the culture-medium also increased the rapidity of the process.

The methods now generally employed by bacteriologists for the anaërobic cultivations embrace all the essentials of the foregoing methods. One of the best arrangements for the purpose is that devised by Dr. Ravenel. His inoculations are deeply made in culture-media as free from air as possible. The tubes are loosely plugged, and are placed in an air-tight chamber the bottom of which contains pyrogallic acid—pyrogallic acid 1, solution of caustic potash 1, water 10. The apparatus is connected by two tubes with an exhaust-pump on one side, and with a hydrogen apparatus on the other, by which means the atmosphere is exhausted, and replaced by hydrogen until only pure hydrogen remains, after which the chamber is permanently sealed and the germs allowed to grow. Such a chamber can be constructed to hold a number of tubes or Petri dishes, yet not be too large to be stood in an incubator. Whatever oxygen may have escaped the exhaustion or have entered by the process of leakage is at once absorbed by the pyrogallic acid in the lower chamber of the apparatus.

An apparatus for plating out strictly anaërobic bacteria that has met with great favor is that invented by Botkin (Fig. 34). It combines the replacement of the air by hydrogen and the absorption of the oxygen possibly remaining by alkaline pyrogallic acid. In using the apparatus the uncovered Petri dishes are placed one

above the other in the rack *c*, and covered with the bell-glass *A*. Liquid paraffin is poured in the dish *B* until it is about half full. From a Kipp's apparatus hydrogen gas enters the little rubber tube *a*, subsequently escaping by the tube *b*. When only pure hydrogen escapes the rubber tubes *a* and *b* are withdrawn, and the apparatus remains filled with hydrogen. Lest a little oxygen should remain, it is best to have the dishes at the top and bottom of the neck filled with alkaline pyrogallic acid. Tetanus can be cultivated in this apparatus.

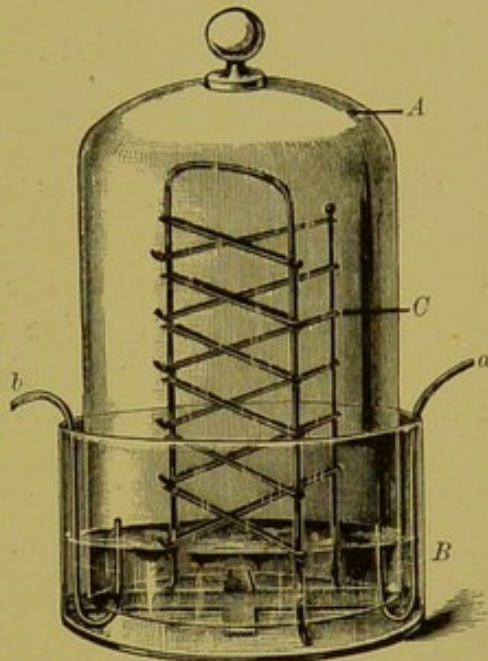


FIG. 34.—Botkin's apparatus for making anaerobic plate-cultures.

Roux has suggested the simplest method of cultivating anaerobic bacteria. The germs are distributed through freshly boiled, still liquid, gelatin or agar-agar, as in making the dilutions for plate-cultures, then drawn into a long, slender sterile piece of glass tubing of small calibre. When the tube is full the ends, which should have been narrowed, are closed in a flame, and the culture is hermetically sealed in an air-tight chamber. The chief difficulty is in transplanting the growing colony. To do this the tube must be opened with a file or a diamond at the point where the colony desired is observed.

CHAPTER IX.

EXPERIMENTATION UPON ANIMALS.

BACTERIOLOGY has to-day become a science whose principal objects are to discover the cause, explain the symptoms, and prepare the cure of diseases. We cannot hope to achieve these objects except by the introduction of bacteria into animals, where their effects and the effects of their products can be studied.

No one should more heartily condemn wanton cruelty to animals than the physician and the naturalist. Indeed, it is hard to imagine a class of men so much of whose lives is spent in relieving pain, and who know so much about pain, being guilty of the wholesale butchery and torture accredited to them by a few of the laity, whose eyes, but not whose brains, have looked over the pages of physiological text-books.

Experimentation upon animals has given us almost all our knowledge of physiology, most of our valuable therapeutics, and the only scientific methods of treating tetanus and diphtheria.

Experiments upon animals we must make, and, as animals differ in their susceptibility to diseases, large numbers and different kinds must be employed.

The bacteriological methods are not cruel. Two principal modes of introducing bacteria are employed: the subcutaneous injection and the intravenous injection.

Subcutaneous injections into animals are made exactly as hypodermic injections are given to man.

The intravenous injections differ only in that the needle of the syringe is introduced into a vein. This is easy in a large animal like a horse, but is very difficult in a small animal, and wellnigh impossible in anything smaller than

a rabbit. Such injections when given to rabbits are generally made into the ear-veins, as those most conspicuous and accessible (Fig. 35). A peculiar and important fact to remember is, that the less conspicuous posterior vein is much better adapted to the purpose than the anterior. The introduction of the needle should be made from the hairy surface of the ear.

Sometimes intra-abdominal and intrapleural injections are made, and in cases where it becomes necessary to determine the presence or absence of tuberculosis or glanders in tissues it may be necessary to introduce small

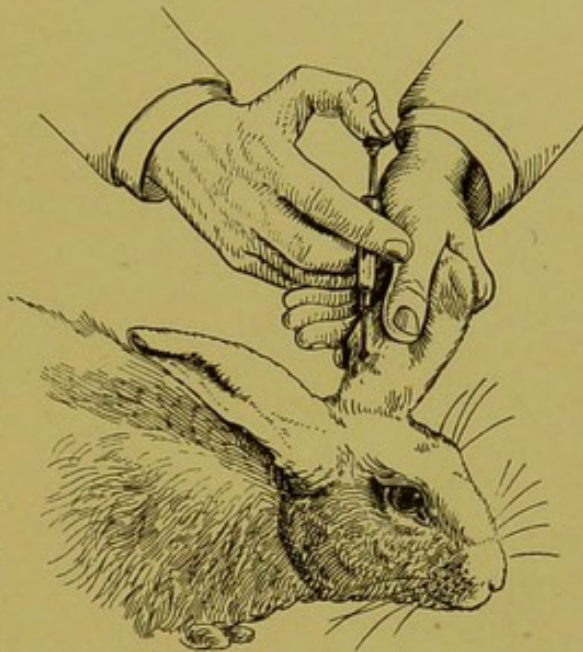


FIG. 35.—Method of making an intravenous injection into a rabbit. Observe that the needle enters the posterior vein from the hairy surface.

pieces of the suspected tissue under the skin or into the abdominal cavities.

Sometimes the inoculation can be made by the platinum wire, a very small opening in the skin being sufficient.

Small animals, like rabbits and guinea-pigs, can be held in the hand, as a rule. Rabbit-holders of various forms can be obtained from dealers. Dogs, cats, sheep, and goats can be tied and held in troughs. A convenient form of mouse-holder, invented by Kitasato, is shown in Figure 36.

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 generally are so crude and destructive as to warrant the comparison drawn by Fränkel, that to inject 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."

The blood of animals, when it is necessary to experiment with it, is best secured from a large vein, generally the jugular. From small animals, such as guinea-pigs, it may be secured by introducing a small cannula into the carotid artery.

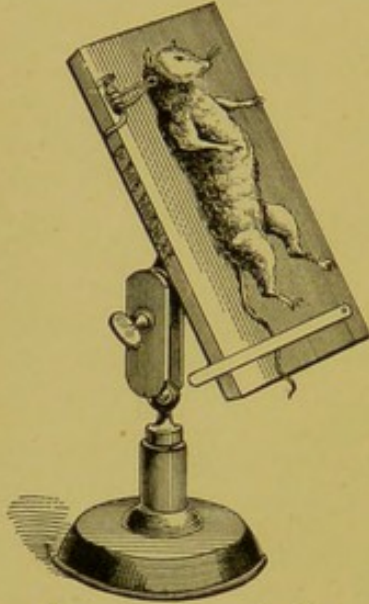


FIG. 36.—Mouse-holder.

Our observations of animals by no means cease 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 the skin, as well as to moisten the hair and enable it to be kept out of the incision.

The animal should be tacked to a board if small, or tied, by cords fastened to the legs, to the corners of a table if large, and should be dissected with sterile knives and scissors. When a culture is to be made from the interior of an organ—say the spleen—it should be incised deeply with a sterile knife and the culture made from its centre.

Fragments intended for subsequent microscopical examination should be cut very small (cubes of 1 c.cm.), placed in absolute alcohol for a few hours, then transferred to weaker alcohol, 80–90 per cent., for preservation. The technique of imbedding and staining the tissues can be found in almost any reliable text-book on pathology or on the special subject of microscopical technique.

CHAPTER X.

THE RECOGNITION OF BACTERIA.

THE most difficult thing in bacteriology is to be able to recognize the bacteria which come under observation.

A certain few micro-organisms are so characteristic in shape and grouping as to be separated by a microscopic examination. Some, as the tubercle bacillus, are characteristic in their reaction to the anilin dyes, and can be differentiated at once by this peculiarity. Some, as the *Bacillus mycoides*, are so characteristic in their agar-agar growth as to eliminate others. The red color of *Bacillus prodigiosus* and the blue of *Bacillus janthinus* will speak almost positively for them. The potato culture of the *Bacillus mesentericus fuscus* and its close relative the *vulgatus* is quite sufficient to enable us to pronounce upon them. Unfortunately, however, there are several hundreds of described species which lack any one distinct character that may be used for differential purposes, and require that for their diagnosis we shall wellnigh exhaust the bacteriological technique in an almost fruitless effort to recognize them.

A series of useful tables has been compiled by Eisenberg, and is now almost indispensable to the worker. Unfortunately, in tabulating bacteria we constantly meet species described so insufficiently as to make them worse than useless on account of the confusion caused.

The only way to recognize a species is to study it thoroughly and compare it, step by step, with the descriptions and tables of known species compiled by Eisenberg and others.

CHAPTER XI.

THE BACTERIOLOGIC EXAMINATION OF THE AIR.

It has been repeatedly emphasized—and indeed at the present time almost every one knows—that micro-organisms float almost everywhere in the air, and that their presence there is a constant source of danger, not only of contamination in our bacteriologic researches, but also a menace to our health.

Such micro-organisms are neither ubiquitous nor equally disseminated, but are much more numerous where the air is dusty than where it is pure—much more so where men and animals are accustomed to live, than upon the ocean or upon high mountain-tops. The purity of the atmosphere bears a distinct relation to the purity of the soil over which its currents blow.

The micro-organisms that occur in the air are for the most part harmless saprophytes which have been separated from their nutrient birthplace and carried about by the wind. They are almost always taken up from dried materials, experiment having shown that they arise from the surfaces of liquids in which they grow with much difficulty. They are by no means all bacteria, and a plate of sterile gelatin exposed for a brief time to the air will generally grow moulds and yeasts as well as bacteria.

The bacteria present are occasionally pathogenic, especially in localities where the discharges of diseased animals have been allowed to collect and dry. For this reason the atmosphere of the wards of hospitals and of rooms in which infectious cases are being treated is much more apt to contain them than the air of the street. However, the dried expectoration of cases of tuberculosis, of in-

fluenza, and sometimes of pneumonia, causes the specific bacteria of these diseases to be far from uncommon in street-dust.

Günther points out that the majority of the bacteria which occur in the air are cocci, sarcina being very abundant. Most of them are chromogenic and do not liquefy gelatin. It is unusual to find a considerable variety of bacteria at a time; generally not more than two or three species are found.

It is an easy matter to determine whether bacteria are present in the air or not, all that is necessary being to expose sterile plates or Petri dishes of gelatin to the air for a while, close them, and observe whether or not bacteria grow upon them.

To make a quantitative estimation is, however, much

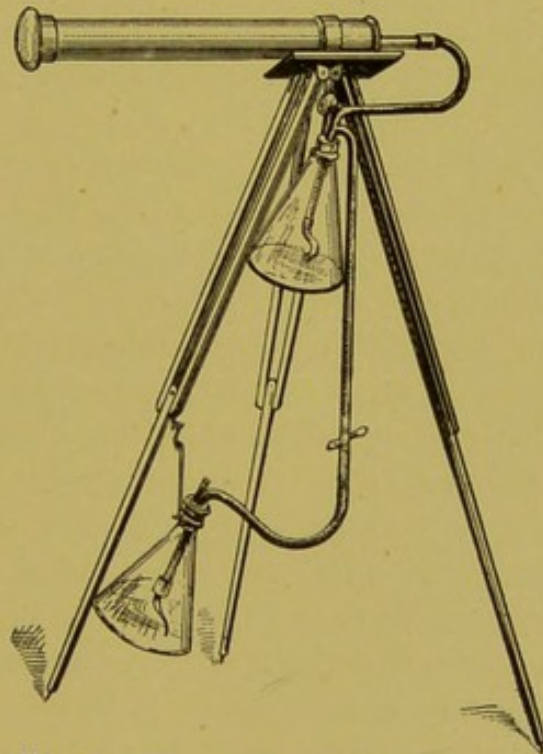


FIG. 37.—Hesse's apparatus for collecting bacteria from the air.

more difficult. Several methods have been suggested, of which the most important may be considered.

The method suggested by Hesse is simple and good. It consists in making a measured quantity of the air to

be examined pass through a horizontal sterile tube about 70 cm. long and 3.5 cm. wide (Fig. 37), the interior of which is coated with gelatin in the same manner as an Esmarch tube. The tube, having been prepared, is closed at both ends with sterile corks carrying smaller glass tubes closed with cotton. When ready for use the tube at one end is attached to a hand-pump, the cotton is removed from the other end, and the air passed through very slowly, the bacteria having time to precipitate 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 amount of air which passed through the tube.



FIG. 38.—
Petri's sand
filter for air-
examination.

In such a cylindrical culture it will be noted that if the air is passed through with the proper slowness, the colonies will be much more numerous near the end of entrance than that of exit. The first to fall will probably be those of heaviest specific gravity—*i. e.* the moulds and yeasts.

A still 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 (Fig. 38). 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 of the tube are closed with corks having a narrow glass tube. The apparatus is heated and sterilized in a hot-air sterilizer, 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 in from ten to twenty minutes. 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. Sternberg remarks that the chief objection to the method is the presence in the gelatin of the slightly opaque sand, which interferes with the recognition and counting of the colonies. This objection has, however, been removed by Sedgwick and Miquel, who use 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, but the principle is the same, and can be modified to suit the experimenter. Petri points out in relation to his method that the filter catches a relatively greater number of bacteria in proportion to moulds than the Hesse apparatus, which depends upon sedimentation.

A particularly useful form of apparatus is a granulated sugar-filter suggested by Sedgwick and Tucker, which 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 lining like that of an Esmarch tube. This cylindrical expansion is divided into squares which make the counting of the colonies very easy (Fig. 39).

The number of germs in the atmosphere will naturally be very variable. Roughly, the number may be estimated at from 100 to 1000 per cubic meter.

In reality, the bacteriologic examination of air is of very little value, as so many possibilities of error may occur. Thus, when the air of a room is quiescent there may be very few bacteria in it; let some one walk across the floor and dust at once rises, and the number



FIG. 39.—Sedgwick's expanded tube for air-examination.

of bacteria is considerably increased: if the person be a woman with skirts, more bacteria will probably be raised from the floor than would be disturbed by a man; 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 valuable examinations are those which aim at the discovery of some definite organism or organisms regardless of the number per cubic meter.

CHAPTER XII.

BACTERIOLOGIC EXAMINATION OF WATER.

UNLESS water has been specially sterilized or distilled and received and kept in sterile vessels, it always contains some bacteria. The number will bear a very distinct relation to the amount of organic matter in the water, though experiment has shown that certain pathogenic and non-pathogenic bacteria can remain vital in perfectly pure distilled water for a considerable length of time. Ultimately, owing to the lack of nutriment, they undergo a granular degeneration.

The majority of the water-bacteria are bacilli, and as a rule they are non-pathogenic. Of course, at times the most virulent forms of pathogenic bacteria—those of cholera and typhoid fever—occur in polluted water, but this is the exception, not the rule.

The method of determining quantitatively the number of bacteria in water is very simple, and can generally be prosecuted without much apparatus. The principle de-

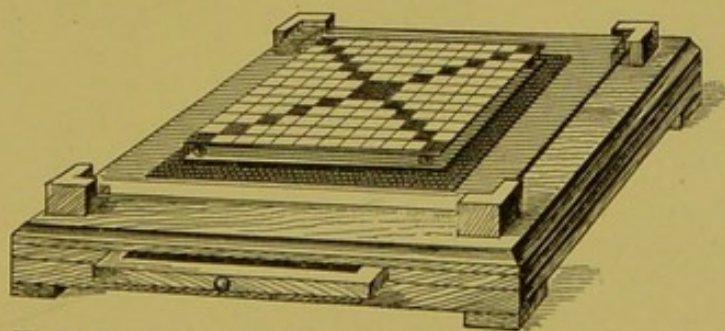


FIG. 40.—Wolfhügel's apparatus for counting colonies of bacteria upon plates.

pend upon the equal distribution of a given quantity of the water to be examined through a sterile liquid medium, and the subsequent solidification of this medium in a

thin layer, so that all the colonies which develop may be counted.

The method, which originated with Koch, may be performed with the Koch plates or with Petri dishes or with Esmarch rolls. It is always best to make a number of these plate-cultures with different amounts of the water to be examined, using, for example, 0.01, 0.1, 0.5, and 1.0 c.cm. added to a tube of gelatin, agar-agar, or glycerin agar-agar.

The exact method must depend somewhat upon the quality of the water to be examined. If the number of bacteria per cubic centimeter is small, large quantities may be used, but if there are 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.

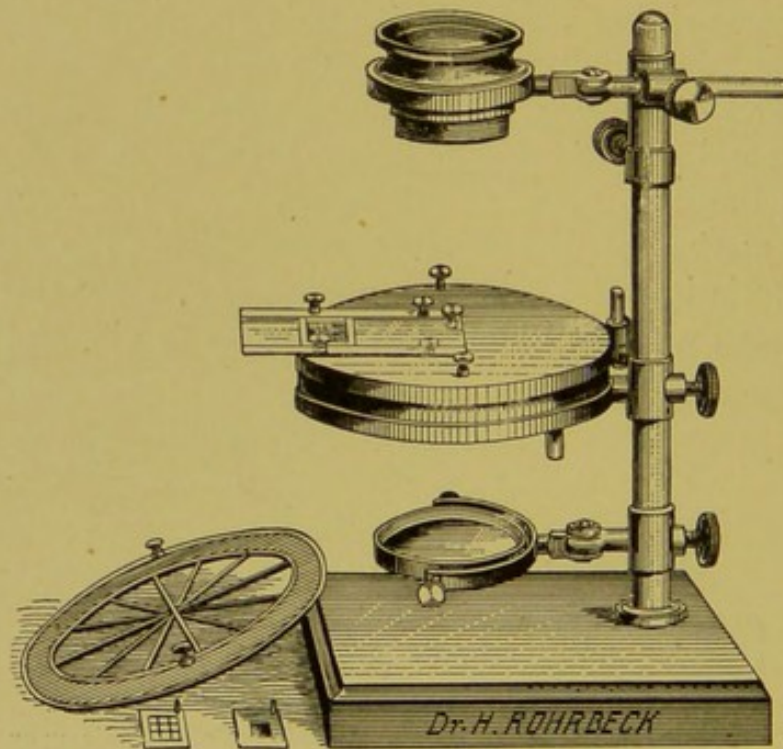


FIG. 41.—Heyroth's instrument for counting colonies of bacteria in Petri dishes.

It is best to count all the colonies if possible, but when there are hundreds or thousands scattered over the plate,

an average estimation of a number of squares ruled upon a glass background (Fig. 40), as suggested by Wolfhügel, is most convenient. In his apparatus a large plate of glass is divided into small square divisions, the diagonals being specially indicated by color. The plate or Petri dish is stood upon the glass, and the number of colonies in a number of small squares is easily counted, and the total number of colonies estimated. In counting the colonies a lens is indispensable. Special apparatuses have been devised for counting the colonies in Petri dishes (Fig. 41) and in Esmarch tubes (Fig. 42).

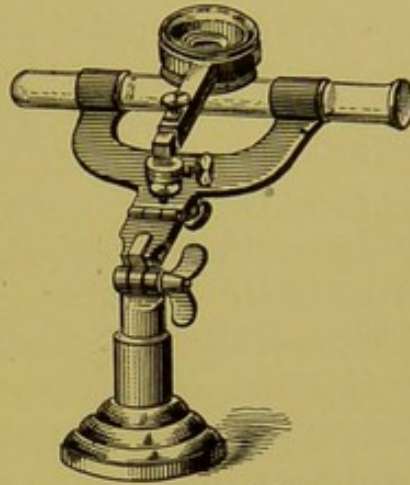


FIG. 42.—Esmarch's instrument for counting colonies of bacteria in tubes.

The majority of the water-bacteria are rapid liquefiers of gelatin, for which reason it seems better to employ agar-agar than gelatin for making the cultures.

In ordinary hydrant-water the bacteria number from 2-50 per cubic centimeter; in good pump-water, 100-500; in filtered water from rivers, according to Günther, 50-200 are present; in unfiltered river-water, 6000-20,000. 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 takes place from the surface of the ground.

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 her-

metically-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. In determining the species of bacteria found in the water reference must be made to the numerous monographs upon the subject, and to tables such as those compiled by Eisenberg.

The discovery of certain important pathogenic bacteria, as those of cholera and typhoid, will be considered under the specific headings.

Unfortunately, the bacteriologic examination of waters does not throw satisfactory light upon their exact hygienic usefulness. Of course, if cholera or typhoid-fever bacteria are present, the water is harmful, but the quality of the water cannot be gauged by the number of bacteria it contains.

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. The filters must frequently be 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 renewal or without firing.

CHAPTER XIII.

BACTERIOLOGIC EXAMINATION OF SOIL.

ALMOST all soil contains bacteria in its upper layers. Their number and character, however, depend somewhat upon the surrounding conditions. 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 being a treatment which not only adds new bacteria to those already present, but also enables those present to grow very much more luxuriantly because of the increased amount of organic matter they receive.

The researches of Flügge, C. Fränkel, and others show that the bacteria of the soil do not penetrate very deeply—that they 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 cease to be found.

Many of the soil-bacteria are anaërobic, and for a careful consideration of them the reader must be referred to monographs upon the subject. The estimation of their number seems to be devoid of any distinct practical importance. C. Fränkel has, however, originated a very accurate method of determining it. By means of a special boring apparatus (Fig. 43) earth can be secured from any depth without digging and without danger of mixing that secured with that of the superficial strata. With sterile liquefied gelatin a definite



FIG. 43.—Fränkel's instrument for obtaining earth from various depths for bacteriologic study.

amount of this soil is mixed thoroughly and the mixture 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 environment, exposure to increased amounts of 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 which are pathogenic for rabbits, guinea-pigs, and mice.

PART II. SPECIFIC DISEASES AND THEIR BACTERIA.

A. THE PHLOGISTIC DISEASES.

I. THE ACUTE INFLAMMATORY DISEASES.

CHAPTER I.

SUPPURATION.

SUPPURATION was at one time supposed to be an inevitable outcome of the majority of wounds, and, although bacteria were observed in the discharges, the old habit of thought and insufficiency of information caused most surgeons to believe that they were spontaneously developed there.

Sir Joseph Lister, whose name we cannot sufficiently honor, conceived that Pasteur's observations upon the germs of life floating in the atmosphere, if they explained the contamination of his sterile infusions, might also explain the changes in wounds, and upon this idea based that most successful system of treatment known as "antiseptic surgery."

The further development of antiseptic surgery, and the extremes into which it was carried by specialists, almost attain to the ridiculous, for not only were the hands of the operator, his instruments, sponges, sutures, ligatures, and dressings kept constantly saturated with irritating germicidal solutions, but at one time the air over the wound was carefully saturated with pulverized antiseptic lotions during the whole operation by means of a steam atomizer. This rather monstrous outcome of the application of Lister's system to surgery was the very natural result of the erroneous idea that the germs which cause

the suppurative changes in wounds entered the exposed tissues principally from the atmosphere, and that the hands and instruments of the operator, while certainly means of infection, were secondary in importance to it.

The researches of more recent date, however, have shown not only that the atmosphere cannot be disinfected, but also that the air of ordinarily quiet rooms, while containing the spores of numerous saprophytic organisms, very rarely contains many pathogenic bacteria. We now also know that a direct stream of air, such as is generated by an atomizer, causes more bacteria to be conveyed into a wound than would ordinarily fall upon it, thereby increasing instead of lessening the danger of infection. It may therefore be stated, with a reasonable amount of certainty, that the atmosphere is rarely an important factor in the process of suppuration.

We have already called attention to the fact that various micro-organisms are so intimate in their relation to the skin that it is almost impossible to get rid of them, and have cited in this relation the experiments of Welch, Robb, and Ghriskey, whose method of disinfecting the hands has been recommended as the best. The investigations of these observers have shown that, no matter how rigid the disinfection of the patient's skin, the cleansing of the operator's hands, the sterilization of the instruments, and the precautions exercised, a certain number of wounds in which sutures are employed will always suppurate. The cause of the suppuration is a matter of vast importance in surgery and in surgical bacteriology, yet it is one which it is impossible to remove. We carry it constantly with us upon our skins.

Welch has described, under the name *Staphylococcus epidermidis albus*, 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 is of the opinion that it is the same organism which is familiar to us under the name of *Staphylococcus pyogenes albus*, but in an attenuated condition. If his opinion be correct,

and we have seated deeply in our derm a coccus which can at times cause abscess-formation, the conclusions of Robb and Ghriskey, that sutures of catgut when tightly drawn may be a cause of skin-abscesses by predisposing to the development of this organism, are certainly justifiable.

Not only does the coccus occur in the attenuated form described, but we have very commonly present upon the skin, generally as a harmless saprophyte, the important *Staphylococcus pyogenes albus*, which is a common cause of suppuration.

Although, as stated, the *Staphylococcus pyogenes albus* is a common cause of suppuration, it rarely occurs alone, the studies of Passet showing that in but 4 out of 33 cases which he investigated was this coccus found by itself. When pure cultures of the coccus are injected subcutaneously into rabbits and guinea-pigs, abscesses sometimes result; sometimes there is no result. Injected into the circulation of these animals, the staphylococci sometimes cause septicemia, and after death can be found

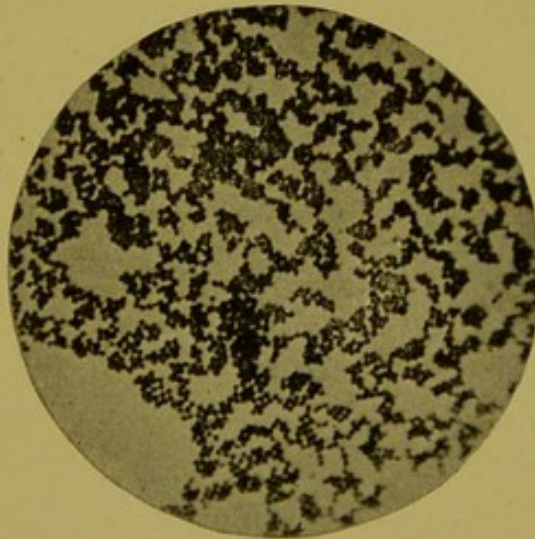


FIG. 44.—*Staphylococcus pyogenes aureus*, from an agar-agar culture; $\times 1000$ (Günther).

in the capillaries, especially of the kidneys. From these illustrations it will be seen that the organism is feebly pathogenic.

In the characteristics of its growth the *Staphylococcus albus* is almost identical with the species next to be described, but differs from it in that there is no golden color produced. Upon the culture-media it grows white.

Generally present upon the skin, though in smaller numbers, is the dangerous and highly virulent *Staphylococcus pyogenes aureus* (Fig. 44), or "golden staphylococcus" of Rosenbach. As the morphology of this organism, and indeed the generality of its characters, are identical with those of the preceding species, it seems convenient to describe them together, pointing out such differences as occur step by step. In doing this, however, it must not be forgotten that, although the *Staphylococcus albus* has been described first, the *Staphylococcus aureus* is the more common organism of the suppurative diseases.

Although they had been seen earlier by several observers, the staphylococci were not isolated and carefully described until 1884, when Rosenbach worked upon them. The results of his study, followed by Passet and a host of others, have now given us pretty accurate information about them.

The cocci are distributed rather sparingly in nature, seeming not to find a purely saprophytic existence a suitable one. They occur, however, wherever man and animals have been, and can be found in the dust of houses, hospitals, and especially surgical wards where proper precautions are not exercised. They are common upon the skin, they live in the nose, mouth, eyes, and ears of man, they are nearly always beneath the fingernails, and they sometimes occur in the feces, especially in children.

The cocci are rather small, measuring about 0.7μ in diameter. When examined in a delicately-stained condition the organisms may be seen to consist of hemispheres separated from each other by a narrow interval. The contiguous surfaces are flat, thus differing from the gonococcus, whose contiguous surfaces are concave.

The grouping is not very characteristic. In both liquid and solid culture-media the organisms either occur in solid masses or are evenly distributed. It is only in the organs or tissues of a diseased animal that it is possible to say that a true staphylococcus grouping is present.

The organism stains brilliantly with aqueous solutions of the anilin dyes. In tissues it can be beautifully stained by Gram's method.

The staphylococci grow well either in the presence or absence of oxygen at a temperature above 18° C., the most rapid development being at about 37° C. Upon the surface of gelatin plates small whitish points can be observed in forty-eight hours (Fig. 45). These rapidly

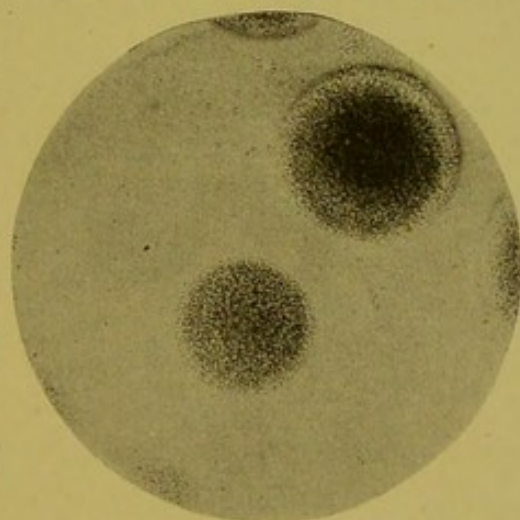


FIG. 45.—*Staphylococcus pyogenes aureus*: colony two days old, seen upon an agar-agar plate; $\times 40$ (Heim).

extend to the surface and cause extensive liquefaction. Hand in hand with the liquefaction is the formation of an orange color, which is best observed at the centre of the colony. 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 much more distinct.

In gelatin punctures the growth occurs along the whole length of the needle-track, and causes an extensive lique-

faction in the form of a long, narrow, blunt-pointed, inverted cone (Fig. 46) full of clouded liquid, at the apex

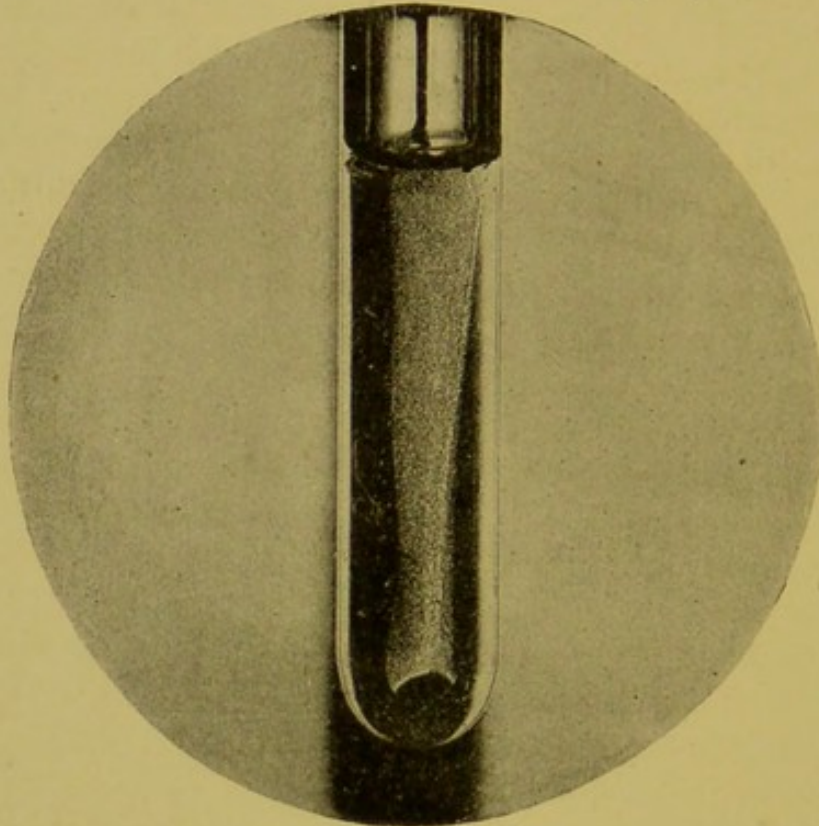


FIG. 46.—*Staphylococcus pyogenes aureus*: puncture-culture three days old in gelatin (Fränkel and Pfeiffer).

of which a collection of golden or orange-yellow precipitate is always present. It is this precipitate in particular that gives the organism its name, "golden staphylococcus."

The most characteristic growth is upon agar-agar. Along the whole line of inoculation an orange-yellow, moist, shining growth occurs. When the growth takes place rapidly, as in the incubator, it exceeds the rapidity of color-production, so that the centre of the growth is distinctly golden; the edges may be white.

Upon potato the growth is luxuriant, producing an orange-yellow coating over a large part of the surface. The potato-cultures give off a sour odor.

When grown in bouillon the organism causes a diffuse cloudiness.

In milk coagulation takes place, and is followed by gradual digestion of the casein.

The *Staphylococcus albus* is exactly the same as the *aureus*, with the exception that in all media it is constantly colorless.

Experiments have shown that the *Staphylococcus aureus*, like its congener, the *albus*, exists in an attenuated form, and there is every reason to believe that in the majority of instances it inhabits the surface of the body in this form.

When virulent the golden staphylococcus is a dangerous and often deadly organism. Its pathogeny among animals is decided. When introduced subcutaneously, abscesses almost invariably follow, except in a certain few comparatively immune species, and not infrequently lead to a fatal termination. In such cases the organisms may be cultivated from the blood of the large vessels, though by far 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 sometimes are full of cocci, and become the centres of small abscesses.

The coccus is almost equally pathogenic for man, though the fatal outcome is much more rare. It enters the system through scratches, punctures, or abrasions, and when virulent generally causes an abscess, as various experimenters who inoculated themselves have discovered to their cost. Garré 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 layers of the skin with his fingernail; 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.

The *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 of the internal organs. Becker and others obtained it from the pus of osteomyelitis, demonstrating that if, after fracturing or crushing a bone, the staphylococcus was injected into the circulation, osteomyelitis would result. Numerous bacteriologists 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 the valvular lesion without the preceding injury.

The *Staphylococcus aureus* is an easy organism to ob-

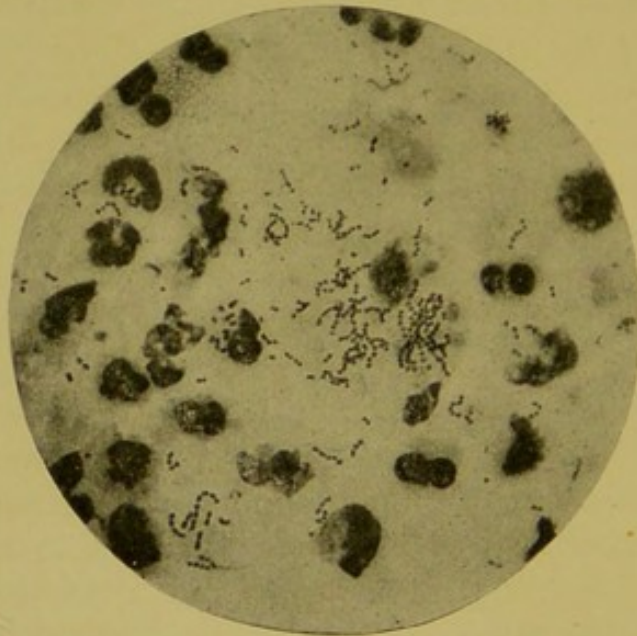


FIG. 47.—*Streptococcus pyogenes*, from the pus taken from an abscess; $\times 1000$ (Fränkel and Pfeiffer).

tain, and can be secured by plating out a drop of pus in gelatin or in agar-agar. Such a preparation, however,

generally does not contain the *Staphylococcus aureus* alone, but shows colonies of the *Staphylococcus albus* as well. In addition to these two principal forms, one sometimes discovers an organism identical with the preceding, except that its growth on agar-agar and potato is of a brilliant lemon-yellow color, and its pathogeny for animals much less. This is the *Staphylococcus citreus* of Passet. It is not quite so common, and not so pathogenic as the others, and consequently much less important.

Another organism whose colonies are frequently obtained from the pus containing the staphylococci is the *Streptococcus pyogenes* of Rosenbach (Fig. 47). It was found by him in 18 of 33 cases studied, fifteen times alone and five times with the *Staphylococcus aureus*. It is a spherical organism of variable size (0.4-1 μ in diameter), constantly associated in pairs and chains of from four to twenty individuals.

The organism stains well with ordinary aqueous solutions of the anilin dyes, and also by Gram's method. Like the coccus already described, it is not motile and does not seem to form spores, though sometimes a large individual—much larger than the others in its chain—may be observed, and may suggest the thought of arthrosporulation.

Upon gelatin plates very small colonies of translucent appearance are observed. 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, and to have numerous irregularities around the edges, due to projecting chains of the cocci. No liquefaction occurs.

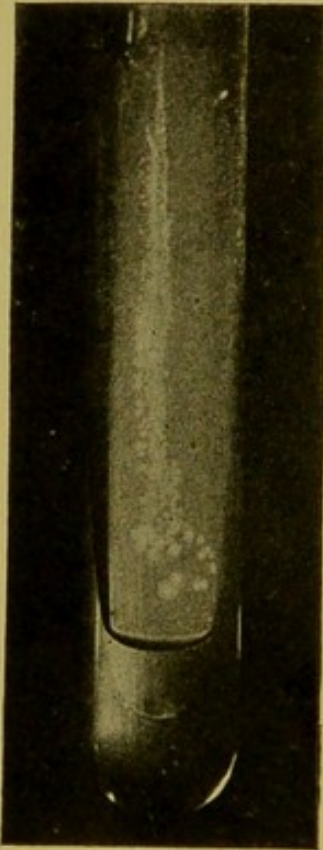


FIG. 48.—*Streptococcus pyogenes*: culture upon agar-agar two days old (Fränkel and Pfeiffer).

In gelatin puncture-cultures no liquefaction is observed. The minute spherical colonies grow along the whole needle-track and form a slightly opaque granular line.

Upon agar-agar an exceedingly delicate transparent growth develops slowly along the line of inoculation. It consists of almost transparent, colorless small colonies which do not become confluent.

The growth upon blood-serum much resembles that upon agar-agar. The streptococcus does not grow upon potato.

The organism seems to grow well in milk which is coagulated and digested.

The Streptococcus is not very sensitive to acids, and can be grown quite well in media with a slightly acid reaction.

Sternberg found that the streptococci succumb to a temperature of 52-54° C. continued for ten minutes.

The streptococcus pyogenes is not very pathogenic for animals. Subcutaneous injections into mice and rabbits are, as a rule, without either general or local manifestations of importance. If, however, an ear of a rabbit is inoculated with a small amount of a pure culture carefully scratched in, a small patch resembling erysipelas usually results. The disturbance passes away in a few days and the animal recovers.

Like the staphylococci, the Streptococcus pyogenes is frequently associated with internal diseases, and has been found in ulcerative endocarditis and in the uterus in cases of infective puerperal endometritis. Its relation to diphtheria is of interest, for, while, in all probability, the great majority of cases of pseudo-membranous angina are caused by the Klebs-Löffler bacillus, yet an undoubted number of cases are met with in which, as in Prudden's 24 cases, no diphtheria bacilli can be found, but which seem to be caused by a streptococcus exactly resembling that under consideration.

There is no clinical difference in the picture of the throat-lesion produced by the two organisms, and the

only positive method of diagnosticating 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. Of course, in streptococcus angina no benefit could be expected from the diphtheria antitoxic serum.

The streptococcus of Rosenbach is thought by many to be identical with a streptococcus described by Fehleisen as the *Streptococcus erysipelatis* (Fig. 49). The two or-

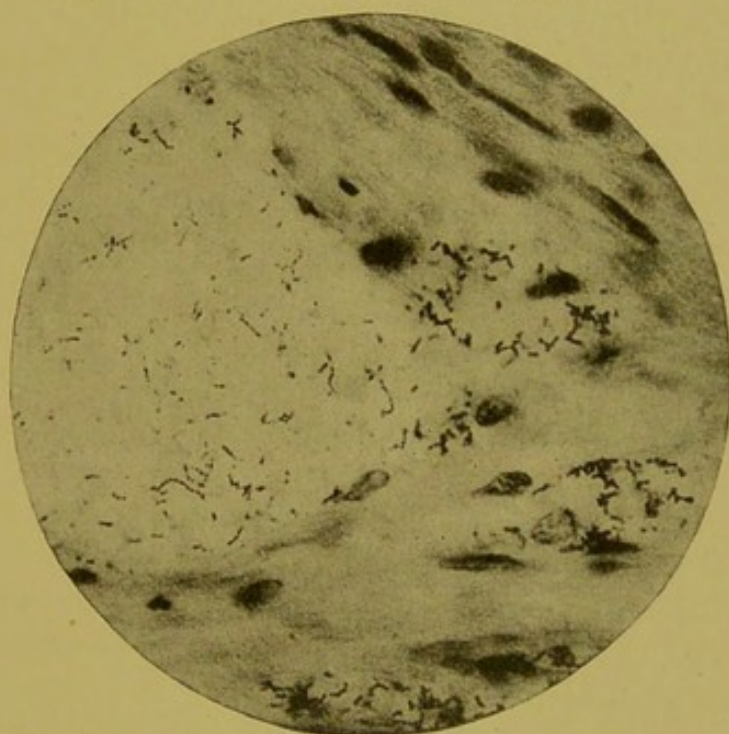


FIG. 49.—*Streptococcus erysipelatis*, seen in a section through human skin; $\times 500$ (Fränkel and Pfeiffer).

ganisms have much in common, but much difference of opinion exists upon the subject of their identity. It may seem unwise to omit the *Streptococcus erysipelatis* as a major topic for discussion, but the similarity of the organism to that just described has caused us to consider them in the same connection.

The streptococci of erysipelas can be obtained in almost pure culture from the serum which oozes from a puncture made in the margin of an erysipelalous patch. They are small cocci, forming long chains—generally from six to

ten individuals, but sometimes reaching a hundred in number. Occasionally the chains can be found collected in tangled masses. They can be cultivated at the room-temperature, but grow much better at 30-37° C. They are not particularly sensitive to the absence of oxygen, but develop a little more rapidly in its presence.

The erysipelas cocci, like the *Streptococcus pyogenes*, are not motile, form no spores, and are destroyed by a low degree of heat. They stain well with aqueous solutions of anilin dyes and also by Gram's method.

The colonies upon gelatin and the development in gelatin tubes, upon agar-agar, and upon blood-serum are identical with the descriptions of the *Streptococcus pyogenes*. No growth occurs on potato.

The growth in bouillon is generally luxuriant, and in a short time causes the medium to be filled with chains of the cocci. As the growth progresses these chains gather in clusters and fall to the bottom as a whitish granular precipitate, above which the liquid remains clear.

When injected into animals Fehleisen's coccus behaves exactly like the *Streptococcus pyogenes*.

Observation has shown that dire results may follow the entrance of this organism into exposed wounds, and that it causes not only local suppuration, but sometimes a general infection.

The empiric experience that the occasional accidental infection of malignant tumors with erysipelas cocci was followed by sloughing and subsequent disappearance of the tumor, suggested inoculation with the *Streptococcus erysipelatis* as a therapeutic measure. The dangerous character of the remedy, however, caused many to refrain from its use, for when one inoculated the living erysipelas germs into the tissues he never could estimate the exact amount of disturbance that would follow. The difficulty seems to have been overcome by Coley, who recommends the toxin instead of the living coccus for injection. A virulent culture is obtained, inoculated

into small flasks of slightly acid bouillon, allowed to grow for three weeks, then reinoculated with *Bacillus prodigiosus*, allowed to grow for ten or twelve days at the room-temperature, well shaken up, poured into bottles of about f3ss capacity, and rendered perfectly sterile by an exposure to from 50-60° C. for an hour. It is claimed that the combined toxins of erysipelas and prodigiosus are much stronger than the simple erysipelas toxin. The best effects are found in cases of sarcoma, where the toxin 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 has been most efficacious; but, although Coley recommends it and Czerny still upholds it, the majority of surgeons have failed to secure the desired results.

Recently (1895) considerable attention has been bestowed upon the development of anti-streptococcus serum, which is said to act specifically upon cases of streptococcus-infection, both general and local. Numerous

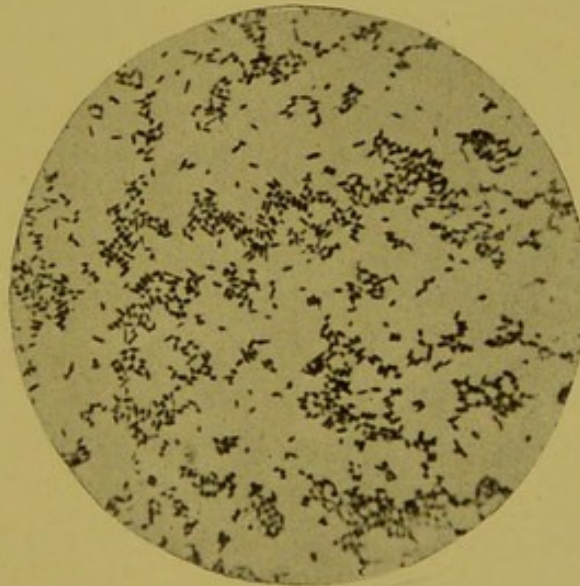


FIG. 50.—*Bacillus pyocyaneus*, from an agar-agar culture; $\times 1000$ (Itzerott and Niemann).

cases are upon record in which the serum exerted a beneficial action, though a case reported by Weatherly

in which Marmorek's serum was used terminated fatally after rather distinct improvement.

It would seem as if an antiphlogistic serum would occupy an important place in the future of medicine.

In some cases the pus evacuated from wounds exhibits a peculiar bluish or greenish color, from the presence of the *Bacillus pyocyaneus* (Figs. 50, 51). This is a short, delicate bacillus of small size, frequently united in chains of four or six. It has round ends, is actively motile, does not form spores, and can exist with or without oxygen.

The superficial colonies upon gelatin plates form small, irregular, ill-defined collections, which produce a fluorescence of the neighboring gelatin. The gelatin softens gradually, and about five days elapse before liquefaction is complete.

The microscope shows the colonies to be round, coarsely-granulated masses

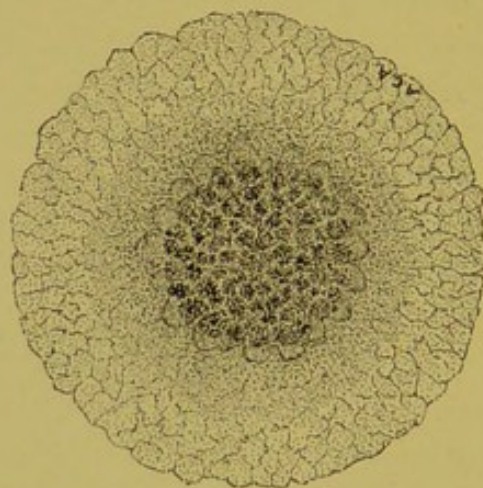


FIG. 51.—*Bacillus pyocyaneus*: colonies upon gelatin (Abbott).

with notched or filamentous borders. They have a yellow-green color. Upon the surface they form a delicate clump with a smooth surface, finely granular, distinctly green in the middle and pale at the edges. The colonies sink into the gelatin as the liquefaction progresses.

In gelatin puncture-cultures most of the development occurs at the upper part of the tube, where a deep saucer of liquefaction forms. The growth slowly descends into the medium, and is the point of origin of a beautiful fluorescence. The bacterial growth sinks to the bottom as it ages. At times a delicate mycoderma forms on the surface.

Upon agar-agar the growth is at first bright green,

developing all along the line of inoculation. The green pigment (fluorescin) is soluble, and soon saturates the culture-medium and makes it very characteristic. As the culture ages, or if the medium upon which it grows contains much peptone, a second pigment (pyocyanin) is developed, and the bright green fades to a deep blue-green, dark-blue, or in some few cases to a deep reddish-brown.

Upon potato a luxuriant greenish, smeary layer is produced.

This bacillus is highly pathogenic for laboratory animals. About 1 c.cm. of a fresh bouillon culture, if injected into the subcutaneous tissue of a guinea-pig or a rabbit, causes a rapid edema, a suppurative inflammation, and death in a short time. The bacilli can be found in the blood and in most of the tissues.

Intraperitoneal injections cause suppurative peritonitis.

It is interesting to observe, in passing, that this pathogeny can be set aside by the immunity which develops

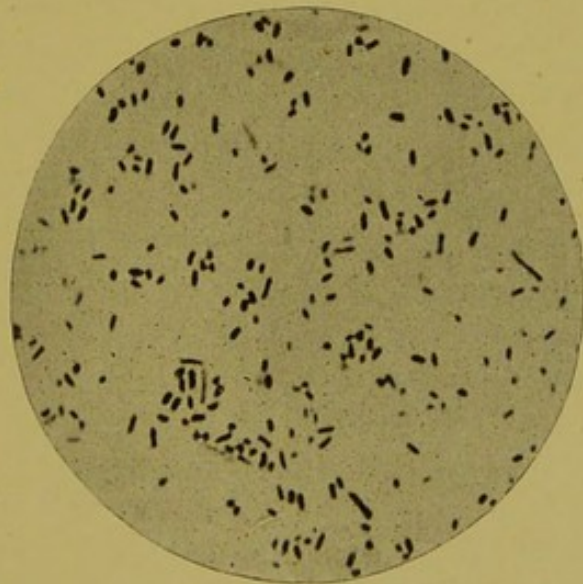


FIG. 52.—*Bacillus pyogenes foetidus*, from agar-agar; $\times 1000$ (Itzerott and Niemann).

after a few inoculations with sterilized cultures. These are easily prepared, as the thermal death-point determined by Sternberg is 56° C.

The bacillus appears to be rather common as a saprophyte, and, as it has been found in the perspiration, probably is not uncommon upon the skin.

The unpleasant odors that sometimes accompany suppuration are probably, in most cases, due to the *Bacillus pyogenes fœtidus* of Passet (Fig. 52). This organism measures about 1.5μ in length and 0.5μ in breadth. It is motile; it probably does not form spores. The colonies are small white disks without distinctive features. In gelatin punctures a thin grayish-white growth occurs upon the surface, and surmounts a collection of spherical confluent colonies in the puncture. It does not cause any liquefaction.

Upon potato an abundant brownish growth takes place.

The cultures all give off an unpleasant putrefactive odor. The organism is only pathogenic for mice and guinea-pigs.

Occasionally other organisms of minor importance are found in pus. Most of these, like the *Bacillus pyocyaneus* and *Bacillus pyogenes fœtidus*, are probably harmless saprophytes accidentally present, so that it will hardly be proper to devote space to their consideration.

Before leaving the subject of suppuration, however, attention must be called to several rather common bacteria which may at times be the cause of troublesome suppuration. Among these are the pneumococcus of Fränkel and Weichselbaum, the *Bacillus coli communis*, and the typhoid bacillus.

The pneumococcus has not infrequently been discovered most unexpectedly in abscesses of the brain and other deep-seated organs, and seems to have powerful chemotactic powers. For a careful consideration of it the reader must be referred to the chapter upon Pneumonia, where it is considered in full.

The *Bacillus coli communis*, which is always present in the intestine, seems at times to enter the blood- or lymph-channels and stimulate suppuration, and numerous cases are on record showing this. The points most frequently

attacked seem to be the bile-ducts and the vermiform appendix, though the significance of the organism in appendicitis has no doubt been overrated. It has also been found in the kidney in scarlatinal nephritis, and is thought to be the exciting cause of some cases. For a more particular study of this organism the reader is referred to the chapter on Typhoid Fever.

The typhoid bacillus is probably less frequently a cause of suppuration than either of the others, yet it seems to be the occasional cause of the purulent sequelæ of typhoid fever. A case has recently been reported by Flexner in which metastatic abscesses were found to be caused by it.

The *Micrococcus tetragenus* has also been found in the pus of acute abscesses: it is quite common in the cavities of pulmonary tuberculosis, and may aid in the destructive processes involved in the general phthisical infection.

Gonorrhœa.—All authorities now accept the “gonococcus” to be the cause of gonorrhœa. It was first observed in the urethral and conjunctival secretions of gon-

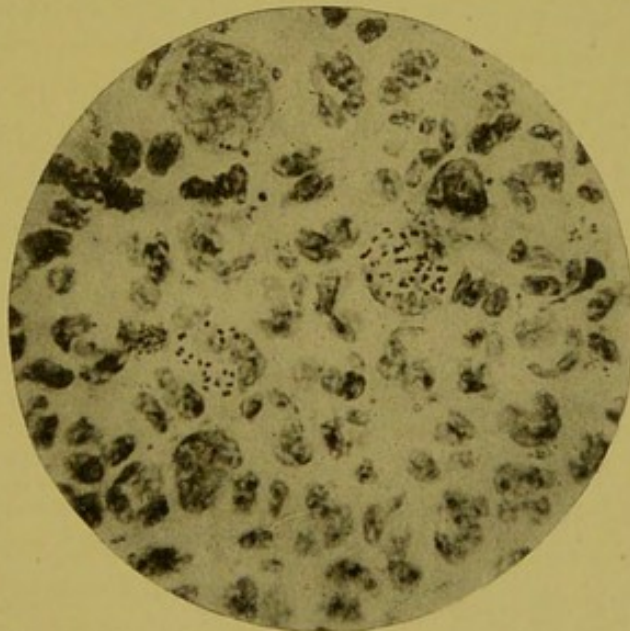


FIG. 53.—Gonococcus in urethral pus; $\times 1000$ (Fränkel and Pfeiffer).

orrhœa and purulent ophthalmia by Neisser in 1879. The organisms are of hemispherical shape, arranged in pairs, so that the inner surfaces are separated from each other

by a narrow interval. Sometimes, instead of pairs of cocci, fours are seen, the group no doubt resulting from the division of a pair.

The described hemispherical shape is not exactly correct, for a good lens generally shows the approximated surfaces to be somewhat concave rather than flat. The Germans see in the organism a resemblance to their popular biscuit called a "semmel."

The gonococcus is small, is not motile, like other cocci, is not provided with flagella, and does not have spores. It stains readily with all the aqueous anilin dyes—best with rather weak solutions—but not by Gram's method. It can be found in the urethral discharges of gonorrhoea from the beginning until the end of the disease, though in the later days its numbers may be outweighed by other organisms. The organisms are generally found within the pus-cells (Fig. 53) or attached to the surface of epithelial cells, and should always be sought for as diagnostic of gonorrhoea, especially as urethritis sometimes is caused by other organisms, as the *Bacillus coli communis*¹ and the *Staphylococcus pyogenes*.

The cultivation of the gonococcus is not an easy task, but one which requires considerable bacteriologic skill. Wertheim accomplished it by diluting a drop of the pus in a little liquid *human blood-serum*, then mixing this with an equal part of melted 2 per cent. agar-agar at 40° C., and pouring into Petri dishes. As soon as the media became firm the dishes were stood in the incubator at 37° C., and in twenty-four hours the colonies could be observed. Those upon the surface showed a dark centre, around which a delicate granular zone could be made out.

When one of these colonies is transferred to a tube of human blood-serum or the above mixture obliquely coagulated, isolated little gray colonies occur; later these become confluent and produce a delicate smeary layer

¹ Van der Pluyn und Loag: *Centralbl. f. Bakt. u. Parasitenk.*, Bd. xvii., Nos. 7, 8, Feb. 28, 1895, p. 233.

upon the medium. The main growth is surrounded by a thin veil-like extension which gradually fades away into the medium. A slight growth occurs upon the water of condensation.

The gonococci may also be cultivated upon acid gelatin, as pointed out by Turro, upon gelatin containing acid urine, and also in acid urine itself, where the gonococci grow near the surface, while the pus-cocci which may be mixed with them sink deeper into the medium.

It is ordinarily presumed that gonorrhœa cannot be communicated to animals, but Turro asserts that the gonococci when grown upon acid gelatin readily communicate urethritis to dogs, and that no *læsio continui* is necessary, the simple introduction of the organisms into the meatus sufficing to produce the disease.

That the gonococcus causes gonorrhœa there is no room to doubt. It is constantly present in the disease, and very frequently also in the sequelæ—endometritis, salpingitis, oöphoritis, cystitis, peritonitis, arthritis, conjunctivitis, etc.—and, so far as can at present be determined, is never found under normal conditions.

In the beginning of their activities the cocci grow in the superficial epithelial cells, but soon penetrate between the cells to the deeper layers, where they continue their irritation as the superficial cells desquamate. Authorities differ as to whether the gonococci can penetrate squamous and columnar epithelium with equal facility.

The periurethral abscesses that occur in the course of gonorrhœa are generally due to the *Staphylococci aureus* and *albus*, not directly to the gonococcus.

As long as the gonococci persist the patient may spread contagion. It must be pointed out that after apparent recovery from the disease the cocci sometimes remain latent in the urethra, and set up a relapse if the patient partake of some substance, as alcohol, irritating to the mucous membranes. Bearing this in mind, patients should not too soon be discharged as cured.

The gonococci are not easily killed, but withstand dry-

ing very well. Kratter was able to demonstrate their presence upon washed clothing six months after the original soiling, and also found that they still stained well.

Bumm found cocci similar to the gonococcus in the urethra, and points out that the shape is not characteristic, that the position in the cells is not positively diagnostic, but that added to these characteristics we must have the refusal to stain by Gram's method before we can say positively that cocci found in urethral pus are gonococci.

II. THE CHRONIC INFLAMMATORY DISEASES.

CHAPTER I.

TUBERCULOSIS.

TUBERCULOSIS is one of the most dreadful and, unfortunately, most common diseases of mankind. It affects alike the young and the old, the rich and the poor, the male and the female, the enlightened and the savage. Nor do its ravages cease with human beings, for it 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 zoölogical 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 even limited to mammals, but occurs in a somewhat modified form in birds, and, it is said, even at times affects reptiles.

It is not a disease of modern times, but one which has persisted through centuries; and though, before the advent of the microscope, not always clearly separated 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 Naples, where its ravages were great and the means taken for its prevention radical.

While the great men of the early days of pathology clearly saw that the time must come when the parasitic

nature of this disease would be proved, and some, as Klebs, Villemin, and Cohnheim, were "within an ace" of the discovery, it remained for Robert Koch to succeed in demonstrating and isolating the specific bacillus, now so well known, and to write so accurate a description of the organism and the lesions it produces as to render it almost unparalleled in medical literature.

The tubercle bacillus (Fig. 54) is a rod-shaped organ-

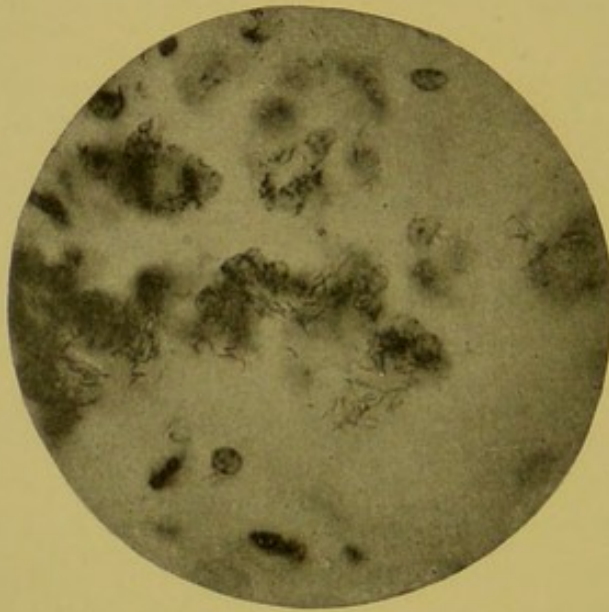


FIG. 54.—Section of a peritoneal tubercle from a cow, showing the tubercle bacilli; $\times 500$ (Fränkel and Pfeiffer).

ism with rounded ends and a slight curve, measuring from $1.5-3.5 \mu$ in length and from $0.2-0.5 \mu$ in breadth. It very commonly occurs in pairs, which may be associated end to end, but generally overlap somewhat and are not attached to each other. It is very common to observe a peculiar beaded appearance in organisms found in pus and sputum (Fig. 55), due to the contraction of fragmented protoplasm within the resisting capsule(?). By some these fragmentations are thought to be bacilli in the stage of sporulation. Koch originally held this view himself, but researches have not been able to substantiate the opinion, and at present the evidences pro

and con. point more strongly in the negative than in the positive direction.

The fragments do not look like the spores of any other organisms. When spores occur in the continuity of bacilli, they are generally discrete oval refracting bodies easily recognized. The fragments seen in the tubercle bacillus are irregular and biconcave instead of oval, have



FIG. 55.—Tubercle bacillus in sputum (Fränkel and Pfeiffer).

ragged surfaces, and are without the refraction peculiar to the ordinary spore.

The spaces between the bacillary fragments cannot be made to stain like the spores of other species. Finally, all known spores resist heat more strongly than the fully-developed bacilli, but experimentation has shown that these degenerative forms are no more capable of resisting heat than the tubercle bacilli themselves.

The organism is not motile, and does not possess flagella.

The tubercle bacillus is peculiar in its reaction to the anilin dyes. It is rather difficult to stain, requiring that the dye used shall contain a mordant (Koch), but it is also very tenacious of the color once assumed, resisting the decolorizing power of strong mineral acids (Ehrlich).

These peculiarities delayed the discovery of the bacillus for a considerable time, but now that we are familiar with them they give us a most valuable diagnostic character, for with the exception of the bacillus of lepra no known bacillus reacts in exactly the same way.

Koch first stained the bacillus with an aqueous solution of a basic anilin dye to which some potassium hydrate was added, subsequently washing with water and counter-staining 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 is at the present time acknowledged to be the best method of staining the bacillus. Many other methods have been suggested, all of them, perhaps, more convenient than Ehrlich's, but none so good.

As being that most frequently performed by the physician, we will first describe the method of seeking the bacillus in sputum.

If one desires to be very exact in his examination, it may be well to 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 object of this is to avoid the presence of fragments of food in the sputum.

The physician will secure a better result if the examination be made on the same day than if he wait a number of days, because if the bacilli are few they occur most plentifully in the small caseous flakes to be described farther on, which are easily found at first, but which break up and become part of a granular sediment that always forms in decomposed sputum.

The fresh sputum when held over a black surface generally shows a number of grayish-yellow, irregular, translucent granules somewhat smaller than the head of a pin. These consist principally of the caseous material

from tuberculous tissue, and are the most valuable part of the sputum for examination. One of the granules is picked up with a pointed match-stick and spread over the surface of a perfectly clean cover-glass. If no such fragment can be found, the purulent part is next best for examination. The mucus itself rarely contains bacilli when free from scraps of tissue and pus.

In cases in which this ordinary procedure fails to reveal bacilli whose presence is strongly indicated by the clinical signs, the exact method of searching for them is to partially digest the sputum with caustic potash, and then collect the solid matter with a centrifugal apparatus. If a very few bacilli are present in the sputum, this method will often secure them.

The material spread upon the cover-glasses 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 too few bacilli upon the glass to enable one to make a satisfactory diagnosis.

As usual, the material is allowed to dry thoroughly, and is then passed three times through the flame for purposes of fixation.

Ehrlich's Method, or the Koch-Ehrlich Method.—The cover-glasses thus prepared are floated, smeared side down, upon, 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 placed in an incubator or a paraffin oven, and kept for twenty-four hours at about the temperature of the body. When removed from the stain they are washed momentarily in water, and then alternately in 25-33 per cent. nitric acid and 60 per cent. alcohol, until the blue color of the gentian violet is almost entirely lost. It must be remembered that the action of the strong acid

is a powerful one, and that too long a time must not be allowed for its application. A total immersion of thirty seconds is quite enough in most cases. After final thorough washing in 60 per cent. alcohol the specimen is counter-stained in a dilute aqueous solution of Bismarck brown or vesuvin. The excess of stain is then washed off in water, and the specimen is dried and mounted in balsam. The tubercle bacilli will appear of a fine dark blue, while the pus-corpuscles, epithelial cells, and other bacteria, having been decolorized by the acid, will be colored brown by the counter-stain.

This method, requiring twenty-four hours for its completion, is naturally one which has fallen into disuse for practitioners who desire in the briefest possible time to know simply whether bacilli are present in the sputum or not.

Among clinicians Ziehl's method with carbol-fuchsin has met with great favor. After having been spread, dried, and fired, the cover-glass is held in the bite of an appropriate forceps (cover-glass forceps), and the stain¹ dropped upon it from a pipette. As soon as the entire cover-glass is covered with stain it is held over the flame of a spirit-lamp or a Bunsen burner until the stain begins to volatilize a little, as indicated by a white vapor. When this is observed, the heating is sufficient, and the temperature can be subsequently maintained by intermittent heating.

If evaporation is allowed to take place, a ring of incrustation occurs at the edge of the area covered by the stain and prevents the proper 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, the excess of water absorbed with paper, and 25 per cent.

¹ Carbol-fuchsin (see p. 86):

Fuchsin,	1;
Alcohol,	10;
5 per cent. phenol in water,	100.

sulphuric or 33 per cent. nitric acid dropped upon it for thirty seconds. The acid is washed off with water, and the specimen is dried and mounted in Canada balsam. Nothing will be colored except the tubercle bacilli, which will appear red.

Gabbett modified the staining by adding methylene blue to the acid solution, which he makes according to this formula:

Methyl blue,	2 ;
Sulphuric acid,	25 ;
Water,	75.

In Gabbett's method, after staining with carbol-fuchsin the specimen is washed with water, acted upon by the methylene-blue solution for exactly thirty seconds, washed with water until only a very faint blue remains, dried, and finally mounted in Canada balsam. By this method the tubercle bacilli are colored red, and the pus-corporuscles, epithelial cells, and the unimportant bacteria blue.

When the tubercle bacilli are to be sought for in sections of tissue, considerable difficulty is at once encountered, partly because of the thickness of the section and partly because of the presence of nuclei which color intensely.

Again, Ehrlich's method must be recommended as the most certain and best method of staining a large number of bacilli.

The sections of tissue, if imbedded in celloidin or paraffin, should be freed from the foreign substances. Like the cover-glasses, they are placed in the stain for twelve to twenty-four hours at a temperature of 37° C. Upon removal they are allowed to lie in water for about ten minutes to wash away the excess of stain and to soften the tissue, which often shrinks and becomes brittle. 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 counter-stained, washed, dehydrated

in 95 per cent. and absolute alcohol, cleared in xylol, and mounted in Canada balsam.

A method which has attained great and deserved praise is Unna's. It is as follows: The sections are placed in a dish of twenty-four-hours-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 next 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 are 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 are 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 as quickly as those stained by Ehrlich's method.

The tubercle bacillus also stains well by Gram's method, but as this is a general method by which many different bacteria are colored, it is ill adapted for purposes of differentiation, especially when the prosecution of the characteristic methods is not more difficult.

So far as is known, the tubercle bacillus is a purely parasitic organism. It has never been found except in the bodies and excretions of animals affected with tuberculosis, and in dusts of which these are component parts. This purely parasitic nature greatly interferes with the isolation of the organism, which cannot be grown upon the ordinary culture-media. Koch first achieved its artificial cultivation by the use of blood-serum. When planted upon this medium the bacilli are first apparent to the naked eye in about two weeks, and occur in the form of small dry, whitish flakes, not unlike fragments

of chalk. These slowly increase at the edges, and gradually form scale-like masses of small size, which under the microscope are seen to consist of tangled masses of bacilli, many of which are in a condition of involution.

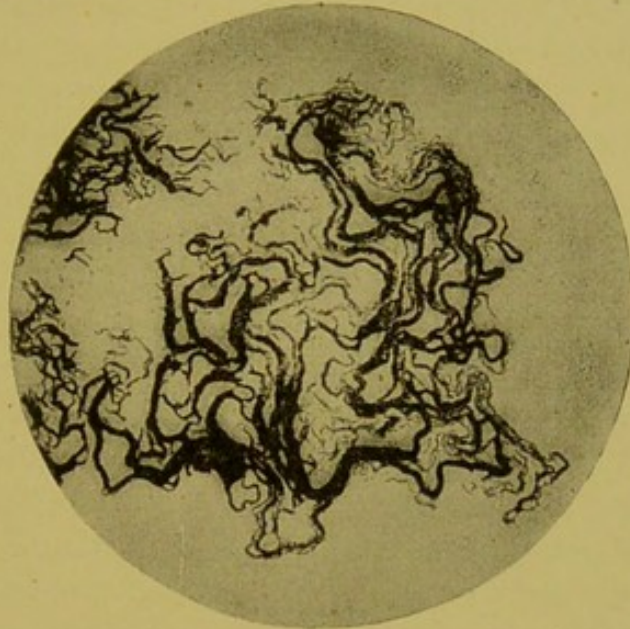


FIG. 56.—*Bacillus tuberculosis*: adhesive cover-glass preparation from a fourteen-day-old blood-serum culture; $\times 100$ (Fränkel and Pfeiffer).

The best method of obtaining a culture is to inoculate a guinea-pig with tuberculous material, allow an artificial tuberculosis to develop, kill the animal after a couple of months, and make the cultures from the centre of one of the tuberculous glands.

Of course many technical difficulties must be overcome. The tuberculous material used for inoculation may be sputum, injected beneath the skin by a hypodermic syringe. The animal is allowed to live for a month or six weeks, then killed. The autopsy is performed according to directions already given. A large lymphatic gland with softened contents or a nodule in the spleen being selected for the culture, an incision is made into it with a sterile knife, or a rigid sterile platinum wire is introduced; some of the contents are removed and planted upon blood-serum. After receiving the inoculated material the tubes are closed, either by a rub-

ber cap placed over the cotton stopper, which is cut off and pushed in, or by a rubber cork above the cotton, the idea of this rubber corking being simply to prevent evaporation. The tubes must be kept in an incubator at the temperature of 37-38° C.

Kitasato has published a method by which Koch has been able to secure the tubercle bacillus in pure culture from sputum. After carefully cleansing the mouth the patient is allowed to expectorate into a sterile Petri dish. By this method the contaminating bacteria from the mouth and the receptacle are excluded, and the expectorated material is made to contain only such bacteria as were present in the lungs. The material is carefully washed a great many times in renewed distilled sterile water until all bacteria not enclosed in the muco-purulent material are removed; it is then carefully opened with sterile instruments, and the culture-medium—glycerin agar-agar or blood-serum—is inoculated from the centre. Kitasato has been able by this method to demonstrate that many of the bacilli ordinarily present in tubercular sputum are dead, although they continue to stain well.

In 1887, Nocard and Roux gave a great impetus to investigations upon tuberculosis by their discovery that the addition of 4-8 per cent. of glycerin to bouillon and agar-agar would make them suitable for the development of the bacillus, and that a much more luxuriant development could be obtained upon these media than upon blood-serum. The growth upon such "glycerin agar-agar" much resembles that upon blood-serum (Fig. 56). The growth upon bouillon with 4 per cent. of glycerin is also luxuriant. As tubercle bacilli require considerable oxygen for their proper development, they grow only upon the surface of the bouillon, where a rather thick mycoderma forms. The surface-growth is rather brittle, and after a time gradually subsides fragment by fragment.

The tubercle bacillus can be grown in gelatin to which glycerin is added, but as its development only takes place at 37-38° C., a temperature at which gelatin is always

liquid, its use for the purpose is disadvantageous rather than useful.

Pawlowski was able to cultivate the bacillus upon potato, but Sander, who found that it could be readily grown upon various vegetable compounds, especially upon acid potato mixed with glycerin, also found that upon such compounds its virulence was constantly lost.

It has also been shown that the continued cultivation of the tubercle bacillus upon such culture-media as are appropriate so lessens its parasitic nature that in the course of time it can be induced to grow feebly upon the ordinary agar-agar.

It is really surprising to note the extremely simple compounds in which the tubercle bacillus can be grown. Instead of requiring the most concentrated albuminous media, as was once supposed, Proskauer and Beck have shown that the organism can 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. It was even found that tuberculin was produced in this inorganic mixture.

The tubercle bacillus seems to require a considerable amount of oxygen for its development. It is also peculiarly sensitive to temperatures, not growing at a temperature below 29° C. or above 42° C. Temperatures above 75° C. kill it after a short exposure.

The tubercle bacillus 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 exposed to its influence.

The widespread character of tuberculosis at one time suggested the idea 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 must be given the credit of having shown that such an idea is untrue, and that tubercle bacilli only exist in the atmospheres frequented by consumptives. His experiments were made by collecting dusts from numerous places—streets, sidewalks, houses, rooms, walls, etc. Injecting them into guinea-pigs, whose constant susceptibility to the disease makes them a very delicate reagent for its detection, Cornet showed the bacilli to be present only in the dust with which pulverized sputum was mixed, and found such infectious dust to be most common where the greatest carelessness in respect to cleanliness prevailed.

Our present knowledge of the life-history of the tubercle bacillus, by showing its indisposition to multiply outside the bodies of animals, the deleterious influence of sunlight upon it, the absence of positive permanent forms, and its sensitivity to temperatures beyond a certain range, confirms all that Cornet has pointed out, and shows us why the expectoration of millions of consumptives has not rendered our atmospheres pestilential.

As long as tuberculosis exists among men or cattle, it shows that the existing hygienic precautions are insufficient. While not so radical as to suggest the unreasonable isolation of patients and destruction of property once practised in the kingdom of Naples, the author would favor the registration of all tuberculous cases as a means of collecting accurate data concerning their origin, would insist upon domestic sterilization and disinfection, and would have special hospitals for as many, especially of the poorer classes, among whom hygienic measures are almost always opposed, as could be persuaded to occupy them.

It has already been declared the duty of the physician to use every means in his power to prevent the spread of infection in the households in his care, and no disease is more deserving of attention than this neglected one. Patients should cease to kiss the members of their fam-

ily and friends; their individual knives, forks, spoons, cups, etc. should be carefully kept apart—secretly if the patient be sensitive upon the subject—from those of the family, and scalded after each meal; the napkins and handkerchiefs, as well as whatever clothing or bed-clothing is soiled by the discharges, should be kept apart from the common wash, and boiled; and of course the expectoration should be carefully attended to, received in a suitable receptacle, sterilized or disinfected, and never allowed to dry, for it has been shown that the tubercle bacillus can remain vital in dried sputum for as long as nine months. A very neat arrangement for collecting and disposing of the expectoration is recommended by some boards of health. It consists of a metal case into which a pasteboard box is fitted. When the box is to be emptied the whole of the pasteboard portion is removed, and, together with the expectoration, burned. The metal part is disinfected, provided with a new pasteboard box, and is again ready for use. (See Fig. 16, page 102.) The physician should also give directions for disinfecting the bedroom occupied by a consumptive before it becomes the chamber of a healthy person.

Boards of health are now becoming more and more interested in tuberculosis, and, though exceedingly slow and conservative in their movements, are disseminating literature among doctors for distribution to their patients, with the hope of achieving by volition that which they would otherwise regard as cruel compulsion.

The channels by which the tubercle bacillus enters the organism are varied. A few cases are on record where the micro-organisms have passed through the *placenta*, so that a tuberculous mother was able to infect her unborn child. It is not impossible that the passage of bacilli in this manner through the placenta causes the development of tuberculosis in infants after birth, the disease having remained latent during fetal life, for Birch-Hirschfeld has shown that fragments of a fetus, itself showing no tubercular lesions, but coming from a

tuberculous woman, were fatal to guinea-pigs into which they were inoculated.

The most frequent channel of infection is the *respiratory tract*, into which the finely-pulverized dust of rooms and streets enters. Probably all of us at some time in our lives inhale living virulent tubercle bacilli, yet not all of us suffer from tuberculosis. Personal predisposition seems of great importance, for it has been shown that without the formation of tubercles virulent bacilli may 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. The tubercle bacillus is a foreign body of irritating properties, and, lodging upon a cell, is soon engulfed in its protoplasm, or, arrested by a leucocyte, is dragged off to some other region in whose narrow passages a most hostile strife doubtless takes place.

Infection also commonly takes place through the *gastro-intestinal tract* by infected food. At present an overwhelming weight of evidence points to the presence of bacilli in the milk of cattle affected with tuberculosis. It does not seem necessary that tuberculous ulcers shall be present in the udders; indeed, the bacilli have been demonstrated in considerable numbers in milk from udders without tubercular lesions discoverable to the naked eye.

The meat from tuberculous animals is less dangerous than the milk, because the meat is nearly always cooked before being eaten, while the milk is generally taken uncooked. The bacilli enter the intestinal lymphatics, sometimes produce lesions immediately beneath the mucous membrane, and lead later on to the formation of ulcers; but generally they first involve the mesenteric lymphatic glands. The thoracic duct is sometimes affected, and from such a lesion it is easy to understand the

development of a general miliary tuberculosis. The occasional absorption of tubercle bacilli by the lacteals, and their entrance into the systemic circulation and subsequent deposition in the brain, bones, joints, etc., are supposed to explain primary lesions of these tissues.

Infection is said also to take place occasionally through the *sexual apparatus*. In sexual intercourse tubercle bacilli from tuberculous testicles may be discharged into the female organs, with resulting tuberculous lesions. The infection in this way generally is from the male to the female, primary tuberculosis of the testicle being much more common than primary tuberculosis of the uterus or ovaries.

While most probably rare, in comparison with the preceding, *wounds* also are avenues of entrance for the tubercle bacilli. Anatomical tubercles are not uncommon upon the hands of anatomists and pathologists, most of these growths being tuberculous in character. An interesting fact concerning these dermal lesions is the exceedingly small number of bacilli which they contain.

The macroscopic lesions of tuberculosis are too familiar to require a description of any considerable length. They consist in nodes, nodules, or collections of agminated nodules, called tubercles, scattered irregularly through the tissues, which are devitalized or disorganized by their presence. When tubercle bacilli are introduced beneath the skin of a guinea-pig, the animal shows no sign of disease for a week or two; it then begins to lose appetite and gradually to diminish in flesh and weight. Examination at this time will show a nodule at the point of injection and enlargement of the neighboring lymphatic glands. The atrophy increases, the animal shows a febrile reaction, and at the end of a varying period of time, averaging about twelve weeks, dies. Post-mortem examination shows a cluster of tubercles at the point of inoculation, enlargement of lymphatic glands both near and remote from the primary lesion (due to the presence

of tubercles), and a widespread invasion of the lungs, liver, kidneys, peritoneum, and other organs and tissues, with tuberculous tissue in a more or less advanced condition of necrosis. Sometimes there are no tubercles discoverable at the point of inoculation. There is no regularity in the distribution of the disease. Tubercle bacilli are demonstrable in immense numbers in all the diseased tissues. The disease as seen in the guinea-pig is more extended than in other animals because of its greater susceptibility, and the death of the animal is more rapid than in other species for the same reason. In rabbits the lesion runs a longer course with similar lesions. In bovines and sheep the infection is generally first seen in, and is principally confined to, the alimentary apparatus and the associated organs, though pulmonary disease also occurs. In man the disease is chiefly pulmonary, though gastro-intestinal and general miliary forms are also 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, and is first inflammatory, then degenerative, in type.

The experiments of Koch, Prudden and Hodenphyl, 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 are chemotactically potent.

While it is extremely interesting to observe that this chemotactic property exists, it seems to be by some other irritant that most of the lesions of tuberculosis are caused. When the dead tubercle bacilli, instead of being injected *en masse* into the areolar tissue, are so introduced into the body—as by intravenous injection—as to disseminate themselves or remain in small groups, the result is quite different, and much more closely resembles that of the action of the living organism.

Baumgarten, whose researches were made upon minute tubercles of the iris, has shown that the first manifesta-

tion of the irritation caused by the bacillus is not the attraction of leucocytes, but the stimulation of the fixed connective-tissue cells of the part affected. These cells increase in number by karyokinesis, and form about the irritating bacterium a minute focus which is the primitive tubercle.

The leucocytes are of secondary advent, and are no doubt attracted both by the substance shown by Prudden and Hodenphyl to exist in the bodies of the dead bacilli and by the necrotic changes which already affect the primary cells. For reasons not understood, the amount of chemotaxis varies greatly in different cases. Sometimes the tubercles will be sufficiently purulent in type almost to justify the name "tubercular abscess;" sometimes there will be a marked absence of cellular elements derived from the blood.

The important toxic substance produced by the bacillus is evidently not associated with chemotaxis, for when the leucocytes are absent the necrosis which is so characteristic persists.

The groups of cells constituting the primitive tubercle have scarcely reached microscopic proportions before a distinct coagulation-necrosis is observable. The protoplasm of the cells affected takes on a hyaline character, and seems abnormally viscid, so that contiguous cells have a tendency to become partially confluent. The chromatin of their nuclei becomes dissolved in the nuclear juice and gives stained nuclei a pale but homogeneous appearance. Sometimes this nuclear change is only observed very late. As the necrosis advances the contiguous cells flow together and form large protoplasmic masses—giant-cells—which contain as many nuclei as there were component cells. It may be that these nuclei multiply by karyokinesis after the protoplasmic coalescence, but only one observer, Baumgarten, has found signs of this process in giant-cells. While these changes are in progress in the cells of the primary focus, the leucocytes may collect in such numbers as to obscure

them and make themselves appear to constitute the primitive cells. When the irritant substance is produced in considerable quantities, the most delicate cells die first; and it is not infrequent to find a tubercle rich in leucocytes suddenly showing degeneration of these cells, with recurring prominence of the original epithelioid cells.

It has been taught by some that the giant-cells are produced by the union of the leucocytes, but a careful observation of the rôle played by these cells will convince one that such an origin for these monstrous cells must be very rare.

Giant-cells are not always produced, for sometimes the necrotic changes are so violent and widespread as to convert the whole cellular mass into a granular detritus of unrecognizable fragments.

Tubercles are constantly avascular, as would be expected of a process which is a combination of progressive irritation and necrosis. The avascularity may be a factor in the necrosis of the larger tuberculous masses, but it plays no part in the degeneration of the smallest tubercles, which is purely toxic.

Tubercles may be developed in any tissue and in any organ. In whatever situation they occur, space is occupied at the expense of the tissue, whose component cells are pushed aside or else included in the nodule. In miliary tuberculosis of the kidney it is not unusual to find a tubercle including a whole glomerule, and resolving its component thrombosed capillaries and epithelium into necrotic fragments.

As almost all tissues contain a supporting tissue-framework of connective-tissue fragments, some of these must be embodied in the new growth. The fibres which possess little vitality are more resistant than cells, and, after all the cells of a tubercle have been destroyed, will be distinctly visible among the granules, so that the tubercle has a reticulated appearance.

As a rule, tubercles steadily increase in size by the invasion of fresh tissue. The tubercle bacillus does not seem

to find the necrotic centres of the tubercles adapted to its growth, and completes its life-cycle with the tissue-cells. It is unusual to find healthy-looking bacilli in the necrotic areas, most of them being observed at the edges of the tubercle, where the nutrition is good. From such edges the bacilli are occasionally picked up by leucocytes and transported through the lymph-spaces, until the phagocyte falls a prey to its prisoner, dies, and sows the seed of a new tubercle. However, for the spread of tubercle bacilli from place to place phagocytes are not always necessary, for the bacilli seem capable of transportation by streams of lymph alone.

Notwithstanding the steady advance which takes place in most observed cases of tuberculosis, and the thoroughly comprehensible microscopic explanation of it, many cases of tuberculosis make quite perfect recoveries.

The periphery of every tubercle is a zone of reaction, with a marked tendency to granulation and organization. If the vital condition is such that through inappropriate nutriment or through unusually active phagocytosis the activity of the bacilli is checked or their death is brought about, this tendency to cicatrization is allowed to progress unmolested, and the necrosed mass is soon surrounded with a zone of newly-formed contracting fibrillar tissue, by which it is perfectly isolated. In such isolated masses lime-salts are commonly deposited. Sometimes this process is perfected without the destruction of the bacilli, but with their incarceration and inhibition. Such a condition is called *latent tuberculosis*, and may at any time be the starting-point of a new infection and lead to a fatal termination.

In 1890, Koch announced some observations upon toxic products of the tubercle bacillus and their relation to the diagnosis and treatment of tuberculosis, which at once aroused an enormous but, unfortunately, a transitory enthusiasm.

These observations, however, are of capital importance. Koch observed that when guinea-pigs are inoculated

with a mixture containing 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 there occurs at the point of inoculation a slight induration which develops into a hard nodule, then ulcerates, and remains until the death of the animal. If, however, in the course of a short time the animals are reinoculated, the course of the process is altogether changed, for, instead of healing, the wound and the tissue surrounding it assume a dark color and become obviously necrotic, and ultimately slough away, leaving an ulcer which rapidly and permanently heals without enlargement of the lymph-glands.

Having made this observation with injected cultures of the living bacillus, Koch next observed that the same change occurred when the secondary inoculation was made with pure cultures of the dead bacilli.

It was also observed that if the material used for the secondary injection was not too concentrated and not too often repeated (only every six to forty-eight hours), the animals thus treated improved in condition, and, instead of dying of the tuberculosis induced by the primary injection in from six to ten weeks, continued to live, sometimes (Pfuhl) as long as nineteen weeks.

Koch also discovered that a 50 per cent. glycerin extract of cultures of the tubercle bacillus produced the same effect as the dead cultures originally used, and gave this substance, *tuberculin*, to the scientific world for experimental purposes, in the hope that the prolongation of life observed in the guinea-pig might be true in the case of man.

The active substance of the "tuberculin" seems to be an albuminous derivative insoluble in absolute alcohol. It is not a toxalbumin.

The action of the tuberculin upon the animal organism is peculiar, but readily understandable. *It does not exert the slightest influence upon the tubercle bacillus,*

but acts upon the living tuberculous tissue. In the description of the tissue-changes already given it has been shown that the tubercle bacillus effects the coagulation-necrosis of the cells, but does not derive its nutriment from the dead tissue. As the cells die and are incorporated in the necrotic mass, the bacilli find the conditions of life unfavorable, and likewise seem to die. The active bacilli, therefore, are always found at the margins of the tuberculous tissues, where the cells are fairly active. The necrosis is due to bacillary poisons. When tuberculin is injected into the organism the result is to double the amount of poisonous influence upon the cells surrounding the bacilli, to destroy their vitality, to remove the favorable conditions of growth from the organism, and to leave it for a time checkmated.

Virchow, who well understood the action of the tuberculin, soon showed that as a diagnostic and therapeutic agent in man its use was attended with great danger. The destroyed tissue was absorbed, and with it the bacilli were likewise absorbed and transported to new areas, where a rapid invasion occurred. Old tuberculous lesions which had been encapsulated were softened, broken down, and became sources of dangerous infection to the individual, so that, a short time after its enthusiastic reception as a "gift of the gods," tuberculin was placed upon its proper footing as a diagnostic agent valuable in veterinary practice, but dangerous in human medicine, except in cases of lupus and other external forms of the disease where the destroyed tissue could be discharged from the surface of the body.

The method of preparation of tuberculin is rather simple. Small flasks exposing a considerable surface of liquid are filled with about 25 c.cm. of bouillon containing about 4 per cent. of glycerin. The bouillon is preferably made with calf- instead of ox-meat. When thoroughly sterile the surfaces are inoculated with pure cultures of the tubercle bacillus and are stood in an incubator. In the course of two weeks a slight surface

growth is apparent, which in the course of time develops into a pretty firm pellicle and gradually subsides. At the end of four or six weeks development ceases and the pellicle sinks. The contents of a number of flasks are then collected in an appropriate vessel and evaporated over a water-bath to one-tenth their volume, then filtered through a Pasteur-Chamberland filter. This is crude tuberculin.

When such a product is injected in doses of a fraction of a cubic centimeter an inflammatory and febrile reaction occurs. The inflammation sometimes causes superficial tuberculous lesions (lupus) to ulcerate and slough away, and for this reason is of some value in therapeutics, although attended with the dangers mentioned above. The fever is sufficiently characteristic to be of diagnostic value, though the tuberculin can only be used as a diagnostic agent in practice upon animals.

Numerous experimenters, prominent among whom are Tizzoni, Cattani, Bernheim, and Paquin, have experimented with the tubercle bacillus and tuberculin, hoping that the principles of serum-therapy might be applicable to the disease. Nothing positive has, however, been achieved. The first-named observers claim to have immunized guinea-pigs in whose blood an antitoxin formed; the last-named thinks the serum of immunized horses a specific for tuberculosis. The field of experimentation is an inviting one, though the chronic course of the disease lessens the certainty with which the results can be estimated.

The Bacillus of Fowl-tuberculosis (*Tuberculosis galinarum*).—The cases of tuberculosis which occasionally occur spontaneously in chickens, parrots, ducks, and other birds were originally attributed to the Bacillus tuberculosis, but the recent works of Rivolta, Mafucci, Cadio, Gilbert, Roget, and others have shown that, while very similar in many respects to the Bacillus tuberculosis, the organism found in the disease of birds has distinct peculiarities which make it a different variety, if not a

separate species. Morphologically, the organisms are similar, the bacillus of fowl-tuberculosis being a little longer and more slender than its ally.

Upon culture-media a distinct rapidity of growth is observable, and we find that, instead of growing only where glycerin is present, the *Bacillus tuberculosis gallinarum* will grow upon blood-serum, agar-agar, and bouillon as ordinarily prepared. It will not grow upon potato. The bacillus will grow at 42-43° C. quite as well as at 37° C., while the growth of the tubercle bacillus ceases at 42° C. Moreover, the temperature of 43° C. does not attenuate its virulence. The thermal death-point is 70° C. Upon culture-media it can retain its virulence for two years.

The growth upon artificial culture-media is luxuriant, and lacks the dry quality characteristic of ordinary tubercle-bacillus cultures. As it becomes old a culture of fowl-tuberculosis turns slightly yellow.

Birds are the most susceptible animals for experimental inoculation, the embryos and young more so than the adults; guinea-pigs are quite immune. Artificial inoculation can only be made in the subcutaneous tissue, never through the intestine. The chief seat of the disease is the liver, where cellular nodes, lacking the central coagulation and the giant-cells of mammalian tuberculosis, and enormously rich in bacilli, are found. The disease never begins in the lungs, and the fowls which are diseased never show bacilli in the sputum or the dung.

Rabbits are easily infected, an abscess forming at the seat of inoculation, and later nodules forming in the lung, so that the distribution is quite different from that seen in birds.

The bacillus stains like the tubercle bacillus, but takes the stain rather more easily. The resistance to acids is about the same.

Pseudo-tuberculosis.—Eberth, Chantemesse, Charrin, and Roger have reported certain cases of so-called pseudo-tuberculosis. The disease occurred spontaneously in

guinea-pigs, and was 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 (Fig. 55). When introduced

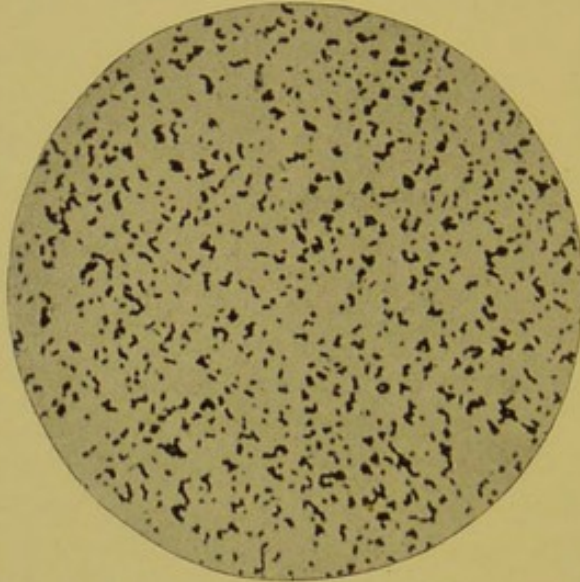


FIG. 57.—*Bacillus pseudo-tuberculosis* from agar-agar; $\times 1000$ (Itzerott and Niemann).

subcutaneously into guinea-pigs the original disease was produced.

Pseudo-tuberculosis seems to be an indefinite affection of which we have very little knowledge, and which is certainly in no way connected with or related to true tuberculosis.

CHAPTER II.

LEPROSY.

LEPROSY is a disease of great antiquity, and very early received much attention and study. In giving the laws to Israel, Moses included a large number of rules for its recognition, the isolation of the sufferers, the determination of recovery, and observances to be fulfilled before the convalescent could once more mingle with his people. The Bible is replete with accounts of miracles wrought upon lepers, and during the times of biblical tradition it must have been an exceedingly common and malignant disease.

At the present time, although we in the Northern United States hear very little about it, leprosy is still a widespread disease. It exists in much the same form as two thousand years ago in Palestine, Syria, Egypt, and the adjacent countries. It is exceedingly common in China, Siam, and parts of India. Cape Colony has many cases. In Europe, Norway, Sweden, and parts of the Mediterranean coast furnish a considerable number of cases. Certain islands, especially the Sandwich Islands, are regular hot-beds for its maintenance. The United States is not exempt, the Gulf coast being chiefly affected.

At one time the view was prevalent that the disease was spread only by contagion, at another that it was miasmatic. At present the tendency is to view it as contagious to a degree rather less than tuberculosis. Sometimes it is hereditary.

The cause of leprosy is now pretty certainly determined to be the lepra bacillus (Fig. 58), which was dis-

covered by Hansen, and subsequently clearly described by Neisser.

The bacillus is almost the same size as the tubercle bacillus—perhaps a little shorter—but lacks the curve which is so constant in the latter. It stains in very much the same way as the tubercle bacillus, but permits of a rather more rapid penetration of the stain, so that

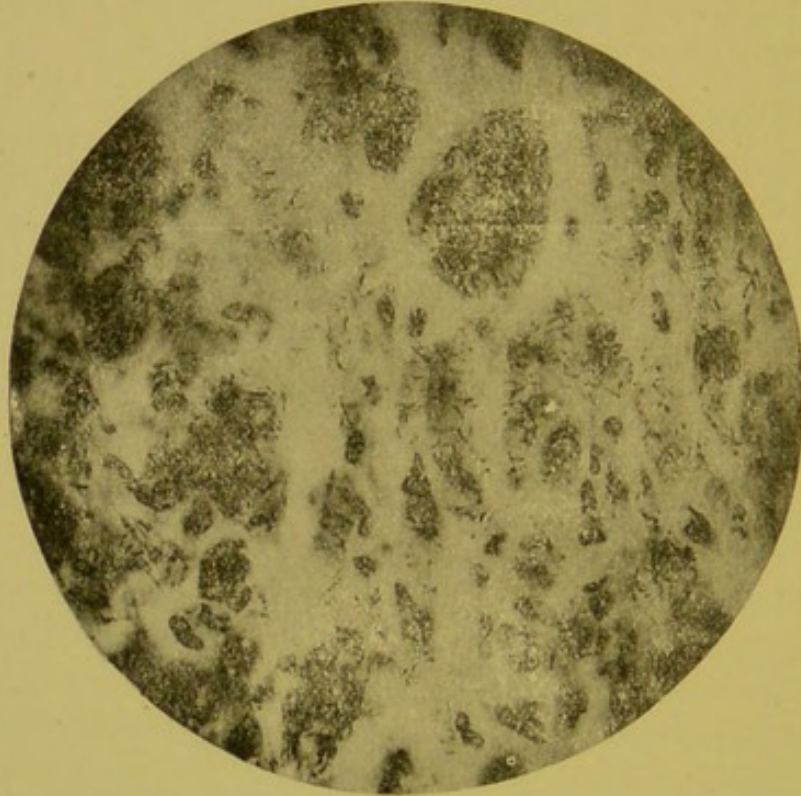


FIG. 58.—*Bacillus lepræ*, seen in a section through a subcutaneous node; $\times 500$ (Fränkel and Pfeiffer).

the ordinary aqueous solutions of the anilin dyes color it quite readily. It stains well by Gram's method, by which beautiful tissue specimens can be prepared. The peculiar property of retaining the color in the presence of the mineral acids which characterizes the tubercle bacillus also characterizes the lepra bacillus, and the methods of Ehrlich, Gabbett, and Unna can be used for its detection.

Like that of the tubercle bacillus, its protoplasm often presents open spaces or fractures, which have been re-

garded by some as spores, but which are even less likely to be spores than the similar appearances in the tubercle bacillus.

The organism almost always occurs singly or in irregular groups, filaments being unknown. It is not motile.

Many experimenters have endeavored to make this bacillus, which is so distinctly present in the nodes of lepra, grow upon artificially-prepared substances, but, in spite of modern methods, improved apparatus, and refined media, all, with the exception of Bordoni-Uffredoizzi, have met with failure. The observer named was able to grow upon a blood-serum-glycerin mixture a bacillus which partook of the staining peculiarities of the bacillus as it appears in the tissues, but differed very much in morphology. After numerous generations this bacillus was induced to grow upon ordinary culture-media. It commonly presented a club-like form, which was regarded by Baumgarten as an involution appearance. Fränkel points out that the bacillus of Bordoni is possessed of none of the essential characters of the lepra bacillus except its staining, and does not see in the large, thick organism which he cultivated anything to suggest the lepra bacillus. Absolute confirmation of the specific nature of the lepra bacillus by means of experiments upon animals is wanting. The lepra bacillus not only refuses to allow itself to be cultivated, but also refuses to be successfully transplanted from animal to animal. Only a very few instances are recorded in which actual inoculation has produced leprosy in either men or animals. Arning was able to secure permission to experiment upon a condemned criminal in the Sandwich Islands. The man was of a family entirely free from the disease. Arning introduced beneath his skin fragments of tissue freshly excised from a lepra nodule, and kept the man under observation. In the course of some months typical lesions began to develop at the points of inoculation and spread gradually, ending in general lepra in the course of about five years.

Melcher and Artmann 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 typical lepra lesions of all the viscera, especially the cecum.

While the lepra bacillus has much in common with the tubercle bacillus, there is not the slightest evidence of any real identity. It has already been shown that lepra bacilli do not grow upon artificial media, and that they cannot be readily transmitted by inoculation. The following description will show that the relation of the bacilli to the lesions is entirely different from that of the tubercle bacilli to the tubercles.

Like the *Bacillus tuberculosis*, the *Bacillus lepræ* probably only occurs in places frequented by persons suffering from the disease. That individuals are infected by the latter less readily than by the former bacilli probably depends upon the fact that leprous infection seems to take place most commonly by the entrance of the organisms into the individual through cracks or fissures in the skin, while the tuberculous infection occurs through the more accessible respiratory and digestive apparatus. Once established in the body, the bacillus by its growth produces chronic inflammatory nodes—the analogues of tubercles.

The nodes of lepra consist of various kinds of cells and of fibres. Unlike the tubercles, the lepra nodes are vascular, and much of the embryonal tissue completes its formative function by the production of fibres. The bacilli are not distributed through the nodes like tubercle bacilli, but are found in groups enclosed within the protoplasm of certain large cells—the “lepra cells.” These cells seem to be overgrown and partly degenerated lymphoid cells. Sometimes they are anuclear, sometimes they contain several nuclei (giant-cells).

Lepra nodules do not degenerate like tubercles, and the formation of ulcers, which constitutes a large part of the disease, seems largely due to the action of external

agencies upon the feebly vital pathological tissue, which is unable to recover itself when injured.

In that form known as anesthetic leprosy, nodules form upon the peripheral nerves, and by connective-tissue formation, as well as the entrance of the bacilli into the nerve-sheaths, cause irritation, then degeneration, of the nerves. The anesthesia which follows these peripheral nervous lesions is one of the conditions predisposing to the formation of ulcers, etc. by allowing injuries to occur without detection and to progress without observation. The ulcerations and occasional loss of phalanges that follow these lesions occur, probably, in the same manner as in syringomyelia.

The disease advances, having first manifested itself upon the face, extensor surfaces, elbows, and knees, to the lymphatics and the internal viscera. Death ultimately occurs from exhaustion, if not from the frequent inter-current affections to which the conditions predispose.

CHAPTER III.

GLANDERS.

GLANDERS is an infectious mycotic disease which, very fortunately, is almost confined to the lower animals. Only occasionally does it secure a victim from hostlers, drovers, soldiers, and bacteriologists, whose frequent association with and experimentation upon animals bring them in frequent contact with those which are diseased. Of all the infectious diseases studied by scientists, none has caused the havoc which glanders has wrought. Several men of prominence have succumbed to accidental infection.

Glanders was first known to us as a disease of the horse and ass characterized by the occurrence of discrete, cleanly-cut ulcers upon the mucous membrane of the nose. These ulcers are formed by the breaking down of nodules which can be detected upon the diseased membranes, and show no tendency to recover, but slowly spread and discharge a virulent pus. The edges of the ulcers are indurated and elevated, the surfaces often smooth. The disease does not progress to any great extent before the submaxillary lymphatic glands begin to enlarge. Later on these glands form large lobulated masses, which may soften, open, and become discharging ulcers. The lungs may also become infected by inspiration of the infectious material, and contain small foci not unlike tubercles in appearance. The animals ultimately die of exhaustion.

In 1882, shortly after the discovery of the tubercle bacillus, Löffler and Schütz discovered in the discharges and tissues of this disease the specific micro-organism, the glanders bacillus (*Bacillus mallei*; Fig. 59), which is its cause.

The glanders bacillus is somewhat shorter and distinctly thicker than the tubercle bacillus. It has rounded ends, and it generally occurs singly, though upon blood-

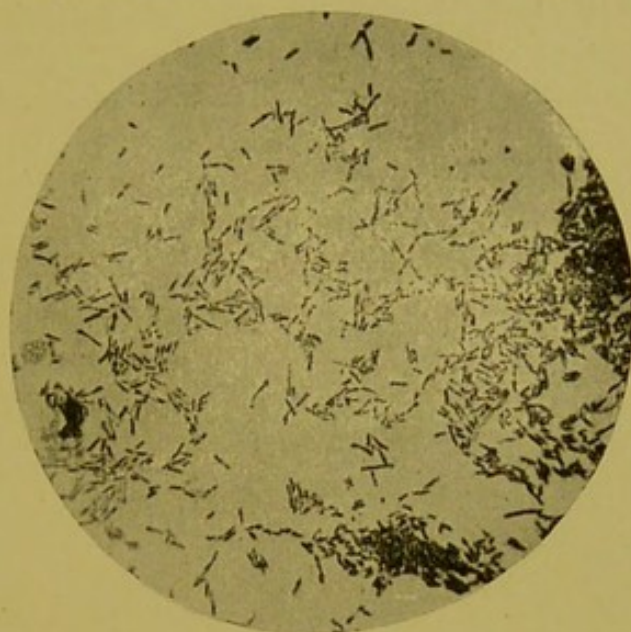


FIG. 59.—*Bacillus mallei*, from a culture upon glycerin agar-agar; $\times 1000$ (Fränkel and Pfeiffer).

serum, and especially upon potato, several joined individuals may be found. Long threads are never formed.

The bacillus is non-motile. Various observers have claimed the discovery of spores, but although in the interior of the bacilli there have been observed irregular spaces like the similar spaces in the continuity of the tubercle bacillus not colored by the stains, they have not yet been definitely proven to be spores. The observation of Löffler that the bacilli can be cultivated after being kept in a dry state for three months makes it appear as if some permanent form (spore) occurs. No flagella have been demonstrated upon the bacillus.

Like the tubercle bacillus, the glanders bacillus does not seem to find conditions outside the animal body suitable for its existence, and probably does not occur except as a parasite.

The organism only grows between 25° and 42° C., and generally grows very slowly, so that attempts at its isola-

tion and cultivation by the usual plate method are apt to fail, because the numerous other organisms in the material grow much more rapidly.

The best method of isolation seems to be the use of an animal reagent. It has been said that glanders principally affects horses and asses. Recent observations, however, have shown the goat, cat, hog (slightly), field-mouse, wood-mouse, marmot, rabbit, guinea-pig, and hedgehog all to be susceptible animals. Cattle, house-mice, white mice, and rats are immune.

The guinea-pig, being a highly susceptible as well as a readily procurable animal, naturally becomes the reagent for the detection and isolation of the bacillus. When a subcutaneous inoculation of some glanders pus is made, the disease can be observed in guinea-pigs by a tumefaction in from four to five days. Somewhat later this tumefaction changes to a caseous nodule, which ruptures and leaves a chronic ulcer with irregular margins. The lymph-glands speedily become involved, and in a month to five weeks signs of general infection are present. The lymph-glands suppurate, the testicles undergo the same process, and still later the joints exhibit a suppurative arthritis containing the bacilli. The animal finally dies of exhaustion. In guinea-pigs no nasal ulcers form. In field-mice, which are even more susceptible, the disease is much more rapid. No local lesions are visible. In two or three days the animal seems unwell, the breathing is hurried, it sits still with closed eyes, and without any other preliminaries tumbles over on its side, dead.

From the tissues of the inoculated animals the pure cultures are most easily made. Perhaps the best places to secure the culture are from softened nodes which have not ruptured or from the suppurating joints. Strauss has, however, given us a method which is of great use, because of the short time 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. The testicles enlarge a little; the skin over them becomes red and shining. The testicles themselves begin to suppurate, and often discharge through the skin. The animal dies in about two weeks. If such an animal 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 purulent discharges from the noses of horses and from other lesions of large animals generally contain very few bacilli, so that a method of isolation by an animal is very advantageous by greatly increasing the number of bacteria.

The bacillus is an aërobic 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. It grows in gelatin, but this is not an appropriate medium, because the bacillus develops best at temperatures at which the gelatin is liquid.

Upon 4 per cent. glycerin agar-agar plates the colonies appear upon the second day as pale-yellow or whitish, shining round dots. Under the microscope they appear as brownish-yellow, thick granular masses with sharp borders.

The culture upon agar-agar and glycerin agar-agar occurs as a moist, shining layer not possessed of distinct peculiarities. Upon blood-serum the growth is rather characteristic. The colonies along the line of inoculation first develop as circumscribed, clear, transparent drops, which later become confluent and form a transparent layer unaccompanied by liquefaction.

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-temperature and soon becoming reddish-brown. As this brown color of the colony develops, the potato for

a considerable distance around it becomes greenish-brown. (See *Frontispiece*.) No other known organism produces the same appearance upon potato.

The organism loses its virulence if cultivated for many generations upon artificial media.

That this bacillus is the cause of glanders there is no room to doubt. Löffler and Schütz have succeeded by the inoculation of horses and asses in producing the well-known disease.

The organisms when in cultures can be stained with the watery anilin-dye solutions, but are difficult to stain in tissues. They do not stain by Gram's method.

The chief difficulty in staining the bacillus in tissues is the readiness with which it gives up the stain in the presence of decolorizing agents. Löffler at first 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 ;
5 per cent. oxalic-acid solution,	1 drop ;
Distilled water,	10 c. cm.

for five seconds, then transferring 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 carbol-methylene blue :

Methylene blue,	1.5
Alcohol,	10.
5 per cent. aqueous phenol solution,	100.

Kühne's method of staining is to place the section in the stain for about half an hour, wash in water, decolorize carefully in hydrochloric acid (10 drops to 500 c. cm. of water), immerse at once in a solution of lithium carbonate (8 drops of a saturated solution of lithium carbonate in 10 c. cm. of water), place in a bath of distilled water for a few

minutes, dip into absolute alcohol colored with a little methylene blue, dehydrate in anilin oil containing a little methylene blue in solution, wash in pure anilin oil, not colored, then in a light ethereal oil, clear in xylol, and mount in balsam.

When stained in sections of tissue the bacilli are found to occupy the interior of small inflammatory zones not unlike tubercles in appearance. These nodules can be seen with the naked eye scattered through the livers, kidneys, and spleens of animals dead of experimental glanders. The nodules consist principally of leucocytes, but also contain numerous epithelioid cells. As is the case with tubercles, the centres of the nodules are prone to degenerate, soften, and also to suppurate. The retrogressive processes upon exposed surfaces, where the breaking down of the nodules allows their contents to escape, are the sources of the typical ulcerations. At times the process is progressive, and some of the lesions heal by the formation of a stellate scar.

As has been mentioned, cultures of the bacillus lose their virulence more or less after four or five generations in artificial media. While this is true, attempts to attenuate fresh cultures by heat, etc. have so far failed.

Leo has pointed out that white rats, which are immune to the disease, may be made susceptible by feeding with phloridzin and causing a glycosuria.

Kalning, Preusse, Pearson, and others have prepared a substance, "mallein," from cultures of the bacillus, and suggested its employment for diagnostic purposes. It seems to be quite useful in veterinary medicine, the reaction occasioned by its injection being similar to that caused by the injection of tuberculin in tuberculous patients. The manufacture of mallein is not attended with great difficulty. The bacilli are grown in glycerin bouillon for several weeks, killed by heat, the culture filtered through porcelain and evaporated to one-tenth of its volume. It has also been prepared from potato cultures, which are said to produce a stronger

toxin. A febrile reaction of more than 1.5° C. following the injection is said to be specific of the disease. Babes has asserted that the injection of this toxic product into susceptible animals will protect them from the disease.

Various experiments have been made with curative objects in view. Certain observers claim to have seen good results follow the injection of mallein in repeated small doses. Others, as Chenot and Picq, find the blood-serum from immune animals like the ox to be curative when injected into infected guinea-pigs.

CHAPTER IV.

SYPHILIS.

ALTHOUGH syphilis is almost as well known as it is widespread, we have not yet discovered for it a definite specific cause. Whether it is due to a protozoan parasite, or whether it is due to a bacterium, the future must decide. Numerous claims have been made by those whose studies have revealed organisms of one kind or another in syphilitic tissues, but no one has yet succeeded either in isolating, cultivating, or successfully inoculating them.

In 1884 and 1885, Lustgarten published a method for the staining of bacilli which he had found in syphilitic tissues and assumed to be the cause of the disease. The staining, which is very complicated, requires that the sections of tissue be stained in Ehrlich's anilin-water gentian-violet solution for twelve to twenty-four hours at the temperature of the room, or for two hours at 40° C.; washed for a few minutes in absolute alcohol; then immersed for about ten seconds in a 1½ per cent. permanganate-of-potassium solution, after which they are placed in an aqueous solution of sulphurous acid for one to two seconds, thoroughly washed in water, run through alcohol and oil of cloves, and finally mounted in Canada balsam dissolved in xylol.

If the bacilli are supposed to be present in pus or discharges from syphilitic lesions, the cover-glasses spread with the material are stained in the same manner, except that for the first washing distilled water instead of absolute alcohol is used.

This method undergoes a modification in the hands of De Giacomo, who prefers to stain the cover-glasses in hot

anilin-water-fuchsin solution for a few moments, sections in the same solution cold for twenty-four hours; then immerse them first in a weak, then in a strong, solution of chlorid of iron. The cover-glasses are washed in water, sections in alcohol, and subsequently passed through the usual reagents for dehydration and clearing.

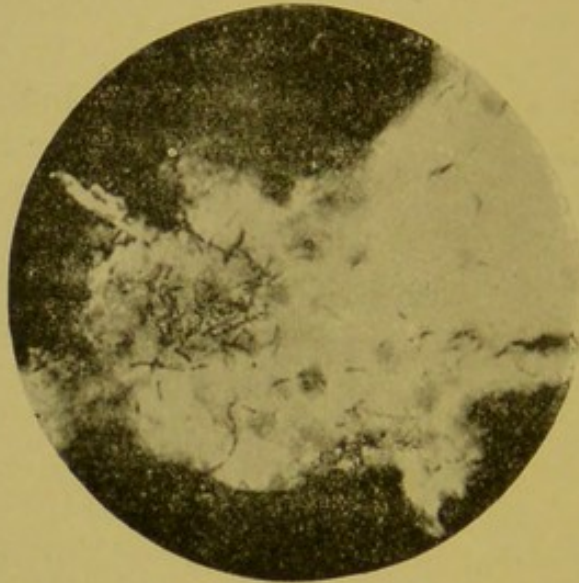


FIG. 60.—Bacillus of syphilis (Lustgarten), from a condyloma; $\times 1000$ (Itzerott and Niemann).

In some syphilitic tissues these methods suffice to define distinct bacilli with a remarkable similarity to the tubercle bacillus. The organism is about the same size as, and even more frequently curved than, the tubercle bacillus, but often presents a club-like enlargement of one end (involution-form?). The bacilli very frequently occur singly, though more often in groups, and never lie free, but are always enclosed in cells. These bacilli are not always found in syphilitic lesions, nor is their demonstration easy under the most favorable circumstances. Lustgarten emphasizes particularly that they are only demonstrable after the most painstaking technical procedures.

The probability of the specificity of this organism was considerably lessened by the observation by Matterstock, Travel, and Alvarez that in preputial smegma, and also

in vulvar smegma from healthy individuals, a similar organism, identical both in morphology and staining peculiarities, could be demonstrated. Of course the occurrence of Lustgarten's bacillus in the internal organs could not but argue against the probability of its identity with the smegma bacillus; but Lustgarten himself pointed out that the bacilli of both tuberculosis and leprosy stain by his method, and thus gave Baumgarten the right to suggest that the few cases well adapted for the demonstration of the Lustgarten bacilli might be cases of mixed infection of tuberculosis and syphilis.

The bacillus has not been isolated or cultivated, and its proper relation to syphilis is a matter which must be decided by future experimentation.

CHAPTER V.
ACTINOMYCOSIS.

IN 1845, Langenbeck discovered that the specific disease of cattle known as actinomycosis could be communicated to man. His observations, however, were not given to the world until 1878, one year after Bollinger had discovered the cause of the disease in animals.

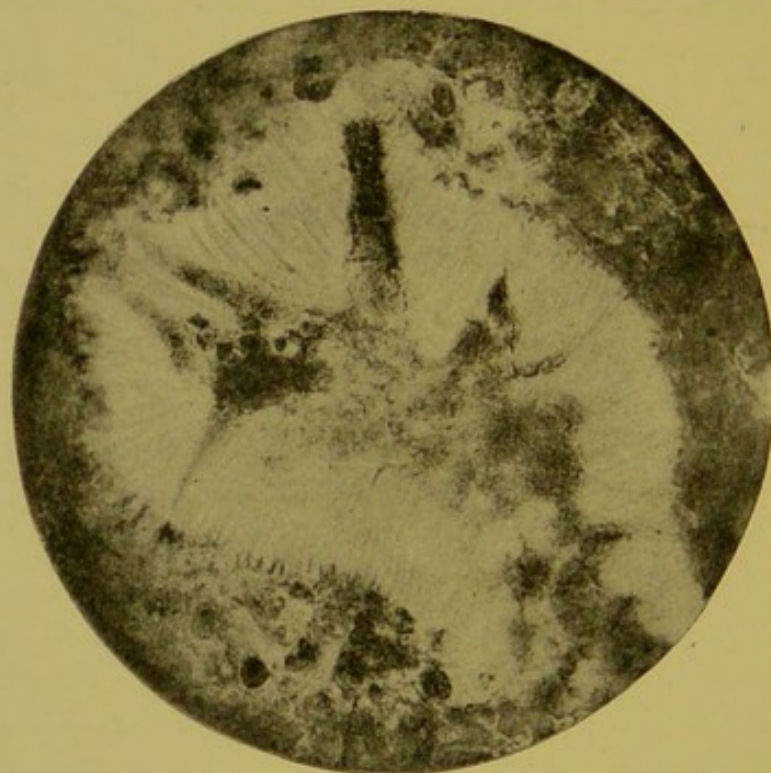


FIG. 61.—*Actinomyces bovis*, from the tongue of a calf; $\times 500$ (Fränkel and Pfeiffer).

Actinomycosis is a disease almost peculiar to the bovine animals, though sometimes occurring in hogs, horses, men, and other animals.

The first manifestations of the disease are usually found either about the jaw or in the tongue, in either of which

localities there are produced considerable enlargements which are sometimes dense and fibrous (wooden tongue) and sometimes suppurative. In sections of these nodular formations small yellowish granules surrounded by some pus can be found. These granules when viewed beneath the microscope exhibit a peculiar rosette-like body—the ray-fungus or actinomyces.

The fungus is of sufficient size to be detected by the naked eye. It can be colored, in sections of tissue, by the use of Gram's method, or better by Weigert's fibrin stain. Tissues pre-stained with carmin, then stained by Weigert's method, give beautiful pictures.

The entire fungus-mass consists of several distinct zones embracing entirely different elements. At the centre of the mass there is found a granular substance containing numerous bodies resembling micrococci. Extending from this centre into the neighboring tissue is a radiating, apparently branched, thickly-tangled mass of mycelial threads. These threads seem to terminate in a zone of conspicuous club-shaped radiating forms which give the colonies the rosette-like appearance. The cells of the tissues affected and a larger or smaller collection of leucocytes form the surrounding resisting tissue-zone.

The degree of chemotactic influence exerted by the organism seems to depend partly upon the tissue affected and partly upon the individuality of the animal. When the animal is but slightly susceptible, and when the tongue is the part affected, the disease is characterized by the production of enlargement due to the formation of cicatricial tissue. If, on the other hand, the animal is highly susceptible or the jaw is affected, the chief symptom is suppuration, with the formation of cavities communicating by sinuses.

Before the nature of the affection was understood it was confounded with various diseases of the bones, principally with osteosarcoma.

From the tissues primarily affected the disease spreads to the lymphatic glands, and not infrequently to the

lungs. Israel has pointed out certain cases of human actinomycosis beginning in the peribronchial tissues, probably from inhalation of the fungi.

The occurrence of three distinct elements as components of the rays served to class this organism among the pleomorphous bacteria in the genus *Cladothrix*, where it has remained undisturbed for at least a decade. Recent researches have, however, changed the view held by some bacteriologists in regard to the actinomyces, and caused them to regard the organism as a bacillus. If it be a bacillus, the central zone of granular cocci-like elements is to be regarded as consisting of individuals in process of rapid division and spore(?)-formation, the mycelial zone as consisting of perfect individuals, and the peripheral zone, with the rosette-like, club-shaped elements, as consisting of individuals partly degenerated through the activity of the cells and tissue-juices (involution-forms).

Jones is of the opinion that the disease, if not identical with, is closely allied to, tuberculosis, and that the occasional branched forms of tubercle bacilli prove the tendency of the individual bacillus to form a reticulum.

When the mycelial threads are carefully examined, the branchings, which appear distinct upon hasty inspection, are found to be more the effect of a peculiar relation which the threads bear to one another than actual bifurcations, so that it must be regarded as very questionable whether these threads ever so divide.

The organism may be grown upon artificial culture-media, as has been proven by Israel and Wolff.

Upon agar-agar or glycerin agar-agar it forms translucent colonies, about the size of a pin's head, of firm, almost cartilaginous, consistence. These colonies consist of bacillary individuals, sometimes seemingly branched. In bouillon similar dense globular organisms can be grown. The blood-serum colonies, which grow similarly to the agar-agar colonies, are rather more luxuriant, and slowly liquefy the medium.

When the actinomyces are grown upon artificial media their virulence is retained for a considerable length of time. If introduced into the abdominal cavities of rabbits, there are produced in the peritoneum, mesentery, and omentum typical nodules containing the actinomyces rays.

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 egg the organism forms peculiar long mycelial threads quite unlike the short forms developing upon agar-agar.

The characteristic rosettes which are constantly found in the tissues are never seen in artificial cultures.

The exact manner by which the organism enters the body is unknown. In some cases it may be by direct inoculation with pus, but there is 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 records a case where a primary mediastinal actinomycosis in the human subject was supposed to be traced to perforation of the posterior pharyngeal wall by a barley spikelet swallowed by the patient.

CHAPTER VI.

MYCETOMA, OR MADURA-FOOT.

A CURIOUS disease of not infrequent occurrence in the Indian province of Scinde is one known as mycetoma, Madura-foot, or *pied de Madura*. It almost invariably affects natives of the agriculturist class, and in most cases begins in or is referred by the patient to the prick of a thorn. It generally affects the foot, more rarely the hand, and in one instance was seen by Boyce in the shoulder and hip. It is more common in men than in women, individuals between twenty and forty years of age suffering most frequently, but persons of any age or sex may suffer from the disease. It is insidious in its onset, as has been said, generally following a slight injury, such as the prick of a thorn. No symptoms are observed in what might be called an incubation stage of a couple of weeks' duration, but after this time elapses a nodular growth gradually forms, attaining in the course of time the size of a marble. Its deep attachments are indistinct and diffuse. The skin becomes purplish, thickened, indurated, and adherent. The points most frequently invaded at the onset are the ball of the great toe and the pads under the bases of the fingers and toes.

In the course of months, although progressing slowly, the lesions attain very perceptible size, distinct tumors being present. Later, sometimes not until after a year or two, the nodes begin to soften, break down, discharge their purulent contents, and originate ulcers and communicating sinuses. The discharge at this stage is a thin sero-pus, and is always mixed with a number of fine round black or pink bodies, described, when black, as resembling gunpowder; when pink, as resembling

fish-roe. It is the detection of these particles upon which the diagnosis rests, and upon which the division of the disease into the *melanoid* and *pale* varieties depends.

The progress of the disease causes an enormous size and a peculiar deformity of the affected foot or hand. The malady is generally painless.

The micro-organismal nature of the disease was early suspected. In spite of the confusion caused by some who confounded the disease with and described it as "Guinea-worm," Carter held that it was due to some indigenous fungus as early as 1874. Boyce and Surveyor believe that the black particles of the melanoid variety represent a curious metamorphosis of a large branching septate fungus, and that the white particles of the other variety are the remains of a lowly-organized fungus and of caseous particles.

Kanthack tried to prove the identity of the fungus with the well-known actinomyces, but there seems to be considerable doubt about the correctness of his view.

Vincent succeeded in isolating the micro-organism by puncturing one of the nodes with a sterile pipette, and has cultivated it upon artificial media. Acid vegetable infusions seem suitable to its growth. It develops scantily in bouillon at the room-temperature, better at 37° C.—in from four to five days. In twenty to thirty days the colony attains the size of a little pea.

In the liquid media the colonies which cling to the glass, and thus remain near the surface of the medium, develop a rose- or bright-red color.

Cultures in gelatin are not very abundant, are colorless, and are unaccompanied by liquefaction.

Upon the surface of agar-agar strikingly 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 generally 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. The colonies are very adherent to the surface of the medium, and are said to be of cartilaginous consistence. The organism also grows in milk without coagulation.

Upon potato the development is meagre, slow, and with very little tendency to chromogenesis. The color-production is more marked if the potato be acid in reac-



FIG. 62.—*Streptothrix Maduræ* in a section of diseased tissue (Vincent).

tion. Some of the colonies upon agar-agar and potato have a powdery surface, no doubt from the occurrence of spores. It is, of course, an aërobic organism.

Under the microscope the organism is found by Vincent to be a streptothrix—a true branched fungus consisting of long bacillary branching threads in a tangled mass. In many of the threads spores could be made out.

Vincent was unable to communicate the disease to animals by inoculation.

Microscopic study of the diseased tissues in cases of mycetoma is not without interest. The healthy tissue is said to be sharply separated from the diseased masses. The latter appear as large degenerated tubercles, except that they are extremely vascular. The mycelial or filamentous fungous mass occupies the centre of the degeneration, where its long filaments can be beautifully demonstrated by the use of appropriate stains, Gram's method being excellent for the purpose. The tissue surrounding the disease-nodes is infiltrated with small round cells. The youngest nodules are seen to consist of granulation-tissue, which in its organization is checked by the coagulation-necrosis which is sure to overtake it. Giant-cells are few.

Not infrequently small hemorrhages occur from the ulcers and sinuses of the diseased tissues; the hemorrhages can be explained from the abundance of small blood-vessels in the diseased tissue.

Although the disease has been described as occurring in Scinde, it is not limited to that province, having been met with in Madura, Hissar, Bicanir, Dehli, Bombay, Baratpur, Morocco, Algeria, one case by Bastini and Campana in Italy, and one by Kempner in America.

CHAPTER VII.

FARCIN DU BŒUF.

THE peculiar disease which sometimes affects numbers of cattle in Guadeloupe, and which was described by the older writers as *farcin du bœuf*, has been carefully studied by Nocard. It is a disease of cattle characterized by a superficial lymphangitis and lymphadenitis, affecting the tracheal, axillary, prescapular, and other glands. The affected glands enlarge, suppurate, and discharge a creamy, sometimes a grumous, pus. The internal organs are often affected with a pseudo-tuberculosis whose central areas undergo a purulent or caseous degeneration.

In the researches of Nocard it was discovered, by staining by Gram's and by Kühne's methods, that in the centres of the tubercles micro-organisms could be defined. They resembled long delicate filaments rather intricately woven, characterized by distinct ramifications which made clear the proper classification of the organism as a *streptothrix*. The organism was successfully cultivated by Nocard upon various culture-media at the temperature of the body. It is aërobic.

In bouillon the organism develops in the form of colorless masses irregular in size and shape, some of which float upon the surface, others of which sink to the bottom of the liquid. Sometimes the surface is covered by an irregular fenestrated pellicle of a gray color.

Upon agar-agar the growth develops in small, rather discrete, irregularly rounded, opaque masses of a yellowish-white color. The surfaces of the colonies are tuberculated, and an appearance somewhat like a lichen is observed.

Upon potato very dry scales of a pale-yellow color rapidly develop.

The growth upon blood-serum is less luxuriant, but similar to that upon agar-agar.

In milk the organism produces no coagulation by its growth, and does not alter the reaction.

Microscopic study always reveals the organism as the same tangled mass of filaments seen in the tissues. The old cultures are rich in spores, which are very small and develop upon the most superficial portions of the growth. These spores resist the penetration of stains to a rather unusual extent.

Cultures retain their virulence for a long time: Nocard found one virulent after it had been kept for four months in an incubating oven at 40° C.

The streptothrix of *farcin du bœuf* is pathogenic for guinea-pigs, cattle, and sheep; dogs, rabbits, horses, and asses are immune.

When the culture or some pus containing the micro-organism is injected subcutaneously into a guinea-pig, a voluminous abscess results. Not long afterward the lymphatic vessels and glands of the region are the seat of swelling and induration, and extensive phlegmons form, which rupture externally and discharge considerable pus. The animal, of course, becomes extremely ill and seems about to die; instead, it slowly recovers its normal condition.

In other animals, as the cow and the sheep, the subcutaneous inoculation results in an abscess relatively less extensive. This ulcerates, then indurates, and seems to disappear, but after the lapse of several weeks or months opens again in the form of a new abscess.

In animals which are immune or nearly immune, like the horse, the ass, the dog, and the rabbit, the subcutaneous inoculation is followed by the formation of a small abscess which speedily cicatrizes.

Intraperitoneal inoculation in the guinea-pig gives rise to an appearance resembling tuberculosis. The omentum may be extensively involved and full of softened nodes.

The liver, spleen, and kidneys appear full of tubercles, but careful examination will satisfy the observer that the tubercles are only upon the peritoneal surfaces, not in the organs.

Intravenous introduction of the cultures produces a condition much resembling general miliary tuberculosis. All the organs contain the pseudo-tubercles in considerable numbers.

CHAPTER VIII.

RHINOSCLEROMA.

IN Austria, Hungary, Italy, and some parts of Germany there sometimes occurs a peculiar disease of the anterior nares, characterized by the occurrence of circumscribed tumors, known as rhinoscleroma. The tumor-masses are somewhat flattened, isolated or coalescent, grow with great slowness, and recur if excised. The disease commences in the mucous membrane and the adjoining skin, and spreads to the skin in the neighborhood by a slow invasion, involving the upper lip, jaw, hard palate, and sometimes the pharynx. The growths are without evidences of inflammation, do not ulcerate, and consist microscopically of infiltration of the papilla and corium of the skin, with round cells which change in part to fibrillar tissue. The tumors possess a well-developed lymph-vascular system. Sometimes the cells undergo hyaline degeneration.

In these little tumors the researches of Von Frisch discovered little bacilli much resembling both in morphology and vegetation the pneumo-bacilli of Friedländer, and, like them, surrounded by capsules. The only marked difference between the so-called bacillus of rhinoscleroma and the *Bacillus pneumoniae* of Friedländer is that the former stains well by Gram's method, while the latter does not, and that the former is rather more distinctly rod-shaped than the latter, and more often shows its capsule in culture-media.

The bacillus can easily be cultivated, and in all media resembles the bacillus of Friedländer too closely to be distinguished from it. Even when inoculated into animals the bacillus behaves much like Friedländer's bacillus.

Inoculation has, so far, failed to produce the disease either in men or in the lower animals.

B. THE TOXIC DISEASES.

CHAPTER I.

DIPHTHERIA.

IN 1883, Klebs pointed out the existence 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 the organism, which is now known by both their names—the Klebs-Löffler bacillus.

The bacillus as described by Löffler is about the length of the tubercle bacillus, about twice its diameter, has a



FIG. 63.—*Bacillus diphtheriæ*, from a culture upon blood-serum; $\times 1000$ (Fränkel and Pfeiffer).

curve similar to that which characterizes the tubercle bacillus, and has rounded ends (Fig. 63). It does not form chains, though two, three, and rarely four individ-

uals may be found joined; generally the individuals are all separate from one another. The morphology of the bacillus is peculiar in its considerable irregularity, for among the well-formed individuals which abound in fresh cultures a large number of peculiar organisms are to be found, some much larger than normal, some with one end enlarged to a club-shape, some greatly elongated, with both ends expanded into club-shaped enlargements. These bizarre forms seem to represent an involution-form of the organism, for, while present in perfectly fresh cultures, they are so abundant in old cultures that scarcely a single well-formed bacillus can be found. It not infrequently happens that in unstained bacilli distinct granules can be defined at the ends—polar granules—thus giving the organism somewhat the appearance of a diplococcus.

The bacillus can be readily stained by aqueous solutions of the anilin colors, but more beautifully and characteristically with Löffler's alkaline methylene blue:

Saturated alcoholic solution of methylene blue, 30;
1:10,000 aqueous solution of caustic potash, 100;

and an aqueous solution of dahlia, as recommended by Roux.

When cover-glass preparations are stained with these solutions, the bizarre forms already mentioned are much more obvious than in the unstained individuals, and the contrast between the polar granules, which color intensely, and the remainder of the bacillus, which tinges slightly, is marked. Through good lenses the organisms are always distinct bacilli, notwithstanding the fact that the ends stain more deeply than the centres, and it is only through poor lenses that the organisms can be mistaken for diplococci. The bacilli stain well by Gram's method, this being a good method to employ for their definition in sections of tissue, though Welch and Abbott assert that Weigert's fibrin method and picro-carmin give the most beautiful results.

The diphtheria bacillus does not form spores, and is delicate in its thermal range. Löffler found that it could not endure a temperature of 60° C., and Abbott has shown that a temperature of 58° C. for ten minutes is fatal to it. Notwithstanding this susceptibility, the organism can be kept alive for several weeks after being dried upon shreds of silk or when surrounded by dried diphtheria membrane.

No flagella have been demonstrated upon the bacillus. It is non-motile.

Fernbach has shown that when the organisms are grown in a medium exposed to a passing current of air, the luxuriance of their development is increased, though their life-cycle is shorter. The growth can also take place when the air is excluded, so that the bacillus must be classed among the optional anaërobic organisms.

The diphtheria bacillus grows readily upon all the ordinary media, and is a very easy organism to obtain in pure culture. Löffler has shown that the addition of a small amount of glucose to the culture-medium increases the rapidity of the 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.

This mixture is filled into tubes, coagulated, and sterilized like blood-serum, and is one of the best-known media in connection with the study of diphtheria.

The clinical impossibility of making an accurate diagnosis of diphtheria without a bacteriologic examination has made many private physicians and many medical societies and boards of health equip laboratories where accurate examinations can be made. The method requires some apparatus, though a competent bacteriologist can often make shift with a bake-oven, a wash-boiler, and other household furniture instead of the regular sterilizers and incubators, which are expensive.

When it is desired to make a bacteriologic diagnosis of a suspected case of diphtheria or to secure the bacillus in pure culture, a sterile platinum wire having a small loop at the end, or a swab made by wrapping a little cotton around the end of a piece of wire and carefully sterilizing in a test-tube, is introduced into the throat and touched to the false membrane, after which it is smeared carefully over the surface of at least three of the blood-serum-mixture tubes, without either again touching the throat or being sterilized. The tubes thus inoculated are stood away in an incubating oven at the temperature of 37° C. for twelve hours, then examined. If the diphtheria bacillus is present upon the first and second tubes, there will be a smeary yellowish-white layer, with outlying colonies on the second tube, while the third tube will show rather large isolated whitish or slightly yellowish colonies, smooth in appearance, but rather irregular in outline. Very often the colonies are china-white in appearance. These colonies, *if found by microscopic examination to be made up of diphtheria bacilli*, will confirm the diagnosis of diphtheria, and will at the same time give pure cultures when transplanted. There are very few other bacilli which grow so rapidly upon Löffler's mixture, and scarcely one other which is found in the throat.

Ohlmacher recommends the microscopic examination of the still invisible growth in five hours. A platinum loop is rubbed over the inoculated surface; the material secured is then mixed with distilled water, dried on a cover-glass, stained with methylene blue, and examined. This method, if reliable, will be very valuable in making an early diagnosis preparatory to the use of the antitoxin.

The presence of diphtheria bacilli in material taken from the throat does not necessarily prove the patient to be diseased. Virulent bacilli can often be discovered in the throats of healthy persons who have knowingly or unknowingly come in contact with the disease. The bacteriologic examination is only an adjunct to the

clinical diagnosis, and must never be taken as positive in itself.

The bacillus grows similarly upon blood-serum and Löffler's mixture. Upon glycerin agar-agar and agar-agar the colonies are much larger, more translucent, always

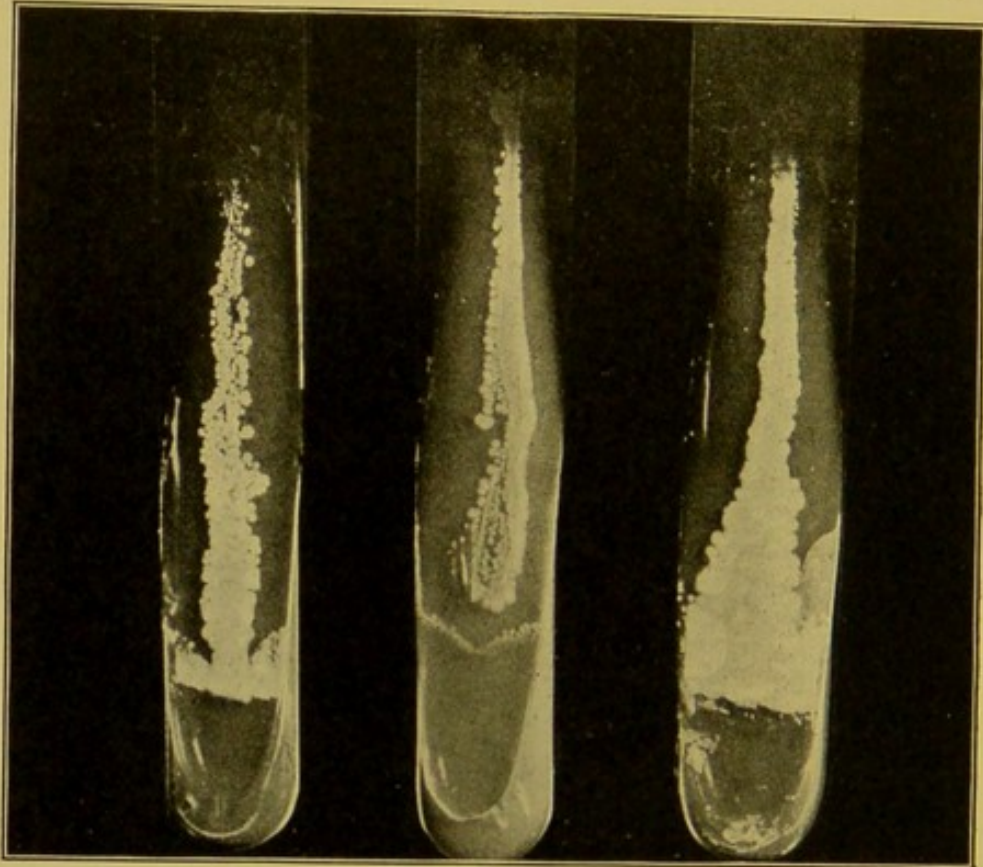


FIG. 64.—Diphtheria bacilli (from photographs taken by Prof. E. K. Dunham, Carnegie Laboratory, New York): *a*, pseudo-bacillus; *b*, true bacillus; *c*, pseudo-bacillus.

without the yellowish-white or china-white color of the blood-serum cultures, and generally are distinctly divided into a small elevated centre and a flatter surrounding zone with indented edges, sometimes with a distinctly radiated appearance. It must be remarked that when sudden transplantations are made from blood-serum to agar-agar the growth resulting is meagre, but the oftener this growth is transplanted to fresh agar-agar the more luxuriant it becomes.

The growth in gelatin puncture-cultures is characterized by small spherical colonies which develop along the entire length of the needle-track. The gelatin is not liquefied.

Upon the surface of gelatin plates the colonies that develop do not attain anything like the size of the colonies upon Löffler's mixture. They appear to the naked

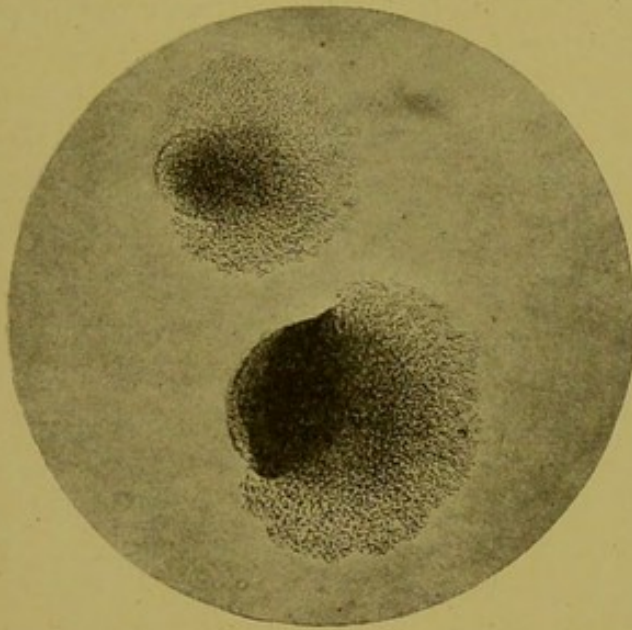


FIG. 65.—*Bacillus diphtheriæ*, colony twenty-four hours old upon agar-agar; $\times 100$ (Fränkel and Pfeiffer).

eye as whitish points with smooth contents and regular though sometimes indented borders. Under the microscope they appear as granular, yellowish-brown colonies with irregular borders (Fig. 65).

When planted in bouillon the organism causes a diffuse cloudiness at first, but, not being motile, soon settles to the bottom in the form of a rather flocculent precipitate which has a tendency to cling to the sides of the glass. Sometimes a delicate irregular mycoderma forms upon the surface, especially when the cultivation is made by the method of Fernbach with a passing current of air. This mycoderma, which may appear quite regular when the flask is undisturbed, is so brittle that it at once falls to pieces if the flask be moved.

Spronck has recently determined that the characteristics of the growth of the diphtheria bacillus in bouillon, as well as the amount of toxin-production, vary according to the amount of glucose in the bouillon. He divides the cultures into three types :

Type A. The reaction of the bouillon becomes acid and remains acid, the acidity increasing. The bacilli accumulate at the bottom of the clear liquid. The toxin-production is meagre.

Type B. There is no change from alkalinity to acidity, but the original alkalinity of the bouillon steadily increases. The culture is very rich, the bottom of the flask shows a considerable sediment, the liquid is cloudy, and a delicate growth occupies the surface. The toxicity is very great.

Type C. In a few days the reaction of the culture becomes acid, and then later on changes to alkaline. During the acid period the liquid is clear, with a white surface-growth. When the alkalinity returns the bouillon clouds and the surface-growth increases in thickness. Sediment accumulates at the bottom of the flask. The toxicity of the culture is much less than in Type B.

Spronck regards the varying reaction as due to the fermentation of the glucose, and asserts that the most luxuriant and toxic cultures are those in which no glucose is present. To exclude as much of the undesirable sugar as possible, he makes the bouillon from the stalest meat obtainable, preferring it when just about to putrefy. Of the meats experimented with, beef was found to be the best.

Upon potato the bacillus only develops when the reaction is alkaline. The potato growth is not characteristic. Welch and Abbott always secured a growth of the organism when planted upon potato, but do not mention the reaction of the medium they employed.

Milk is an excellent medium for the cultivation of the *Bacillus diphtheriæ*, and is possibly at times a means of infection. Litmus milk is an excellent medium for ob-

erving the changes of reaction brought about by the growth of the bacillus. At first the alkalinity, which is always favorable to the development of the bacillus, is destroyed by the production of an acid. When the culture is old the acid is replaced by a strong alkaline reaction.

Diphtheria as it occurs in man is generally a disease characterized by the formation of a pseudo-membrane upon the fauces. It is a local infection, due to the presence and development of the bacilli on the pseudo-membrane, but is accompanied by a general toxemia resulting from the absorption of a violently poisonous substance produced by the bacilli. The bacilli are found only in the membranous exudation, and most plentifully in its older portions. As a rule, they do not distribute themselves through the circulation of the animal, though at times they may be found in the heart's blood.

The most malignant cases of the disease seem to be due to pure infection by the diphtheria bacillus, though such cases are more rare than those in which the *Streptococcus pyogenes* and *Staphylococcus aureus* and *albus* are found in association with it.

It may be well to remark that all pseudo-membranous diseases of the throat are not diphtheria, but that some of them result from the activity of the pyogenic organisms alone.

No more convincing proof of the existence of a powerful poison in diphtheria could be desired than the evidences of general toxemia resulting from the absorption of material from a comparatively small number of bacilli situated upon a little patch of mucous membrane.

In animals artificially inoculated the lesions produced are not identical with those seen in the human subject, yet they have the same general features of local infection with general toxemia.

Guinea-pigs, kittens, and young pups are susceptible animals. When half a cubic centimeter of a twenty-four-hour-old bouillon culture is injected beneath the skin of

such an animal, the bacilli multiply at the point of inoculation, with the production of a patch of inflammation associated with a distinct fibrinous exudation and the presence of an extensive edema. The animal dies in twenty-four to thirty-six hours. The liver is enlarged, and sometimes shows minute whitish points, which in microscopic sections prove to be necrotic areas in which the cells are completely degenerated and the chromatin of their nuclei is 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 the toxin. The bacilli are constantly absent from these lesions. Flexner has shown these foci to be common to numerous irritant poisonings, and not peculiar to diphtheria alone.

The lymphatic glands are usually enlarged; the adrenals are also enlarged, and, in cases into which the live bacilli have been injected, are hemorrhagic.

Sometimes the bacilli themselves are present in the internal organs, and even in the blood, but generally this is not the case.

It might be argued, from the different clinical pictures presented by the disease as it occurs in man and in animals, that they were not expressions of the same thing. A careful study, however, together with the evidences adduced by Roux and Yersin, who found that when the bacilli were introduced into the trachea of animals opened by operation a typical false membrane was produced, and that diphtheritic palsy often followed, and of hundreds of investigators, who find the bacilli constantly present in the disease as it occurs in man, must satisfy us that the doubt of the etiological rôle of the bacillus rests on a very slight foundation.

One reason for skepticism in this particular is the supposed existence of a *pseudo-diphtheria bacillus*, which has so many points in common with the real diphtheria bacillus that it is difficult to distinguish between them. We have, however, come to regard this pseudo-bacillus as

an attenuated form of the real bacillus. The chief points of difference between the bacilli are that the pseudo-bacillus is shorter than the diphtheria bacillus when grown upon blood-serum; that the cultures in bouillon progress much more rapidly at a temperature of from 20–22° C. than those of the true bacillus; and that the pseudo-bacillus is not pathogenic for animals. These slight distinctions are all exactly what might be expected of an organism whose virulence had been lost, and whose vegetative powers had been altered, by persistent manipulation or by unfavorable surroundings.

The diphtheria bacilli are always present in the throats of patients suffering from diphtheria, and constitute the element of contagion by being accidentally discharged by the nose or mouth by coughing, sneezing, vomiting, etc. Whoever comes in contact with such material is in danger of infection.

It is of great interest to notice the remarkable results obtained by Biggs, Parke, and Beebe in New York, where the bacteriological examinations conducted in connection with diphtheria show that the virulent bacilli may be found in the throats of convalescents 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 the throats of their care-takers, who, while not themselves infected, may be the means of conveying the disease from the sick-room to the outer world. The importance of this observation must be apparent to all readers, and serves as further evidence why most thorough isolation should be practised in connection with this dreadful disease.

From time to time reference has been made to the toxin elaborated by the diphtheria bacillus. Roux and Yersin first demonstrated the existence of this substance in cultures passed through a Pasteur porcelain filter. The toxin is intensely poisonous, and by the modern improved methods can be secured in such concentration that 0.1 c.cm. will kill a guinea-pig in twenty-four hours.

The toxin is not an albuminous substance, and 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 or third week.

In addition to the toxin, a toxalbumin has been isolated by Brieger and Fränkel.

Behring discovered that the blood of animals rendered immune to diphtheria by inoculation, first with attenuated and then with virulent organisms, contained a neutralizing substance which was capable of annulling the effects of the bacilli or the toxin when simultaneously or subsequently inoculated into non-protected animals. This substance, in solution in the blood-serum of the immunized animals, is the *diphtheria antitoxin*.

The preparation of the antitoxin for therapeutic purposes received a further elaboration in the hands of Roux. The subject is one of great interest, but must be considered briefly in a work of this kind.

The antitoxin is manufactured commercially at present, the method being the immunization of large animals to great quantities of the toxin, and the withdrawal of their antitoxic blood when the proper degree of immunity has been attained. The details are as follows:

The Preparation of the Toxin.—The method employed by Roux and others at the present time was first suggested by Fernbach, and consists in growing the most virulent bacilli obtainable in alkaline bouillon exposed in a thin layer to the passage of a current of air.

The cultures are allowed to grow for three or four weeks at a temperature of 37° C., with a stream of moist air constantly passing over them. After the given time has passed, it will be found that the acidity primarily produced by the bacillus gives place to a much more intense alkalinity than originally existed. The acme of the toxin-production seems to keep pace with this alkaline production. When "ripe," 0.4 per cent. of trikresol

is added to the cultures, which are then filtered through porcelain. If the toxin must be kept before using, it is best to preserve it unfiltered, as it deteriorates more rapidly after filtration. Unfiltered toxin causes too much local irritation. If the bacillus employed was virulent and the conditions of culture were favorable, the filtered culture should be so toxic that 0.1 c.cm. would be fatal to a 500-gram guinea-pig in twenty-four hours (Roux). Even under the most favorable circumstances it is difficult to obtain a toxin which will kill in less than thirty hours.

The experience of the author with Fernbach's apparatus has not been satisfactory. The passing current of air is a frequent source of contamination to the culture, and requires great care. In the end it is questionable whether the toxin thus produced is better than that obtained from an ordinary flask exposing a large surface to the air.

The Immunization of the Animal.—The animals chosen to furnish the antitoxic serum should be animals which present a distinct natural immunity to ordinary doses of the toxin, and should be sufficiently large to furnish large quantities of the finished serum. Behring originally employed dogs and sheep; Aronson at first preferred the goat; but Roux introduced the horse, which is more easily immunized than the other animals mentioned, and, being large enough to furnish a considerable quantity of serum, recommends itself strongly for the purpose.

The animal chosen should be free from tuberculosis and glanders, as tested by tuberculin and mallein, but need not be expensive. A horse with a disabled foot will answer well. Rheumatic horses should be rejected. In the beginning a small dose of the toxin—about 1 c.cm.—should be given hypodermically to detect individual susceptibility. Horses vary much in this particular, as Roux has pointed out. The author found light-colored horses to be distinctly more susceptible than dark-colored ones.

If well borne, the preliminary injection is followed in about eight days by a larger dose, in eight days more by

a still larger one, and the increase is continued every eight days or so, according to the condition of the animal, until enormous quantities—300 c.cm.—are introduced at a time.

The toxin causes some local reaction—at first a distinct inflammation, later a painful edema and a febrile reaction. The amount of local irritation is much less marked when the injections are made slowly; and a gravity apparatus, which is filled with the amount of serum to be injected, suspended from the ceiling of the stable so that the toxin is allowed to take its own time to enter the tissues, can be recommended. Sometimes it takes an hour to inject 300 c.cm. in this manner.

The amount of local reaction, edema, etc., the appetite and general condition, the temperature-curve, and the stability of the body-weight, must all be taken into consideration, so that the administration shall not be too rapid and the animal be thrown into a condition of cachexia with toxic instead of antitoxic blood.

One of the principal things to be avoided is haste. Too frequent or too large dosage is almost certain to kill the animal.

Behring found that mixing the toxin with trichlorid of iodine lessened the irritant effect upon susceptible animals.

The suggestion of Prof. Pearson, that the large doses of toxin might with readiness be introduced into the trachea when the absorption is good, has been successfully accomplished by the author. The absorption seems to take place without any change in the toxin, and to be as rapid as from the subcutaneous tissue.

The Preparation of the Serum for Therapeutic Purposes.—When, because of the tolerance to large quantities of toxin, the horse seems to possess antitoxic blood, a "twitch" is applied to the upper lip, the eyes are blindfolded, a small incision is made through the skin, a trocar thrust into the jugular vein, and the blood allowed to flow through a cannulated tube into sterile bottles. It

is allowed to coagulate, and remains upon ice for two days or so, that the clear serum may be pipetted off. This serum is the *antitoxic serum*. It does not always materialize according to the desires of the experimenter, sometimes proving unexpectedly strong in a short time, sometimes unexpectedly weak after months of patient preparation.

The strength of the serum is expressed in what are known as *immunizing units*. This denomination originated with Behring, whose original or *normal serum* was of such strength that 0.1 c.cm. of it would protect against the ten-times fatal dose of toxin when simultaneously injected into guinea-pigs. Each cubic centimeter of this normal serum he called an *immunizing unit*. Later it was shown that the strength of the serum could easily be increased tenfold, so that 0.01 c.cm. of the serum would protect the guinea-pig against the ten-times fatal dose. Each cubic centimeter of this stronger serum was described as an antitoxic unit, and of course contained ten immunizing units. Still later it was shown that the limits were by no means reached, and he succeeded in making serums as much as three hundred times the normal strength, each cubic centimeter of which contained 300 immunizing units or 30 antitoxic units.

The serums ordinarily sold are of three strengths—600 units in 10 c.cm., 1000 units in 10 c.cm., and 1500 units in 10 c.cm. The weaker strength is used for immunizing healthy children and adults who come in contact with the contagium. The stronger serums are for treatment. There is, of course, no way of estimating the amount of toxin in the blood of a child suffering with diphtheria, and therefore no accurate method of determining exactly how much antitoxin should be given. Ehrlich asserts that less than 500 units is valueless: 10 c.cm. is probably an average dose, and, as the remedy seems harmless, it is better to err on the side of too much than on that of too little.

The largest collection of statistics upon the results of

antitoxic treatment in diphtheria in the hospitals of the world are probably those collected by Prof. Welch, who, excluding every possible error in the calculations, "shows an apparent reduction of case-mortality of 55.8 per cent."

One of the most important things in the treatment is to begin it early enough. 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.

After the toxin has set up destructive organic lesions in various organs and tissues of the body, no amount of neutralization will restore the integrity of the parts, so that the antitoxin must fail in these cases.

The urticaria which sometimes follows the injection of antitoxic serum seems to bear a distinct relation to the age of the serum, fresh serums being more liable to produce it than those which have been kept for a week or two.

The erythemata are probably in some way associated with the globulicidal action of the blood. Keeping the serum "until it is ripe" lessens this effect. The serums from different horses probably vary much in both their irritant and globulicidal properties, so that antitoxins prepared by mixing the serums from a number of horses are probably preferable to those from single horses.

Dried serums are much less active than fresh ones.

For purposes of immunization smaller doses than those used for treatment suffice. According to Biggs, 2 cubic centimeters are sufficient to give complete protection. The immunity that results from the injection is of a month or six weeks' duration.

CHAPTER II

TETANUS.

ONE of the most exquisitely toxic bacteria of which we have any knowledge is the bacillus discovered in 1884 by Nicolaier, obtained in pure culture by Kitasato in 1889, and now universally recognized as the cause of tetanus. It is a peculiar organism, whose striking feature is a considerable enlargement of one end, in which a bright round spore is seen (Fig. 66). The bacilli which



FIG. 66.—*Bacillus tetani*; $\times 1000$ (Fränkel and Pfeiffer).

are not sporiferous are long, rather slender, have rounded ends, seldom unite in chains or pairs, are non-motile, and have no flagella. The bacilli stain readily with ordinary aqueous solution of the anilin dyes, and also very readily by Gram's method.

The tetanus bacillus is a common saprophytic organism which can be found in most garden-earth, in dust,

in manure, and sometimes in the intestinal discharges of animals. It is extremely difficult to isolate and cultivate, because it will not grow where the smallest amount of oxygen is present.

The method now generally employed for the isolation of this bacillus is that originated by Kitasato, and based upon his observation that its spores can resist high temper-

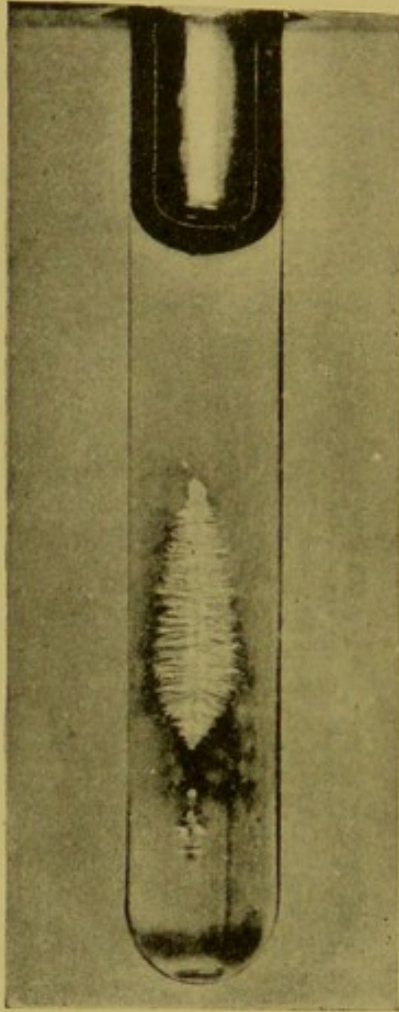


FIG. 67.—*Bacillus tetani*: six-days-old puncture-culture in glucose-gelatin (Fränkel and Pfeiffer).

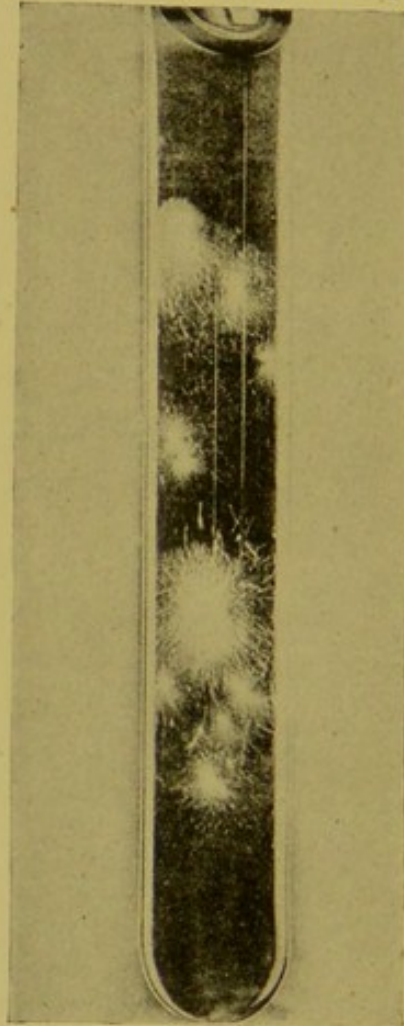


FIG. 68.—*Bacillus tetani*: culture four days old in glucose-gelatin (Fränkel and Pfeiffer).

atures. After finding that the typical bacilli are present in earth or pus, or whatever the material to be investigated was, Kitasato exposed a portion of it for an hour to a temperature of 80° C. By this heating all the fully-developed bacteria, tetanus as well as the others, and the

great majority of the spores except those of tetanus, were destroyed, and, as little other than tetanus spores remained, their cultivation was made comparatively easy.

The resistance which the tetanus bacilli manifest toward heat is only part of a great general resisting power of which they are possessed. It is said that they can retain their vitality in the dried condition for months. Sternberg says they can resist 5 per cent. carbolic solutions for ten hours, but will not grow after fifteen hours' immersion. 5 per cent. carbolic acid, to which 0.5 per cent.

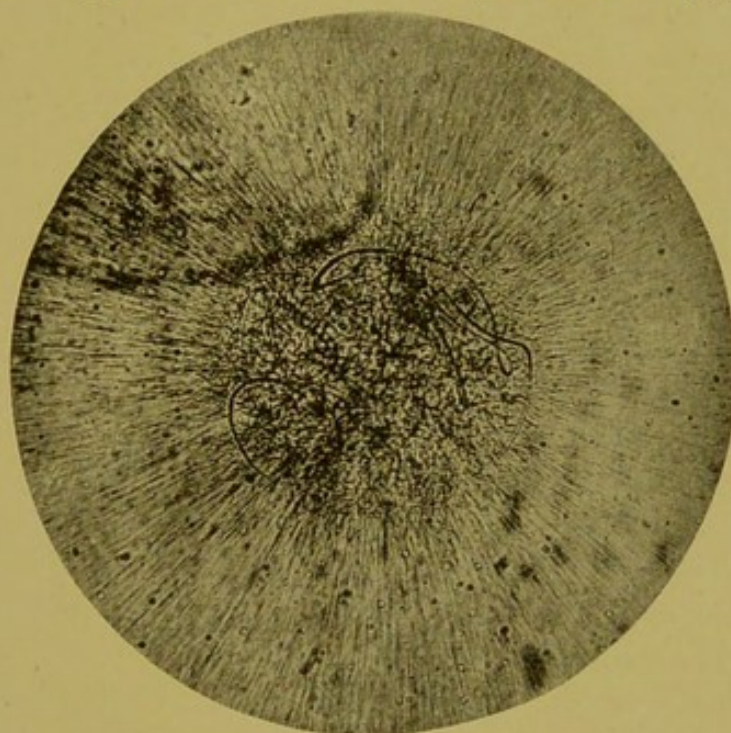


FIG. 69.—*Bacillus tetani*: five-days-old colony upon gelatin containing glucose; $\times 1000$ (Fränkel and Pfeiffer).

of hydrochloric acid has been added, destroys them in two hours. They are also 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 spores are destroyed in thirty minutes. The resistance to heat is only within certain limits, for exposure to passing steam for from five to eight minutes is certain to kill the spores.

The colonies of the tetanus bacillus, when grown in

an atmosphere of hydrogen upon gelatin plates, somewhat resemble those of the well-known hay bacillus. There is a dense rather opaque central mass from which a more transparent zone is readily separable. The margins of this outer zone are made up of a radiating fringe of projecting bacilli (Fig. 69). The liquefaction that occurs is much slower than that caused by bacillus subtilis.

When grown in gelatin puncture-cultures the development occurs deep in the puncture, and consists of multitudes of short-pointed processes radiating from the puncture, somewhat resembling a fir tree (Fig. 67). Liquefaction begins in the second week and causes the disappearance of the radiating processes. 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 much more rapid; that in agar-agar punctures is much slower, but similar to the gelatin cultures except for the absence of liquefaction. The organism can also be grown in bouillon, and attains its maximum development at a temperature of 37° C. Much gas is given off from the cultures.

Cultures of the tetanus bacillus in all media give off a peculiar characteristic odor—a burnt-onion smell, with a suggestion of putrefaction about it.

The methods for excluding the oxygen from the cultures and replacing it by hydrogen, as well as other methods suggested for the cultivation of the strictly anaërobic organisms, are given under the appropriate heading (Anaërobic Cultures), and need not be repeated here.

Tetanus bacilli exist in nature as widely distributed saprophytes. They are quite common in the soil, and the fact that they are most plentiful in manured ground has suggested that they originate in the intestines of horses and reach the earth from their excrement. Le Dentu has, however, shown that the tetanus bacillus is a common organism in New Hebrides, where there are no

horses. In these islands the natives poison their arrows by dipping them into a clay rich in tetanus bacteria.

The work of Kitasato has given us a very exact knowledge of the tetanus bacillus and completely establishes its specific nature.

The organisms generally enter the animal body through a wound caused by some implement which has been in contact with the soil, or enter abrasions from the soil directly. Doubtless many of the wounds are so small that their existence is overlooked, and this, together with the fact that the period of incubation of the disease, especially in man, is of considerable duration, and at times permits the wound to heal before any symptoms of intoxication occur, serves to explain to us at least some of the reported cases in which no wound is said to have existed.

It would seem that in some rare cases tetanus can occur without the previous existence of a wound. Such a case has been reported by Kamen, who found that the intestine of a person dead of the disease was rich in the *Bacillus tetani*. Kamen is of the opinion that the bacilli can grow in the intestine and be absorbed, especially where there are imperfections in the mucosa. It is not impossible, though he does not think it probable, that the bacteria growing in the intestine could elaborate enough toxin to produce the disease by absorption.

All animals are not alike susceptible to the disease. Men, horses, mice, rabbits, and guinea-pigs are all susceptible; dogs are much less so. Most birds are scarcely at all susceptible either to the bacilli or to the poison. Amphibians are immune, though it is said that frogs can be made susceptible by elevation of their body-temperature.

When a white mouse is inoculated with an almost infinitesimal amount of bouillon or solid culture, or is inoculated with garden-earth containing the tetanus bacillus, the disease is almost certain to follow, the first symptoms coming on in from one to two days.

The mouse develops typical tetanic convulsions, which begin first in the neighborhood of the inoculation, but soon become general. Death follows sometimes in a very few hours. In rabbits the period of incubation is nearly two weeks, and in man may be three weeks.

The conditions in the animal body are not favorable for the development of the bacilli, because of the free supply of oxygen contained in the blood, and we find that they grow with great slowness, remain localized at the seat of inoculation, and never enter the blood- or lymph-circulation. Doubtless most cases of tetanus are cases of mixed infection in which the bacillus enters with bacteria, which greatly aid its growth by using up the oxygen in their neighborhood. The amount of poison produced must be exceedingly small and its power tremendous, else so few bacilli growing under adverse conditions could not produce fatal toxemia. The poison is produced rapidly, for Kitasato found that if mice were inoculated at the root of the tail, and afterward the skin and the subcutaneous tissues around the inoculation were either excised or burned out, this treatment would not save the animal unless the operation were performed *within an hour after the inoculation*.

The circulating blood of diseased animals is fatal to susceptible animals because of the toxin which it contains; and that the urine is also toxic to mice proves the excretion of the toxin through the kidneys.

From pure cultures of tetanus bacilli grown in various media, and from the blood and tissues of animals affected with the disease, Brieger has succeeded in separating two poisonous substances—"tetanin" and "tetano-toxin."

The pathology of the disease is of much interest because of its purely toxic nature. There is generally a small wound with a slight amount of suppuration. 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.

An interesting fact contributed to our knowledge of the disease has been presented by Vaillard and Rouget, who found that if the tetanus bacilli were introduced into the body freed from their poison, they were unable to produce any signs of disease because of the promptness with which the phagocytes took them up. If, however, their poison was not removed, or if the body-cells were injured by the simultaneous introduction of lactic acid or other chemical agents, the bacilli would immediately begin to manufacture the toxin and produce symptoms of the disease.

The toxin is easily prepared, being readily soluble in water. The most ready method of preparation is to grow the bacilli in bouillon, keeping the culture-medium at a temperature of 37° C., and allowing it to remain undisturbed for from two to four weeks, by which time it will have attained a toxicity so great that 0.000005 c.cm. will cause the death of a mouse. The toxin is very rapidly destroyed by heat, and cannot bear any temperature above $60-65^{\circ}$ C. It is also decomposed by light. When preserved in the dark in a refrigerator it can be kept indefinitely. The best method of keeping it is to add 0.5 per cent. of phenol, and then store it in a cool, dark place.

By the gradual introduction of such a toxin into animals Behring and Kitasato have been able to produce in their blood a distinctly potent and valuable antitoxic substance.

The method for the production of this tetanus antitoxic serum is very much like that for the diphtheria antitoxic serum (*q. v.*), except that a much longer time is required for its production, that the doses of toxin are of necessity smaller because its toxicity is greater, and that trichlorid of iodine or Gram's solution will probably need to be added to the toxin to prevent too powerful a local reaction. Horses, dogs, and goats may be used.

As tetanus cases are not very common, and the antitoxic serum when produced is not very stable in its properties, Tizzoni and Cattani have successfully prepared it

in a solid form, in which, it is claimed, it can be kept indefinitely, shipped any distance, and used after simple solution in water. Their method is to precipitate the antitoxin from the blood of immunized dogs with alcohol. Numerous cases of the beneficial action of this antitoxin are on record.

As Welch has pointed out, the antitoxin of tetanus has proved to be rather a disappointment in human medicine, and also for the treatment of large animals, such as the horse. The results following its injection, in combination with the sterile toxin, into mice, guinea-pigs, and rabbits are highly satisfactory, but the amount needed, in proportion to the body-weight, to save the animal from the toxin being manufactured in its body by bacilli increases so enormously with the day or hour of the disease as to make the dosage, which increases millions of times where that of diphtheria antitoxin increases but tenfold, a matter of difficulty and uncertainty. Nocard also calls attention to the fact that the existence of tetanus is unknown until there is sufficient toxemia to produce spasms, and that therefore it is impossible to attack the disease in its inception; we are obliged to meet it upon the same grounds as diphtheria in the later days of the disease—a time when it is well known that the chances of improvement are greatly lessened.

Of course, as there is no other remedy that combats the disease at all, the antitoxin is one which, when obtainable, should always be employed.

CHAPTER III.

HYDROPHOBIA, OR RABIES.

No micro-organism of hydrophobia has as yet been discovered, yet the peculiarities of the disease are such as to leave no doubt in the mind of a bacteriologist that one must exist. To find it is now the desideratum.

Although many men have labored upon hydrophobia, no name is so well known or so justly honored as that of the great pioneer in bacteriology, Pasteur. The profession and laity are alike familiar with his name and work, and although at times the newspapers of our country and certain members of the profession have opposed the methods of treatment which he has suggested as the result of his experimentation, we cannot but feel that this skepticism and opposition are due either to ignorance of the principles upon which Pasteur reasoned or to a culpable conservatism. The most vehement opponent that Pasteur has in America seems to disbelieve the existence of rabies. It is impossible to argue with him.

Hydrophobia, or rabies, is a specific toxemia to which dogs, wolves, skunks, and cats are highly susceptible, and which can, through their saliva, be communicated to men, horses, cows, and other animals. The means of communication is almost invariably a bite, hence the inference that the specific organism is present in the saliva.

The animals that are infected manifest no symptoms during a varying incubation-period in which the wound generally heals kindly. This period may last for as long a time as twelve months, but in rare cases may be only some days. An average duration of the period of incubation might be stated as about six weeks.

As the incubation-period comes to an end there is an observable alteration in the wound, which becomes reddened, sometimes may suppurate a little, and is painful. The victim, if a man, is much alarmed and has a sensation of horrible dread. The period of dread passes into one of excitement, which in many cases amounts to a wild delirium and ends in a final stage of convulsion and palsy. The convulsions are tonic, rarely clonic, and subsequently cause death by interfering with the respiration, as do those of tetanus and strychnia.

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 the inconvenience and occasional spasms which the attempt causes.

This description, brief and imperfect as it is, will illustrate the parallelism existing between hydrophobia and tetanus. In the latter we observe the entrance of infectious material through a wound, which, like the bite in hydrophobia, sometimes heals, but often suppurates a little. We see in both affections an incubation-period of varying duration, though in hydrophobia it is much longer than in tetanus, and convulsions of tonic character causing death by asphyxia.

It is maintained by some that the stage of excitement argues against the specific nature of the disease, but these subjective symptoms are like the mental condition of tuberculosis, which leads the patient to make a hopeful prognosis of his case, and the mental condition of anthrax, in which it is stated that no matter how dangerous his condition the patient is seldom much alarmed about it.

Pasteur and his co-workers found that in animals that die of rabies the salivary glands, the pancreas, and the nervous system contain the infection, and are more appropriate for 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 rabies in the rabbit in a couple of weeks. The medulla of this rabbit introduced beneath the dura mater of a second rabbit produced a more violent form of the disease in a shorter time, and by frequently repeated implantations Pasteur found that an extremely virulent material could be obtained.

Inasmuch as the toxins of diphtheria and tetanus circulate in the blood, and not infrequently saturate the nervous systems of animals affected, it might be concluded that the material with which Pasteur worked was a toxin. This is readily disproven, however, not only by the fact that the toxin would weaken instead of strengthen by the method of transfer from animal to animal, it not being a vital entity, but also by the discovery that when an emulsion of the nervous system of an affected animal is filtered through porcelain, or when it is heated for a few moments to 100° C., or exposed for a considerable time to a temperature of 75° or 80° C., its virulence is entirely lost. This would seem to prove that that which is in the nervous system and communicates the disease is a living, active body—a parasite, and in all probability a bacterium. However, all endeavors to discover, isolate, or cultivate this organism have failed.

Pasteur noted that the virulence of the poison was less in animals that had been dead for some time than in the nervous systems of those just killed, and by experimentation showed that when the nervous system was dried in a sterile atmosphere the virulence was attenuated in proportion to the length of time it had been dry. This attenuation of virulence of course suggested to Pasteur the idea of a protective vaccination, and by inoculating a dog with much attenuated, then with less attenuated, then with moderately strong, and finally with strong, virus, the dog developed an immunity which enabled it to resist the infection of an amount of viru-

lent material that would certainly kill an unprotected animal.

It is remarkable that this thought, which was a theory based upon a broad knowledge, but experience with comparatively few bacteria, should every day find more and more grounds for confirmation as our knowledge of immunity, of toxins, and of antitoxins progresses. What Pasteur did with rabies is what we now do in producing the antitoxin of diphtheria—*i. e.* gradually accommodate the animal to the poison until its body-cells are able to neutralize or resist it. As the poison cannot be secured outside of the body because the bacilli, micrococci, or whatever they may be cannot be secured outside of the body, he does what Behring originally did in diphtheria—introduces attenuated poison-producers—bacilli crippled by heat or drying, and capable of producing only a little poison—accustoms the animal to these, and then to stronger and stronger ones until immunity is established.

The genius of Pasteur did not cease with the production of immunity, but, we rejoice to add, extended to the kindred subject of therapy, and has now given us a *cure for hydrophobia*.

For the production of a cure in infected cases very much the same treatment is followed as has been described for the production of immunity. The patient must come under observation early. The treatment consists of the subcutaneous injection of about 2 grams of an emulsion of a rabbit's spinal cord which had been dried for from seven to ten days. This beginning dose is not increased in size, but each day the emulsion used is from a cord which has not been dried so long, until, when the twenty-fifth day of treatment is reached, the patient receives 2 grams of emulsion of spinal cord dried only three days, and is considered immune or cured.

It will be observed that this treatment is really no more than the immunization of the individual during the incubation stadium, and the generation of a vital force—shall we call it an antitoxin?—in the blood of the animal

in advance of the time when the organism is expected to saturate the body with its toxic products.

This, in brief, is the theory and practice of Pasteur's system of treating hydrophobia. It is exactly in keeping with the ideas of the present, and is most extraordinary in its reasonings and details when we remember that the first application of the method to human medicine was made October 26, 1885, nearly ten years before the time we began to understand the production and use of anti-toxins.

CHAPTER IV.

SYMPTOMATIC ANTHRAX.

"SYMPTOMATIC ANTHRAX," *charbon symptomatique*, *Rauschbrand*, "quarter-evil," and "black-leg" are the various names applied to a peculiar disease of cattle common during the summer season in the Bavarian Alps, Baden, Schleswig-Holstein, and some parts of the United States, characterized by the occurrence of irregular, emphysematous, crepitating subcutaneous pustules. Diseased areas are also found in the muscles, and are most common over the quarters, hence the name "quarter-evil." When incised the affected tissues have a dark color and contain a dark, bloody serum.

The micro-organismal nature of the disease had been suspected from an early date, but until the work of Faser and Bollinger the disease was confounded with anthrax. Still later, Arloing, Thomas, Cornevin, and Kitasato studied the disease, and succeeded in demonstrating the specific micro-organism, which Kitasato successfully cultivated upon artificial media.

The bacillus which the results of these labors brought to light is a rather large individual (3-5 μ in length, 0.5-0.6 μ in breadth) with rounded ends. The bacilli are occasionally united in twos, but are never united in long chains (Fig. 70). They are actively motile (Thoinot and Masselin say scarcely at all motile) when examined in the hanging drop, but after a short time, perhaps because of the exposure to the oxygen required in the hanging-drop preparation, the movement is lost and the bacilli die. When stained by Löffler's method a considerable number of flagella can be demonstrated. Large

oval spores are found; by their presence they distort the bacilli in which they occur, causing them to assume a spindle shape (clostridium), or, when two are united and a spore occupies one of them, a drumstick shape. In-



FIG. 70.—Bacillus of symptomatic anthrax, containing spores, from an agar culture; $\times 1000$ (Fränkel and Pfeiffer).

volution-forms are exceedingly common in old cultures, and are of enormous size and of granular appearance.

The bacillus can be stained with the ordinary aqueous solutions of the anilin dyes, but will not retain the color by Gram's method or Weigert's fibrin method. It can be colored in sections of tissue with Löffler's solution, and can be observed in the blood without staining shortly after death.

The spores, which can be stained by ordinary methods, are quite resistant to the action of heat and disinfectants, and withstand the effects of drying for a considerable length of time.

The bacillus of symptomatic anthrax (Fig. 71) is a strictly anaërobic, parasitic bacterium. It grows at temperatures above 18° C., but best at 37° C.

The artificial cultivation which was achieved by Kitasato is not more difficult than that of other an-

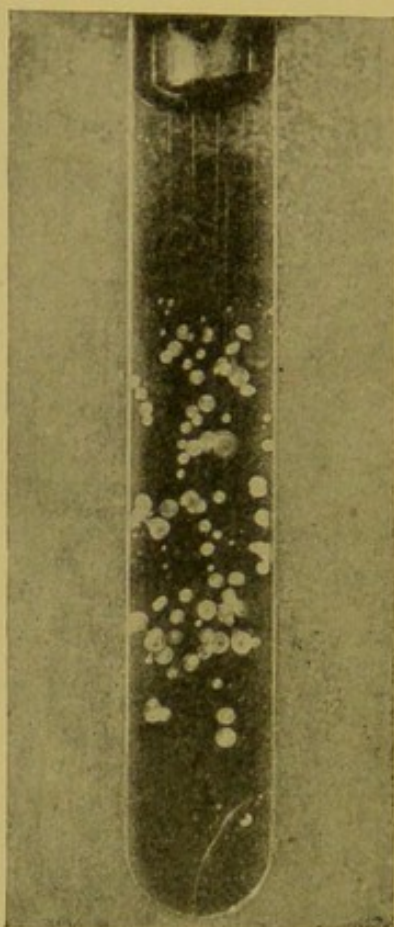


FIG. 71.—Bacillus of symptomatic anthrax : four-days-old culture in glucose-gelatin (Fränkel and Pfeiffer).

aërobic organisms. In gelatin containing 1 to 2 per cent. of glucose or 5 per cent. of glycerin the organism develops quite well, the exact appearance depending somewhat upon the method by which it was planted. If the bacteria are dispersed through the culture-medium, the little colonies will appear in the lower parts of the tube as nearly spherical or slightly irregular, clouded, liquefied areas containing bubbles of gas. If, on the other hand, the inoculation is made by a deep puncture, a stocking-shaped liquefaction forms along the whole lower part of the puncture, leads to considerable gas-production, and finally causes the liquefaction of all the gelatin except a thin superficial stratum. A peculiar acid odor is given off by the cultures.

When the bacteria grow anaërobically in Esmarch tubes, the colonies are irregularly club-shaped or spherical, with a tangled mass of delicate projecting filaments visible upon microscopic examination.

In agar-agar the development is similar to that in gelatin. The gas-production is marked, the liquefaction of course absent, and the same acid odor pronounced.

The bacillus also develops quite well in bouillon, the bacillary masses sinking to the bottom in the form of whitish flakes, while the gas-bubbles collect at the top. In this medium the virulence is unfortunately soon lost.

Milk also seems to be a favorable culture-medium. The development of the bacilli is unaccompanied by coagulation.

The virulence of the organism is soon lost in all culture-media, but it is said that the virulence of the culture can be much increased by the addition to it of 20 per cent. of lactic acid.

When susceptible animals are inoculated with a minute portion of a pure culture in a little subcutaneous pocket, such as is described in connection with tetanus and malignant edema, the bacilli proceed to grow, produce the well-known affection, and lead to a certainly fatal outcome. Cows seem to be the most susceptible animals, especially those between six months and four years old; sheep and goats are also sometimes affected. Curiously enough, animals that are immune to malignant edema are seemingly more susceptible to Rauschbrand. Of the laboratory animals, the guinea-pig is most susceptible; swine, dogs, and rabbits are very slightly susceptible; horses, goats, and birds are immune.

The virulence of the bacillus is capable of ready attenuation by exposure to heat, by previous exposure of its spores to heat, or by drying combined with exposure to increased temperature. The inoculation of animals with the attenuated bacilli causes a very mild affection, followed by complete immunity to the virulent organisms. Upon this principle the "protective vaccination" is based. Kitt has, however, shown that almost the same method as that employed by Pasteur for vaccination against rabies may be employed against this bacillus, and that when muscular tissue from an animal dead of the disease is dried at a temperature of 32-35° C., and then exposed for six hours to a temperature of 85-90° C., and a second portion is exposed in the same manner to a temperature of 100-104° C., an emulsion of this tissue in distilled water, salt-solution, or bouillon, injected into the animals to be protected, will

act in a manner resembling the pulverized spinal cords of the rabbits used in rabies, and give an almost perfect immunity. Roux and Chamberland have found that filtered cultures can also produce immunity when properly introduced into animals.

The immunity to symptomatic anthrax seems, however, to be one of degree, for Arloing, Cornevin, and Thomas found that when the bacillus was introduced into the animal body simultaneously with a 20 per cent. solution of lactic acid, either the virulence of the bacillus or the resistance of the tissues was so changed that natural immunity was destroyed and the bacteria allowed to develop and produce the disease. Roger found also that refractory animals, like the rabbit, mouse, pigeon, and chicken, could be made susceptible by the combined injection of the Rauschbrand bouillon, the *Bacillus prodigiosus*, *Proteus vulgaris*, and other harmless organisms.

When the guinea-pig is inoculated with the bacillus of symptomatic anthrax, it dies in from twenty-four to thirty-six hours. The post-mortem examination shows a bloody serum at the point of inoculation, and the muscles are dark red or black, like those of the "black-leg" of cattle. No changes are apparent in the internal organs. The bacilli are at first found near the point of inoculation in the inflammatory exudations only, but soon after death, being motile, they spread to all parts of the body.

The peculiarities of symptomatic anthrax point to the entrance of the bacteria into the animal body through wounds, but the occurrence of epidemics at certain geographical points, known technically as "Rauschbrand stations," suggests that infection may also take place through the respiratory and alimentary tracts.

At first thought, as Fränkel points out, one might imagine that an animal dead of quarter-evil and the discharges from its body might be harmless, as compared, for example, with the cadavers and discharges of anthrax, because of the purely anaërobic method of the growth of the bacillus of symptomatic anthrax and the rapidity of its

death in the presence of oxygen. This is, however, untrue, for the rapid development of a permanent form in the resisting spores of the bacillus makes the pollution of the soil exceedingly dangerous for cows who subsequently browse upon it. That the spores are of great vitality is shown by the well-known laboratory method of keeping them on hand for experimental purposes, dried in the muscular tissue of a diseased animal.

Every precaution should be exerted to have the affected animals isolated, and their cadavers disinfected and destroyed or buried in such a manner that subsequent infection is impossible.

Statistical results of Guillod and Simon, based upon 3500 protective inoculations, show a distinct reduction of the death-rate from 5-20 per cent. in unprotected animals to 0.5-2 per cent. in protected animals.

CHAPTER V.

TYPHOID FEVER.

THE bacillus of typhoid fever (Fig. 72) was discovered by Eberth in 1880, and was first secured in pure culture

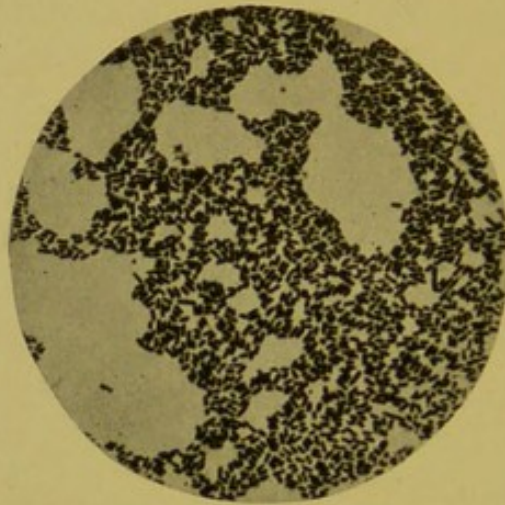


FIG. 72.—*Bacillus typhi*, from a twenty-four-hours-old agar-agar culture; \times 650 (Heim).

from the spleen and affected lymphatic glands by Gaffky four years later.

The organism is a small, short bacillus about $1-3\ \mu$ ($2-4\ \mu$ Chantemesse, Widal) in length and $0.5-0.8\ \mu$ broad (Sternberg). The ends are rounded, and it is rather exceptional for the bacilli to be united in chains, though this arrangement is common in potato cultures. The size and morphology vary distinctly with the nature of the culture-medium and the age of the culture. Thoinot and Masselin in describing these morphological peculiarities mention that when grown in bouillon it is a very slender bacillus; in milk it is a large bacillus; upon agar-agar and potato it is very thick and short; and in old gelatin cultures it forms very long filaments.

The organisms are actively motile, the motility probably being caused by the numerous flagella with which

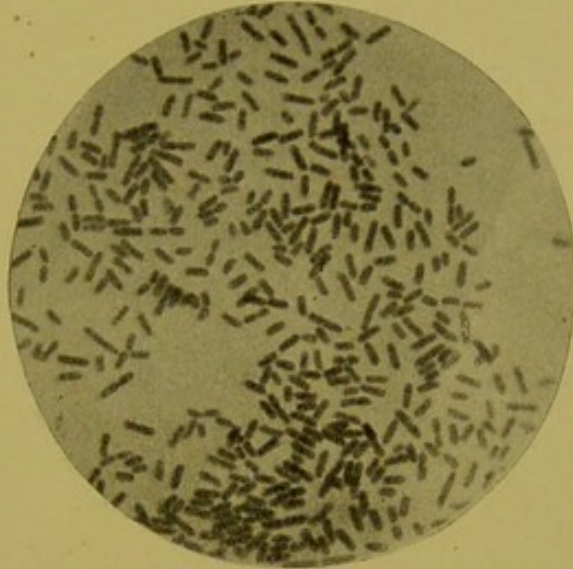


FIG. 73.—*Bacillus coli communis*, from an agar-agar culture; $\times 1000$ (Itzerott and Niemann).

the bacilli are provided. The flagella stain well by Löffler's method, and, as they are numerous (eighteen

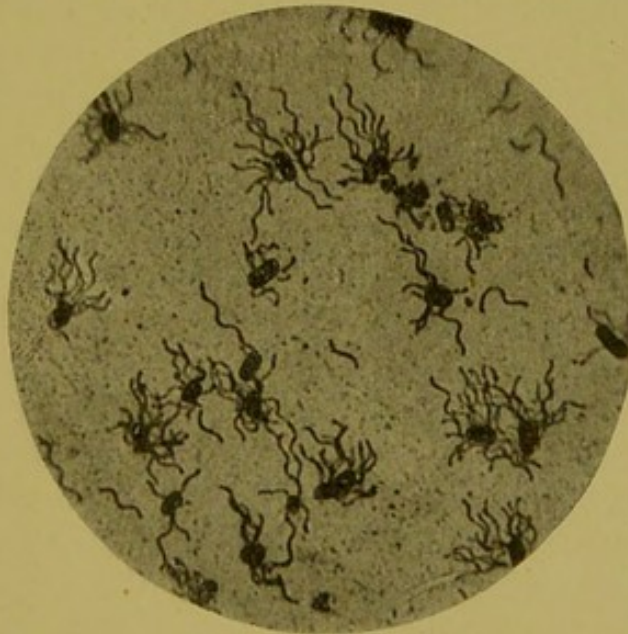


FIG. 74.—*Bacillus typhi*, from an agar-agar culture six hours old, showing the flagella stained by Löffler's method; $\times 1000$ (Fränkel and Pfeiffer).

to twenty) and readily demonstrable, the typhoid bacillus is the favorite subject for their study.

The organism stains quite well by the ordinary methods, but loses the color entirely when stained by Gram's method. Its peculiarity of staining is the readiness with which the bacillus gives up its color in the presence of solvents, so that it is particularly difficult to stain it in tissue.

When sections are to be stained the best method is to allow the tissue to remain in Löffler'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 preparations the bacilli may be found in little groups, which are easily discovered, under a low power of the microscope, as bluish specks, and readily resolved into bacilli with the high power of the oil-immersion lens.

In bacilli stained by this alkaline methylene-blue solution dark-colored dots may sometimes be observed near the ends of the rods. These dots were at first regarded as spores, but are now denominated polar granules, and are thought to be of no importance.

The typhoid bacillus is both saprophytic and parasitic. It finds abundant conditions in nature for its growth and development, and, enjoying strong resisting powers, can accommodate itself to environment much better than the majority of pathogenic bacteria, and can be found in water, air, soiled clothing, dust, sewage, milk, etc. contaminated directly or indirectly by the intestinal discharges of diseased persons.

The bacillus is also occasionally present upon green vegetables sprinkled with water containing it, and an epidemic is reported in which the infection was traced to oysters from a certain place where the water was infected

through sewage. The bacillus probably enters milk occasionally in water used to dilute it.

The resistant powers of the organisms have already been described as great. They can grow well at the room-temperature. The thermal death-point is given by Sternberg as 60° C. The bacilli can, according to Klemperer and Levy, 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. When buried in the upper layers of the soil the bacilli retain their vitality for nearly six months. Cold has no effect upon the typhoid bacilli, for freezing and thawing several times are without injury to them. They have been found to remain alive upon linen for from sixty to seventy-two days, and upon buckskin for from eighty to eighty-five days. Sternberg has succeeded in keeping hermetically-sealed bouillon cultures alive for more than a year. In the presence of chemical agents the bacillus is also able to retain its vitality, 0.1 to 0.2 per cent. of carbolic acid added to the culture-media being 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.

Cultures of the typhoid bacillus may be obtained, but with difficulty, from the alvine discharges of typhoid patients. In examining this material, however, it must be remembered that the bacilli are certain to be present only in the second and third weeks.

As numerous saprophytic bacteria are present in the feces, the resistance which the typhoid bacillus exhibits to carbolic acid can be made use of in obtaining the pure culture. To each of several tubes of melted gelatin 0.05 per cent. of carbolic acid is added. This addition is most easily calculated by supposing the average amount of gelatin contained in a tube to be 10 c.cm. To the average tube $\frac{1}{10}$ c.cm. of a 5 per cent. solution of carbolic acid is added, and gives very nearly the desired quantity. A

minute portion of the feces is broken up with a platinum loop and stirred in the tube of melted gelatin; a drop from this dilution is transferred to the second tube, a drop from it to a third, and then the contents of each tube are poured upon a sterile plate or into a Petri dish,

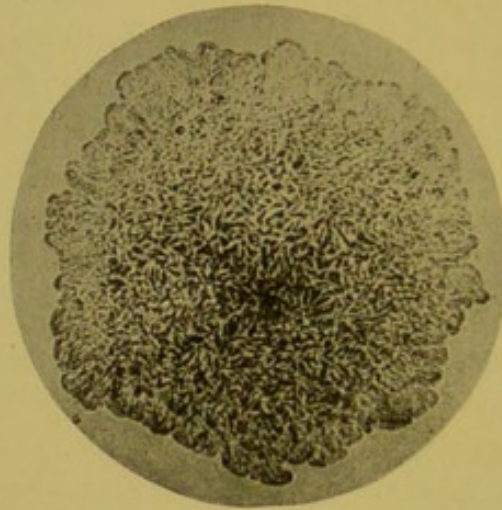


FIG. 75.—*Bacillus typhi abdominalis*: superficial colony two days old, as seen upon the surface of a gelatin plate; $\times 20$ (Heim).

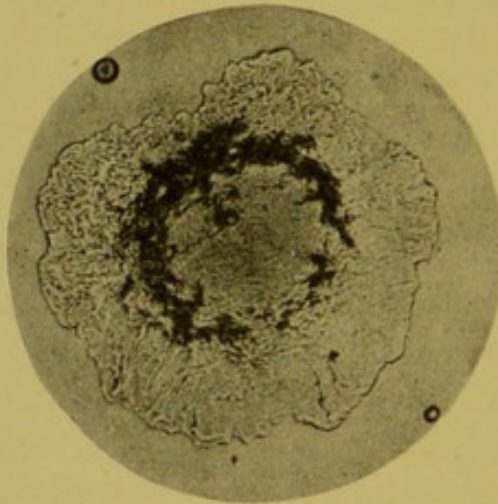


FIG. 76.—*Bacillus coli communis*: superficial colony two days old upon a gelatin plate; $\times 21$ (Heim).

or rolled, according to Esmarch's plan, in the manner already described. The carbolic acid present in these cases prevents the great mass of saprophytes from developing, but allows the perfect development of the

typhoid bacillus (Fig. 75) and its near congener, the *Bacillus coli communis* (Fig. 76).

The colonies that develop upon such gelatin plate-cultures are seen under the microscope to be brownish-yellow in color, spindle-shaped, and sharply circumscribed. When superficial they are larger and form a bluish iridescent layer with notched edges. The centre of the superficial colonies is the only portion which shows the yellowish-brown color. The margins of the colony appear somewhat reticulated. The gelatin is not liquefied.

Unfortunately, the appearances of the colonies of the *Bacillus typhi* and the *Bacillus coli communis* are identical, and make it next to impossible to select a single colony of either with any certainty. The only solution of the problem is to transfer a large number of colonies to some culture-medium in which a characteristic of one or the other species is manifested, and then study the growth.

When transferred to gelatin puncture-cultures the bacilli develop along the entire track of the wire, with the formation of minute confluent spherical colonies. A small thin whitish layer develops upon the surface near the centre. The gelatin is not liquefied, but sometimes is slightly clouded in the neighborhood of the growth. The growth upon the surface of obliquely solidified gelatin, agar-agar, or blood-serum is not very luxuriant. It forms a thin, moist, translucent, non-characteristic band with smooth edges.

Upon potato a characteristic growth takes place. When the potato is inoculated and stood in the incubating oven, no growth can be detected at the end of the second day, unless the observer be skilled and the examination thorough. If, however, the medium be touched with a platinum wire, it is discovered that its entire surface is covered with a rather thick, invisible layer of a sticky vegetation which the microscope shows to be made up of bacilli. No other bacillus gives the same kind of growth upon potato. Unfortunately, it is not constant,

for occasionally there will be encountered a typhoid bacillus which will show a distinct yellowish or brownish color. The typical growth seems to take place only when the reaction of the potato is acid.

In bouillon the only change produced by the growth of the bacillus is a diffuse cloudiness.

In milk a slight and slow acidity is produced. The growth in milk is not accompanied by coagulation.

The chief hindrance to the ready isolation of the typhoid bacillus is the closely-allied species described by Escherich as the *Bacterium coli commune*, by Emmerich as the *Bacillus Neapolitanus*, and now known as the *Bacillus coli communis*. This organism, being habitually present in the intestine, exists there in typhoid fever, and adds no little complication to the bacteriological diagnosis by responding in exactly the same manner as the typhoid bacillus to the action of carbolic acid, by having colonies almost exactly like those of typhoid, by growing in exactly the same manner upon gelatin, agar-agar, and blood-serum, by clouding bouillon in the same way, by being of exactly the same shape and size, by having flagella, by sometimes being motile, and, in fact, by so many pronounced similarities as to warrant the assertion of many that it and the typhoid bacillus are identical.

At the present time we are in more or less of a quandary about this extraordinary resemblance, but base our differentiation of the species upon certain constant, slight, but distinct differences.

The *Bacillus coli communis* grows differently upon acid potato, producing a smeary, elevated, circumscribed, brownish layer which bears a resemblance to the growth of the typhoid bacillus upon alkaline or neutral potato. This bacterium, in addition to a more pronounced acid-production in milk, causes prompt coagulation, which the typhoid bacillus does not.

When the colon bacillus is planted in gelatin or agar-agar containing a small amount of glucose, a beautiful

gas-production is developed, which is unknown to the typhoid bacillus.

Finally, the typhoid bacillus does not produce indol, but the addition of potassium nitrite and sulphuric acid to bouillon cultures of the colon bacillus invariably brings about the rose color which characterizes this product.

Not only are the morphological and vegetative similarities of these organisms great, but their pathogeny bears many points of resemblance. The open lymphatics and vessels of the intestinal ulcers of typhoid favor the absorption of the bacteria in the digestive tract, and the colon bacillus enters the blood no longer to be a saprophyte, but now to be a virulent pus-producer, and in many cases of typhoid we find suppurations and other milder inflammations due to this microbe. This is also a stumbling-block, for the typhoid bacillus when distributed through the blood may act in exactly the same manner.

The typhoid bacillus may enter the body, at times, through dust (Klemperer and Levy), but no doubt, in the great majority of cases, enters the digestive tract at once through the mouth. It may possibly enter through the rectum at times, as illustrated by the mention which Eichhorst makes of the infection of soldiers in military barracks through the wearing of drawers previously worn by comrades who had suffered from typhoid.

When ingested the resisting power of the bacillus permits it to pass uninjured through the acid secretions of the stomach and to enter the intestine, where the chief local disturbances are set up.

The bacilli enter the solitary glands and Peyer's patches, and multiply slowly during the one to three weeks of the incubation of the disease. The immediate result of their residence in these lymphatic structures is increase in the number of cells, and ultimately the necrosis and sloughing which cause the typical post-mortem lesion. From the intestinal lymphatics the bacilli pass, in all probability, to the mesenteric glands, which become enlarged and

softened, and finally extend to the spleen and liver, and sometimes to the kidneys. The growth of the bacilli in the kidneys causes the albuminuria of the disease. Sometimes under these conditions the bacilli can be found in the urine. Occasionally the bacilli succeed in entering the general circulation, and, finding a lodgement at some remote part of the body, set up local inflammatory processes sometimes terminating in suppuration.

The bacilli can be found in the intestinal lesions, in the mesenteric glands, in the spleen, in the liver, in the kidneys, and in any local lesions which may be present. Their scattered distribution and their occurrence in minute clumps have already been alluded to. They should always be sought for at first with a low power of the microscope.

Ordinarily no bacilli can be found in the blood, but it has been shown that the blood in the roseolæ sometimes contains them, so that the eruption may be regarded as one of the local irritative manifestations of the bacillus.

The amount of local disturbance, in proportion to the constitutional disturbance, is, in the majority of cases, slight, and almost always partakes of a necrotic character, which suggests that in typhoid we have to do with a toxic bacterium whose disease-producing capacity resides in the elaboration of a toxic substance. This, indeed, is true, for Brieger and Fränkel have separated from bouillon cultures a toxalbumin which seems to be the specific poison. Klemperer and Levy also point out further clinical proof in certain exceptional cases dying with the typical picture of typhoid, yet without characteristic post-mortem lesions, the only confirmation of the diagnosis being the discovery of the bacilli in the spleen.

As the discovery of the bacilli in the spleen, and especially the securing of a pure culture of the bacilli from the spleen, are sometimes attended with considerable difficulty because of the dissemination of the colonies throughout the organ, 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 may take place.

Typhoid fever is a disease which is communicable to animals with difficulty. They are not affected by bacilli in fecal matter or in pure culture mixed with the food, and are not diseased by the injection into them 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 germs for a considerable time, yet without symptoms. The introduction of pure cultures into the abdominal cavity of most animals is without effect. Fränkel and Simon, however, found that when pure cultures are injected into mice, rabbits, and guinea-pigs the animals die. Many observers attribute the deaths in such cases to the toxin injected with the bacilli, and consider it entirely independent of the living organisms injected. In such fatal cases, however, the bacilli are found in large numbers in the blood, making the condition resemble septicemia.

When animals are treated in the manner described in the chapter upon Cholera—*i. e.* the gastric contents rendered alkaline, a large quantity of laudanum injected into the peritoneal cavity, and the bacilli introduced through an esophageal catheter—Klemperer, Levy, and others found that there was produced an intestinal condition which very much resembled typhoid as it occurs in man. The virulence of the bacillus can be very greatly increased by rapid passage from guinea-pig to guinea-pig.

In the experiments of Chantemesse and Widal the symptoms following the injection of virulent culture into guinea-pigs were briefly as follows: "Very shortly after the inoculation there is a rise of temperature, which continues from one to four hours, and is succeeded by a depression of the temperature, which continues to the

fatal issue. Meteorism and great tenderness of the abdomen are observed. At the autopsy a sero-fibrinous or sero-purulent peritonitis is observed—sometimes hemorrhagic. There is also generally a pleurisy, either serous or hemorrhagic. All the abdominal viscera are congested. The intestine is congested—contains an abundant mucous secretion. The Peyer patches are enlarged. The spleen is enlarged, blackish, and often hemorrhagic. In cases which are prolonged the liver is discolored. The kidneys are congested, the adrenals filled with blood.

“In such cases the bacillus can be found upon the inflamed serous membranes, in the inflammatory exudates, in the spleen in large numbers, in the adrenals, the liver, the kidneys, and sometimes in the lungs. The blood is also infected, but to a rather less degree.

“In cases described as chronic, the bacillus disappears completely in from five to twenty-four hours, and produces but one lesion, a small abscess at the point of inoculation.

“Sanarelli has observed that if some of the poisonous products of the colon bacillus or the *Proteus vulgaris* be injected into the abdominal cavity of an animal recovering from a chronic case, it speedily succumbs to typical typhoid fever.”

The failure to produce a satisfactory combination of symptoms by experimental inoculation into animals is one of the impediments in the way of the production of an antitoxin for use in human medicine. As long as there can be the slightest doubt thrown upon the specificity of the bacillus because of the failure to produce the recognized symptoms in animals, so long an antitoxic substance, if produced at all, will be rejected by many in the profession. Animals can easily be accustomed to this bacillus, and when so accustomed seem, according to Chantemesse and Widal, to develop in their blood an antitoxic substance capable of protecting other animals. Stern has also found that in the blood of recent human convalescents a substance exists which has a

distinct protective effect upon guinea-pigs. His observation is in accordance with an earlier one in the same line by Chantemesse and Widal. There is only the foreshadowing of a useful antitoxic substance in the work which has already been done, but, judging from the success met with in tetanus and diphtheria, we can build exalted hopes of future success.

Rumpf, Kraus, and Buswell report a number of cases of typhoid which were favorably influenced by the introduction hypodermically of small quantities of sterilized cultures of *Bacillus pyocyaneus*, and have thus added somewhat to our knowledge of antagonistic bacteria and neutralizing toxins. These experiments are still too new to deserve prolonged mention.

One of the most important and practical points for the physician to grasp in relation to the subject of typhoid fever is the highly virulent character of the discharges from the bowels. In every case the greatest care should be taken for a proper disinfection of the feces, a rigid attention 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 the large cities with their unmanageable slums, and the distribution of the bacilli to villages and towns by watercourses polluted in their infancy might be checked.

CHAPTER VI.

CHOLERA.

CHOLERA is a disease from which certain parts of India are never free. These areas, in which it is endemic, are the foci from which the great epidemics of the world, as well as the constant smaller epidemics of India, probably spread. No one knows when cholera was first introduced into India, and the probabilities are that it is indigenous to that country, as yellow fever is to Cuba. Very early mention of it is made in the letters of travellers, in books and papers on medicine of a century ago, and in the governmental statistics, yet we find that little is said about the disease except in a general way, most attention being directed to the effect upon the armies, native and European, of India and adjacent countries. The opening up of India by Great Britain in the last half century has made possible much accurate scientific observation of the disease and the relation which its epidemics bear to the manners and customs of the people.

The filthy habits of the people of India, their poverty, their crowded condition, and their religious customs, all serve to aid in the distribution of the disease. We are told that 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. 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 large quantity of this sacred but polluted water, and, as may be imagined, the number of pious Hindoos who leave Benares with comma bacilli in their intestines or upon their clothes is great, for there are few months in the

year when there are not at least some cases of cholera in the city.

The frequent pilgrimages and great festivals of the Hindoos and Moslems, by bringing together an enormous number of people who crowd in close quarters where filth and bad diet are common, cause a rapid increase in the number of cases during these periods and the dispersion of the disease when the festivals break up. The disease extends readily along the regular lines of travel, visiting town after town, until from Asia it has frequently extended into Europe, and by the steamships plying on foreign waters has been several times carried to our own continent and to the islands of the seas. Many cases are on record which show conclusively how a single ship, having a few cholera cases on board, may be the cause of an outbreak of the disease in the port at which it arrives.

It seems strange to us now, with the light of present information illuminating the pages of the past, to observe how the distinctly infectious nature of such a disease could be overlooked in the search for some atmospheric or climatic cause, some miasm, which was to account for it.

The discovery of the organism which seems to be the specific cause of cholera was made by Koch, who was appointed one of a German cholera-commission to study the disease in Egypt and India in 1883-84. Since his discovery, but a decade ago, the works upon cholera and the published investigations to which the spirillum has been subjected have produced an immense literature, a large part of which was stimulated by the Hamburg epidemic of two years ago.

The micro-organism described by Koch, and now generally accepted to be the cause of cholera, is a short individual about half the length of a tubercle bacillus, considerably stouter, and distinctly curved, so that the original name by which it was known was the "comma bacillus" (Figs. 77, 78).

A study of the growth of the organism and the forms which it assumes upon different culture-media soon convinces us that we have to do with an organism in no way related to the bacilli. If the conditions of nutrition are



FIG. 77.—Spirillum of Asiatic cholera, showing the flagella; $\times 1000$ (Günther).

diminished so that the multiplication of the bacteria by simple division does not progress with the usual rapidity, we find a distinct tendency toward—and in some cases, as upon potato, a luxuriant development of—long spiral threads with numerous windings—unmistakable spirilla. Fränkel has found that the exposure of cultures to unusually high temperatures, the addition of small amounts of alcohol to the culture-media, etc., will so vary the growth of the organism as to favor the production of spirals instead of commas. One of the most common of the numerous forms observed is that in which two short curved individuals are so joined as to produce an S-shaped curve.

The cholera spirilla are exceedingly active in their movements, and in hanging-drop cultures can be seen to swim about with great rapidity. Not only do the comma-shaped organisms move, but when distinct spirals exist, they, too, move with the rapid rotary motion so common among the spirilla.

The presence of flagella upon the cholera spirillum can be demonstrated without difficulty by Löffler's method (*q. v.*). Each spirillum possesses a single flagellum attached to one end.

Inoculation-forms of most bizarre appearance are very common in old cultures of the spirillum, and very often

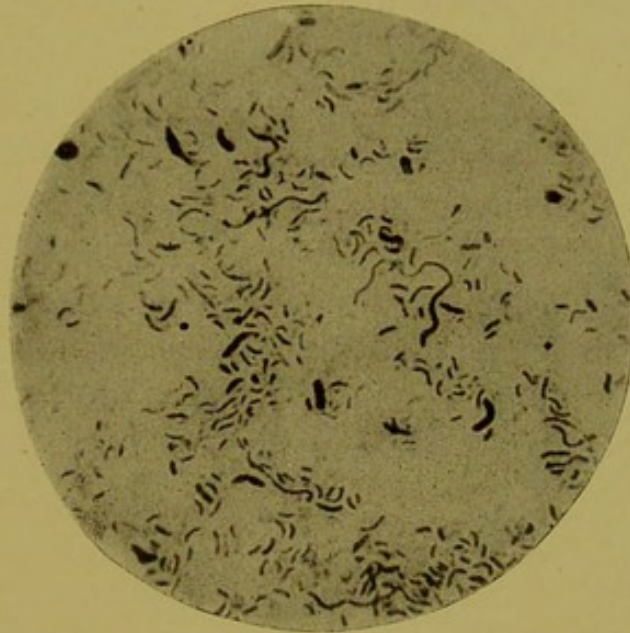


FIG. 78.—Spirillum of Asiatic cholera, from a bouillon culture three weeks old, showing numbers of long spirals; $\times 1000$ (Fränkel and Pfeiffer).

there can be found in fresh cultures many individuals which show by granular protoplasm and irregular outline that they are partly degenerated. Cholera spirilla from various sources seem to differ in this particular, some of the forms being as pronounced in their involution as the diphtheria bacilli.

In partially degenerated cultures in which long spirals are numerous Hüppe observed, by examination in the "hanging drop," in the continuity of the elongate members, certain large spherical bodies which he described as spores. These bodies were not enclosed in the organisms like the spores of anthrax, but seemed to exemplify the form of sporulation in which an entire individual transforms itself into a spore (arthrospore). Koch, and indeed all other observers, failed to find signs of fructification in

the cholera organism, and the true nature of the bodies described by Hüppe must be regarded as doubtful. Most bacteriologists disagree with Hüppe in believing that arthrospores exist at all, and the fact (which will be pointed out later on) that there is very little permanence about cholera cultures throws additional doubt upon the accuracy of Hüppe's conclusion.

The cholera spirillum stains well with the ordinary aqueous solutions of the anilin dyes; fuchsin seems particularly appropriate. At times the staining must be continued for from five to ten minutes to secure homogeneity. The cholera spirillum 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 flowed over it, and it is flattened out by pressing down on it 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. Comma bacilli so prepared and examined with an oil-immersion lens ($\times 700-800$) may then be seen: their characters are the more readily made out because of the slight stain which they take up, and because they still retain their power of vigorous movement, which would be entirely lost if the specimen were dried, stained, and mounted in the ordinary fashion."

The colonies of the spirillum when grown upon gelatin plates are highly characteristic. They appear in the lower strata of the gelatin as small white dots, gradually grow out to the surface, effect a gradual liquefaction of the medium, and then appear to be situated in little pits with sloping sides (Fig. 79). This peculiar appearance, which gives one the suggestion that the plate is full of little holes or air-bubbles, is due to the evaporation of the liquefied gelatin.

One of the best methods of securing pure cultures of

the cholera spirillum, and also of making a diagnosis of the disease in a suspected case, is probably that of Schottelius. The method is very simple: A small quantity of the fecal matter is mixed with bouillon and stood in an incubating oven for twenty-four hours. If the

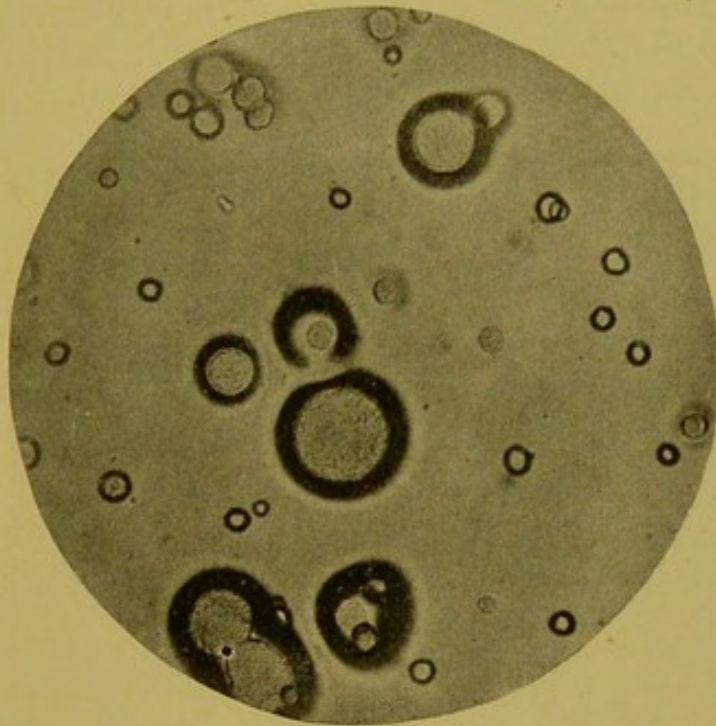


FIG. 79.—Spirillum of Asiatic cholera: colonies two days old upon a gelatin plate; $\times 35$ (Heim).

cholera spirilla are present, they will grow most rapidly at the surface of the liquid when 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.

Under the microscope the principal characteristics can be made out. The colony of the cholera spirillum scarcely resembles that of any other organism. The little colonies which have not yet reached the surface of the gelatin begin very soon to show a pale-yellow color and to exhibit irregularities of contour, so that they are almost never smooth and round. They are coarsely granular, and have the largest granules in the centre. As the colony increases in size the granules also increase

in size, and attain a peculiar transparent character which is suggestive of powdered glass. The commencement of liquefaction causes the colony to be surrounded with a transparent halo. When this occurs the colony begins to sink, from the digestion and evaporation of the medium, and also to take on a peculiar rosy color.

In puncture-cultures in gelatin the growth is again so characteristic that it is quite diagnostic (Fig. 80). The

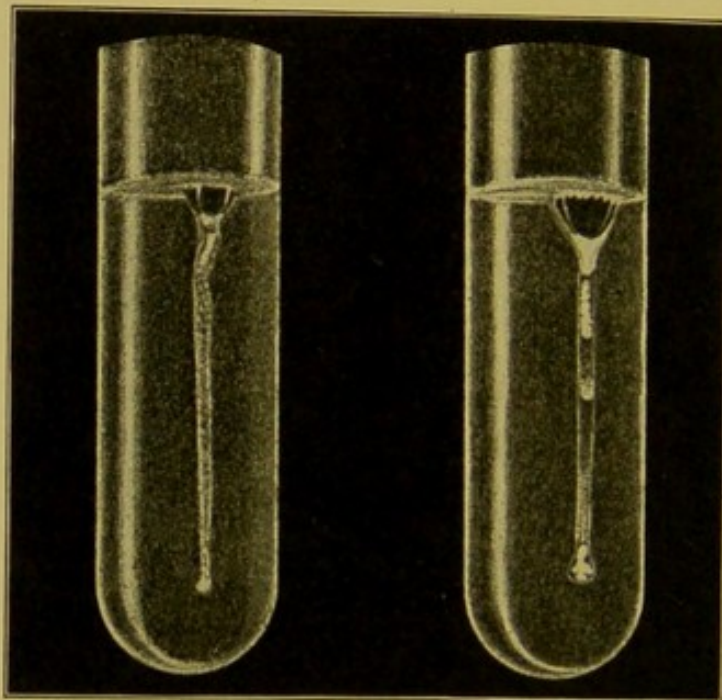


FIG. 80.—*Spirillum cholera Asiatica*; gelatin puncture-cultures aged forty-eight and sixty hours (Shakespeare).

growth takes place along the entire puncture, but develops best at the surface, where it is in contact with the atmosphere. An almost immediate liquefaction of the medium begins, and, keeping pace with the rapidity of the growth, is more marked at the surface than lower down. The result of this is the occurrence 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 solid surrounding portions, and appears to be surmounted by an air-bubble.

The luxuriant development of the spirilla in gelatin produces considerable solid material to sediment and fill up the lower third or lower 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. Liquefaction of the medium is not complete for several weeks. According to Fränkel, in eight weeks the organisms in the liquefied culture all die, and cannot be transplanted. Kitasato, however, has found them living and active on agar-agar after ten to thirty days, and Koch was able to demonstrate their vitality after two years.

When planted upon the surface of agar-agar the spirilla produce a white, shining, translucent growth along the entire line of inoculation. It is in no way peculiar. 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.

The growth upon blood-serum likewise is without distinct peculiarities, and causes gradual liquefaction of the medium.

Upon potato the spirilla grow well, even when the reaction of the potato is acid. In the incubator at a temperature of 37° C. a transparent, slightly brownish or yellowish-brown growth, somewhat resembling the growth of glanders, is produced. It contains large numbers of long spirals.

In bouillon and in peptone solution the cholera organisms grow well, especially upon the surface, where a folded, wrinkled mycoderma is formed. Below the mycoderma the culture fluid generally remains clear. If the glass be shaken and the mycoderma broken up, fragments of it sink to the bottom.

In milk the development is also luxuriant, but takes place in such a manner as not visibly to alter its appear-

ance. The existence of cholera organisms in milk is, however, rather short-lived, for the occurrence of any acidity at once destroys them.

Wolffhügel and Riedel have shown that if the spirilla are plunged in sterilized water they grow with great rapidity after a short time, and can be found alive after months have passed. Fränkel points out that this ability to grow and remain vital for long periods in sterilized water does not guarantee the same power in unsterilized water, for in the latter the simultaneous growth of other bacteria in a few days serves to extinguish the cholera germs.

One of the characteristics of the cholera spirillum is the metabolic production of indol. The detection of this substance is easy if the spirilla are grown in a transparent colorless solution. As the cholera organisms also produce nitrites, all that is necessary is to add a drop or two of chemically pure sulphuric acid to the culture-medium for the production of the well-known reddish color.

Several toxic products of the metabolism of the spirilla have been isolated. Brieger and Fränkel have isolated a toxalbumin; Villiers, a toxic alkaloid fatal to guinea-pigs; and Gamaléia, two substances about equally toxic.

The cholera spirilla can be found with great constancy in the intestinal evacuations of all cholera cases, and can often be found in the drinking-water, milk, and upon vegetables, etc. in cholera-infected districts. There can be little doubt that they find their way into the body through the food and drink. Many cases are reported in the literature upon cholera that show how the disease-germs enter the drinking-water, and are thus distributed; how they are sometimes thoughtlessly sprinkled over vegetables, offered for sale in the streets, with water from polluted gutters; how they enter milk with water used to dilute it; how they are carried about in clothing and upon foodstuffs; how they can be brought to articles of food upon the table by flies which have preyed upon cholera excrement; and how many other interesting in-

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fections are made possible. The literature upon these subjects is so vast that in a sketch of this kind it is scarcely possible to comprise even the most instructive examples. One physician is reported to have been infected with cholera while experimenting with the spirilla in Koch's laboratory.

The evidence of the specificity of the cholera spirillum when collected shows that it is present in the choleraic dejections with great regularity, and that it is as constantly absent from the dejecta of healthy individuals and those suffering from other diseases; but these facts do not admit of satisfactory proof by experimentation upon animals. Animals are never affected by any disease similar to cholera during the epidemics, nor do foods mixed with cholera discharges or with pure cultures of the cholera spirillum affect them. This being true, we are prepared to receive the further information that subcutaneous injections of the spirilla are often without serious consequences, though cultures differ very much in this respect, some always causing a fatal septicemia in guinea-pigs, others being as constantly harmless.

Intraperitoneal injection of the virulent cultures produces a fatal peritonitis in guinea-pigs.

One reason that animals and certain men are immune to the disease seems to be found in the distinct acidity of the normal gastric juice, and the destruction of the spirilla by it. Supposing that this might be the case, Nicati and Rietsch, Von Ermengen 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. The result was that the animals usually died, sometimes with choleraic symptoms; but the excessively grave nature of the operation upon such a small and delicately constituted animal as a guinea-pig greatly lessens the value of the experiment. Koch's method is much more satisfactory. By injecting laudanum into the abdominal cavity

of guinea-pigs the peristaltic movements are checked. The amount given for the purpose is very large, about 1 gram for each 200 grams of body-weight. It generally narcotizes the animals for a short time, but they recover without injury. After administering the opium the contents of the stomach are neutralized by introducing through a pharyngeal catheter 5 c.cm. of a 5 per cent. aqueous solution of sodium carbonate. With the gastric contents thus alkalinized and the peristalsis paralyzed a bouillon culture of the spirilla is introduced. 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 injected animals, the spirilla are never found in the blood or the 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.

Guinea-pigs are also 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-organism, and injection and redness of the peritoneum and viscera.

Although in reading upon cholera at the present time we find very little skepticism in relation to Koch's "comma bacillus," we do find occasional doubters who believe with Von Pettenkoffer that the disease is miasmatic. Pettenkoffer's theory is that the disease has much to do with the ground-water and its drying zone. He regards as the principal cause of the disease the development of germs in the subsoil moisture during the warm months, and their impregnation of the atmosphere as a miasm to be inhaled, instead of ingested with food and drink. This idea of Pettenkoffer's, combined with

his other idea that individual predisposition must precede the inception of the disease, is scarcely compatible with what has gone before, and cannot possibly be made to explain the march of the disease from place to place with caravans, or its distribution over extended areas when fairs and religious gatherings among the Hindoos break up, the people from an infected centre carrying cholera with them to their homes.

While it is an organism that multiplies with great rapidity under proper conditions, the cholera spirillum is not possessed of much resisting power. Sternberg found that it was killed by exposure to a temperature of 52° C. for four minutes. Kitasato, however, found that ten or fifteen minutes' exposure to a temperature of 55° C. was not always fatal. In the 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 was destroyed 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 to thirty days in fecal matter, and often disappears in one to three days. The organism is very susceptible to the influence of carbolic acid, bichlorid of mercury, and other germicides.

This low vital resistance of the microbe is very fortunate, for it enables us to establish safeguards 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 foods are rendered innocuous by boiling or cooking. Vessels may be disinfected by thorough washings with jets of boiling water thrown upon them through hose. Baggage can be sterilized by superheated steam.

It often becomes a matter of importance to detect the presence of cholera in drinking-water, and, as the dilution in which the bacteria exist in such a liquid may be very great, much difficulty is experienced in finding them

by ordinary methods. One of the most expeditious methods that have been recommended is that of Löffler, who adds 200 c.cm. of the water to be examined to 10 c.cm. of bouillon, allows the mixture to stand in an incubator for 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 presence of air. A similar method can be used to detect the spirilla when their presence is suspected in feces.

Gruber and Wiener, Haffkine, Pawlowsky, and Pfeiffer have all succeeded in immunizing animals against the toxic substances removed from cholera cultures or against living cultures properly injected. There seems, according to the researches of Pfeiffer, to be no doubt that in the blood of the protected animals a protective substance is present. In the peritoneal infection of guinea-pigs the spirilla grow vigorously in the peritoneal cavity, and can be found in immense numbers after twelve to twenty-four hours. If, however, together with the culture used for inoculation, a few drops of the protective serum be introduced, Pfeiffer found that instead of multiplying the organisms underwent a peculiar granular degeneration and disappeared, the unprotected animal dying, the protected animal remaining well.

Of the numerous attempts which have from time to time been made, and are still being made, to produce immunity against cholera in man or to cure cholera when once established in the human organism, nothing very favorable can at the present time be said. Experiments in this field are not new: we find Dr. Ferrán administering hypodermic injections of pure virulent cultures of the cholera spirillum in Spain as early as 1885, in the hope of bringing about immunity. The more modern work of Haffkine seems to be followed by a distinct diminution of mortality in protected individuals. According to the work of this investigator, two vaccines are used, one of which, being mild, prepares the animal (or

man) for a powerful vaccine, which, were it not preceded by the weaker form, would bring about extensive tissue-necrosis and perhaps death. Protection certainly seems to follow the operation of these vaccines.

Haffkine's studies embrace more than 40,000 inoculations performed in India. From his latest paper (Dec., 1895) the following extract will show the results:

"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 that the dog is susceptible to cholera, and have utilized the observation to prepare an antitoxic serum in considerable quantities. The dogs were first immunized with attenuated cultures, then with 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 results from the injection of blood-serum from convalescent patients into the diseased. One recovery out of three cases treated is recorded—not a very glittering result.

In all these preliminaries the foreshadowing of a future therapeutics must be evident, but as yet nothing really satisfactory has been achieved.

CHAPTER VII.

SPIRILLA RESEMBLING THE CHOLERA SPIRILLUM.

The Finkler and Prior Spirillum.—Somewhat similar to the spirillum of cholera, and in some respects closely related to it, is the spirillum obtained from the feces of a case of cholera nostras by Finkler and Prior in 1884. It is a rather shorter, stouter organism, with a more pronounced curve, than the cholera spirillum, and rarely forms the long spirals which characterize the latter. The central portion is also somewhat thinner than the ends, which are a little pointed and give the organism a less uniform appearance than that of cholera (Fig. 81).

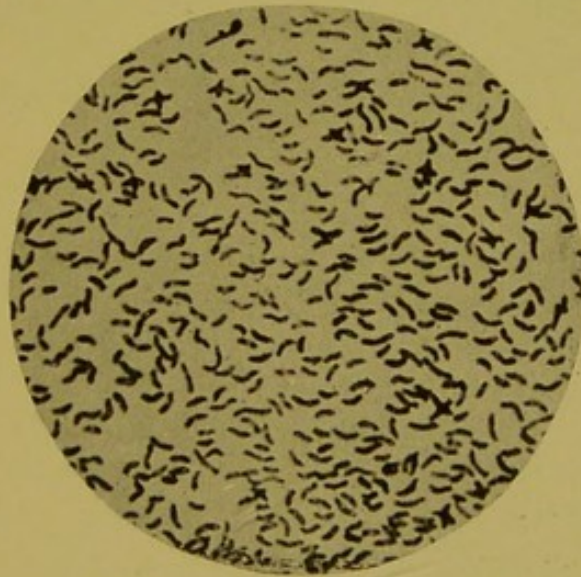


FIG. 81.—Spirillum of Finkler and Prior, from an agar-agar culture; $\times 1000$ (Itzerott and Niemann).

Involution-forms are very common in cultures, and occur 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. Although at first thought to be a variety of the cholera germ, marked differences of growth were soon observed, and showed the organism to be a separate species.

The growth upon gelatin plates is quite rapid, and leads to such extensive liquefaction that four or five dilutions must frequently be made before the growth of a single colony can be observed. To the naked eye the colonies appear as small white points in the depths of the gelatin (Fig. 82). They, however, rapidly reach the surface,

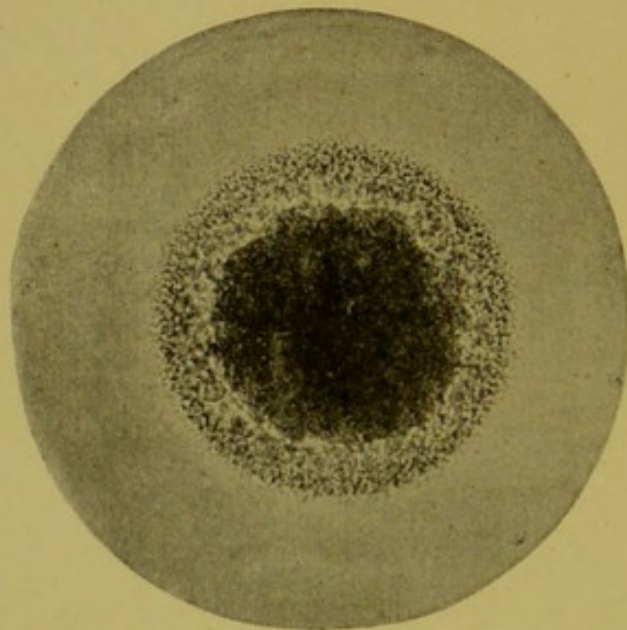


FIG. 82.—*Spirillum* of Finkler and Prior: colony twenty-four hours old, as seen upon a gelatin plate; $\times 100$ (Fränkel and Pfeiffer).

begin liquefaction of the gelatin, and by the second day appear about the size of lentils, and are situated in little depressions. Under the microscope they are of a yellowish-brown color, are finely granular, and are surrounded by a zone of sharply circumscribed liquefied gelatin. Careful examination with a high power of the microscope shows a rapid movement of the granules of the colony.

In gelatin punctures the growth takes place rapidly along the whole puncture, forming a stocking-shaped liquefaction filled with cloudy fluid which does not pre-

precipitate rapidly; a rather smeary, whitish mycoderma is generally formed upon the surface. The much more extensive and more rapid liquefaction of the medium, the wider top to the funnel-shaped liquefaction at the surface,

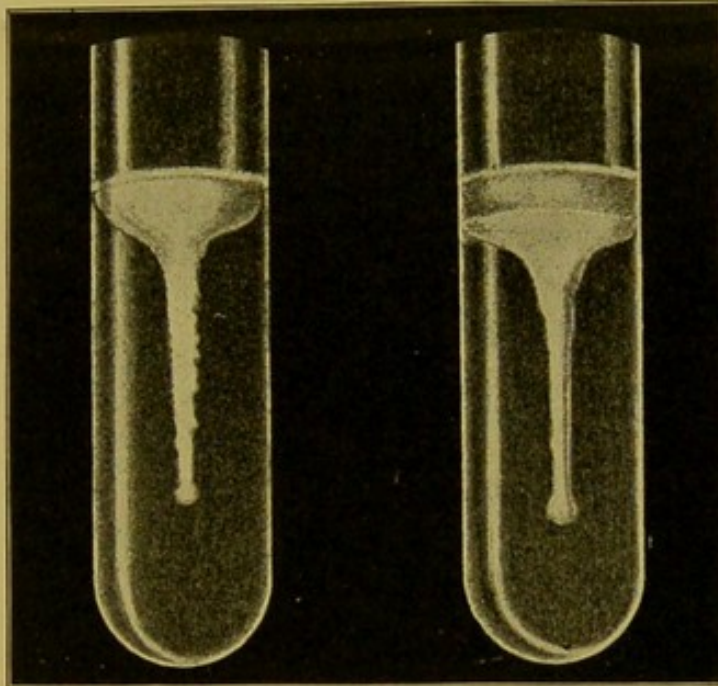


FIG. 83.—Spirillum of Finkler and Prior: gelatin puncture-cultures aged forty-eight and sixty hours (Shakespeare).

the absence of the air-bubble, and the clouded nature of the liquefied material, all serve to differentiate it from the cholera spirillum.

Upon agar-agar the growth is also very 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.

The cultures upon potato are also very different from those of cholera, for instead of a temperature of 37° C. being required for a rapid development, 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 is rapidly liquefied by the growth of the organism.

Buchner has shown that in media containing some glucose an acid reaction is produced.

The spirillum does not grow well, if at all, in milk, and speedily dies in water.

The organism does not produce indol.

The spirillum can be stained well by the ordinary dyes, and seems, like the cholera spirillum, to have a special affinity for the aqueous solution of fuchsin.

In connection with this bacillus the question of pathogenesis is a very important one. At first it was suspected that it was, if not the spirillum of cholera itself, a very closely allied organism. Later it was regarded as the cause of cholera nostras. At present its exact pathological significance is a question. 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 according the method of Koch, about 30 per cent. of the animals die, but the intestinal lesions produced are not the same as 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 very unlikely, from the collected evidence, that the Finkler and Prior spirillum is associated with pathogenesis in the human species. As Fränkel points out, it is probably a frequent and harmless inhabitant of the human intestine.

The Spirillum of Denecke.—Another organism with a distinct resemblance to the cholera spirillum is one described by Denecke as occurring in old cheese (Fig. 84). Its form is much the same as that of the spirillum of cholera, the shorter individuals being of equal diameter throughout. The spirals which are produced are longer than those of the Finkler and Prior spirillum, and are more tightly coiled than those of the cholera spirillum.

Like its related species, this micro-organism is actively motile. 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.

Upon gelatin plates the growth of the colonies is much more rapid than that of the cholera spirillum, but slower than that of the Finkler and Prior spirillum. The col-



FIG. 84.—Spirillum Denecke, from an agar-agar culture; $\times 1000$ (Itzerott and Niemann).

onies appear as small whitish, round points, which soon reach the surface of the gelatin and commence liquefaction. By the second day they are about the size of a pin's head, have a yellow color, and occupy the bottom of a conical depression. The appearance is much like that of a plate of cholera spirilla.

The microscope shows the colonies to be of irregular shape and coarsely granular. The color is yellow, and is pale at the edges, gradually becoming intense toward the centre. The colonies are surrounded at first by distinct lines of circumscription, later by clear zones, which, according to the illumination, are pale or dark. From this description it will be seen that the colonies differ from those of cholera in the prompt liquefaction of the gelatin, their rapid growth, yellow color, irregular form, and distinct lines of circumscription.

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 myco-

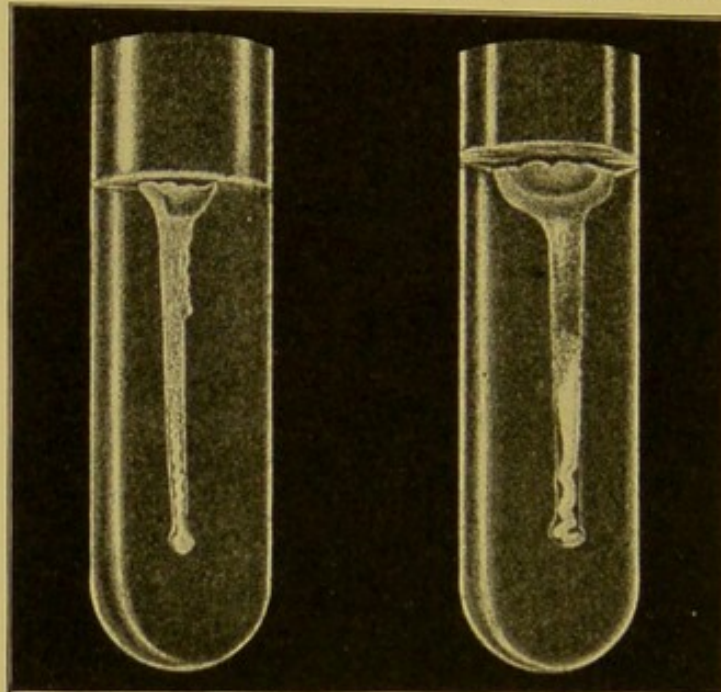


FIG. 85.—*Spirillum Denecke*: gelatin puncture-cultures aged forty-eight and sixty hours (Shakespeare).

derma forms. Liquefaction of the entire gelatin generally requires about two weeks.

Upon agar-agar this spirillum grows as a thin yellowish layer which does not seem inclined to spread widely.

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 long beautiful spirals.

The organism sometimes produces indol, but is irregular in its action in this respect.

The spirillum of Denecke is mentioned only because of its morphological relation to the cholera spirillum, not because of any pathogenesis which it possesses. It probably is not associated with any human disease. Experiments, however, have shown that when the spirilla are introduced into the intestines of guinea-pigs whose gastric contents are alkalinized and whose peristalsis is

paralyzed with opium, about 20 per cent. of the animals die from intestinal disease.

The Spirillum of Gamaléia (Spirillum Metchnikoff).—Very closely related to the cholera spirillum in its morphology and vegetation and possibly, as has been suggested, a descendant of the same original stock, is the spirillum which Gamaléia cultivated from the intestines of chickens affected with a disease similar to chicken-cholera. This spirillum is a curved organism, a trifle shorter and thicker than the cholera spirillum, a little more curved, and with similar rounded ends (Fig. 86).

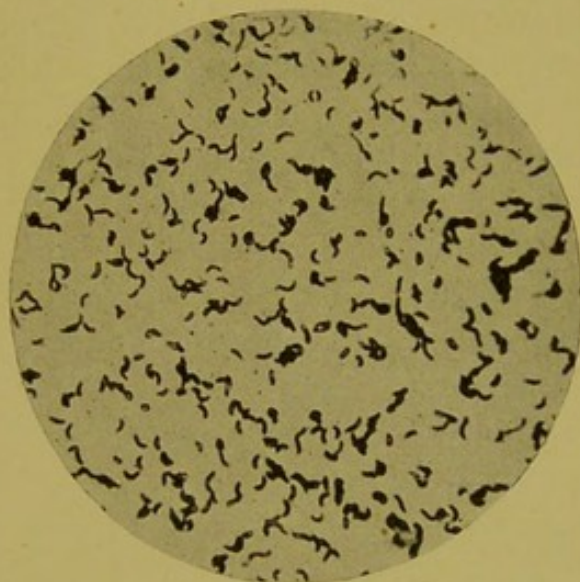


FIG. 86.—Spirillum Metchnikoff, from an agar-agar culture; $\times 1000$ (Itzerott and Niemann).

It forms long spirals in appropriate media, and is actively motile. Each spirillum is provided with a terminal flagellum. No spores have been positively demonstrated.

The organism, like the cholera vibrio, is very susceptible to the influence of acids, high temperatures, and drying, so that spores are probably not formed. It grows well both at the temperature of the room and at that of incubation.

The bacterium stains easily, the ends more deeply than the centre. It is not stained by Gram's method.

Upon gelatin plates a remarkable similarity to the

colonies of the cholera spirillum is developed, yet there is a difference, and Pfeiffer points out that "it is comparatively easy to differentiate between a plate of pure cholera spirillum and a plate of pure *Spirillum Metchnikoff*, 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 bacterium as a kind of intermediate species between the cholera and the Finkler-Prior spirilla.

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 areas of liquefaction resembling colonies of the Finkler-Prior spirilla occur. The liquefaction of the gelatin is quite rapid, the resulting fluid being turbid. Generally there will be upon a plate of *Vibrio Metchnikoff* some colonies which closely resemble cholera by occupying small conical depressions in the gelatin. Under a high power

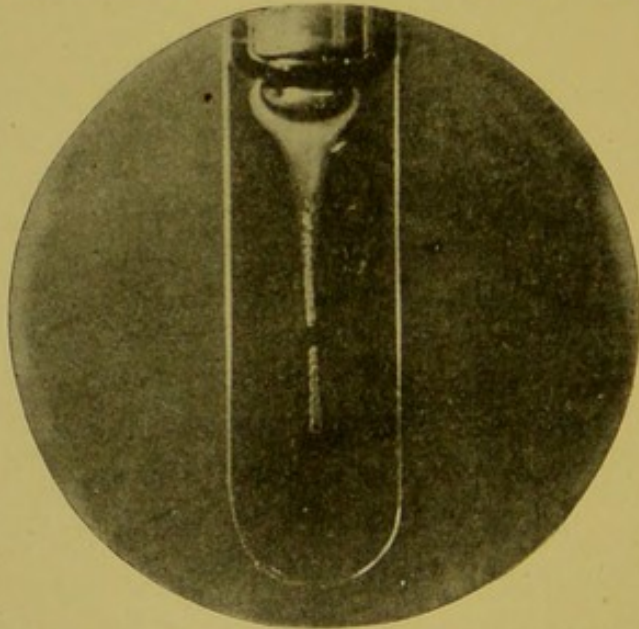


FIG. 87.—*Spirillum Metchnikoff*; puncture-culture in gelatin forty-eight hours old (Fränkel and Pfeiffer).

of the microscope the contents of the colonies, which appear to be of a brownish color, are observed to be in rapid

motion. The edges of the bacterial mass are fringed with radiating organisms (Fig. 87).

In gelatin tubes the culture is very much like that of cholera, but develops more slowly.

Upon the surface of agar-agar a yellowish-brown growth develops along the whole line of inoculation.

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.

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 mycoderma forms upon the surface.

The addition of sulphuric acid to a culture grown in a medium rich in peptone produces the same rose color observed in cholera cultivations.

The organism is pathogenic for animals, but not for man. Pfeiffer has shown that chickens, pigeons, and guinea-pigs are highly susceptible animals. The birds when inoculated under the skin generally die—pigeons always. When guinea-pigs are treated according to the method of Koch for the inoculation of cholera, the temperature of the animal rises for a short time, then abruptly falls to 33° C. or less. Death follows in 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.

In the blood and all the organs of pigeons and young chickens the organisms can be found in such large numbers that Pfeiffer has suggested the term "vibrionensep-

ticæmie" for the condition. In the intestines very few alterations are noticeable, and very few spirilla can be found.

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.

Spirillum Berolinensis.—This organism (Fig. 88),

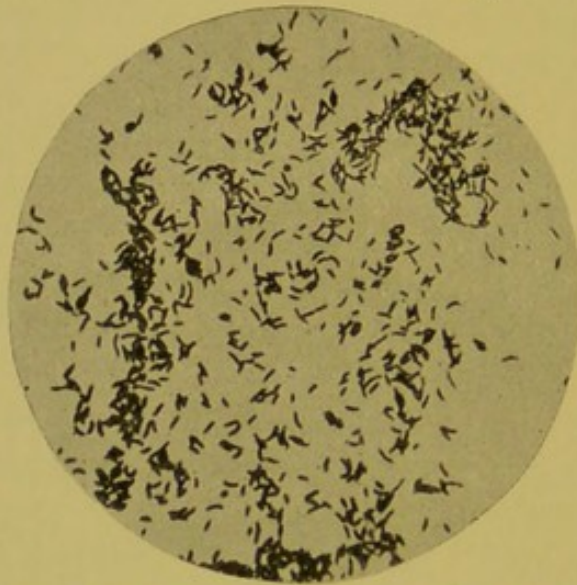


FIG. 88.—*Spirillum Berolinensis*, from an agar-agar culture; $\times 1000$ (Itzerott and Niemann).

which was discovered by Neisser in the summer of 1893, is of great interest in comparison with the spirillum of cholera and its related forms. Its morphology is in every particular exactly like that of the cholera spirillum, but its growth is a little more rapid. It grows upon the same culture-media and at the same temperature. The colonies are, however, quite different.

Upon the second day, when grown upon gelatin plates, the colonies of the *Spirillum Berolinensis* appear finely granular and paler than those of cholera. The borders are generally smooth and circular. As it becomes older the colony takes on a slightly brownish color, and may be nodulated or radiately lobulated. The gelatin is very slowly liquefied.

In puncture-cultures the development takes place along the entire puncture, and causes a gradual liquefaction of the gelatin.

Upon agar-agar the growth is generally similar to that of the cholera spirillum, but at times is copious, dry, and ragged, and suggests leather by its appearance.

When introduced intraperitoneally into guinea-pigs the animals die in from one to two days.

The indol reaction is exactly like that given by cultures of the cholera spirillum. The spirillum does not stain by Gram's method.

Spirillum Dunbar.—This organism (Fig. 89) was de-



FIG. 89.—*Spirillum Dunbar*, from agar-agar; $\times 1000$ (Itzerott and Niemann).

scribed in 1893 by Dunbar and Oergel, who secured it from the water of the Elbe River. It much resembles the cholera spirillum, but it never exhibits sigmoid forms. It stains poorly, the ends taking the color much better than the central portion.

Gelatin is liquefied by the growth of this organism more quickly than by the cholera spirillum. The colonies upon gelatin and the puncture-cultures in gelatin are identical with those of the cholera spirillum.

On agar-agar a luxuriant whitish-yellow layer is produced.

In bouillon and peptone solution the addition of dilute sulphuric acid produces the red color of nitro-indol.

It is said that cultures grown at a temperature of 22° C. phosphoresce in the dark.

The spirillum seems to be pathogenic for guinea-pigs when introduced into the stomach according to Koch's method for cholera.

Spirillum Danubicus.—This organism (Fig. 90) also



FIG. 90.—*Spirillum Danubicus*, from an agar-agar culture; $\times 1000$ (Itzerott and Niemann).

much resembles cholera. It was first isolated by Heider in 1892. In appearance it is rather delicate and decidedly curved. It is often united in sigmoid and semicircular forms, and exhibits long spirals in old cultures. It is actively motile, each organism presenting a terminal flagellum.

The growth upon gelatin plates is rapid. Small light-gray colonies, resembling those of cholera, but exhibiting a dentate margin, are observed. The growth in gelatin punctures also much resembles cholera, and the agar-agar growth can scarcely be distinguished from it.

The potato growth has a distinct yellowish-brown color.

Milk is coagulated in three or four days.

This spirillum does not produce indol.

Heider found the spirillum pathogenic for guinea-pigs.

Spirillum I. of Wernicke.—This organism is about twice as large as the cholera spirillum, liquefies gelatin more rapidly, produces indol, and is feebly pathogenic for guinea-pigs.

Spirillum II. of Wernicke.—This spirillum is smaller than the cholera spirillum, liquefies gelatin more slowly, produces indol, and is highly pathogenic for rabbits, guinea-pigs, pigeons, and mice.

Spirillum Bonhoffi.—This organism (Fig. 91) was found in water by Bonhoff. It has a decided resem-

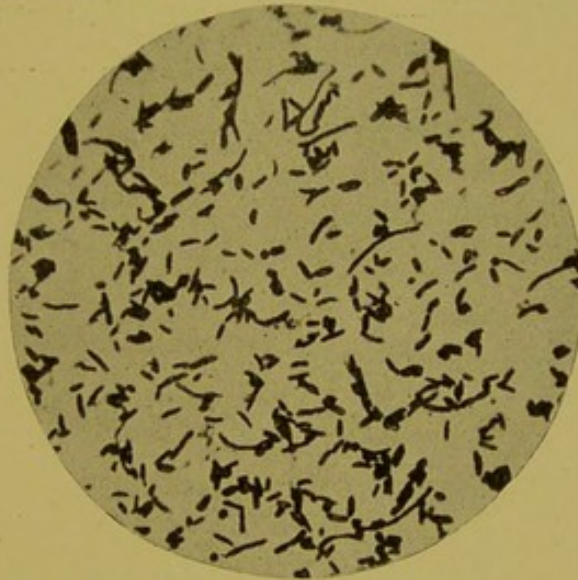


FIG. 91.—Spirillum Bonhoffi, from a culture upon agar-agar; $\times 1000$ (Itzerott and Niemann).

blance to the cholera spirillum, but is rather stouter and less curved. Curved forms—*i. e.* semicircles, sigmoids, and spirals—occur in old cultures especially.

These organisms are colored badly with ordinary stains, dahlia seeming to be the most appropriate color, and accomplishing the process better if warmed. The organism is motile, and has a long flagellum attached to one end.

The colonies develop slowly upon gelatin plates, first appearing in forty-eight hours as little grayish points.

The margin of the colony is sharply circumscribed; the interior is broken up. The gelatin is *not* liquefied. In gelatin punctures there is no liquefaction observable.

Upon agar-agar the development at the temperature of the incubator, which is more rapid than that at the temperature of the room, results in the production of a bluish-gray layer.

The growth upon potato has a brownish color. The growth in bouillon and in peptone solutions is accompanied by the production of indol.

The spirillum is pathogenic for mice, guinea-pigs, and canary birds.

Spirillum Weibeli.—This spirillum (Fig. 92) was found in 1892 by Weibel in spring-water which had a long time

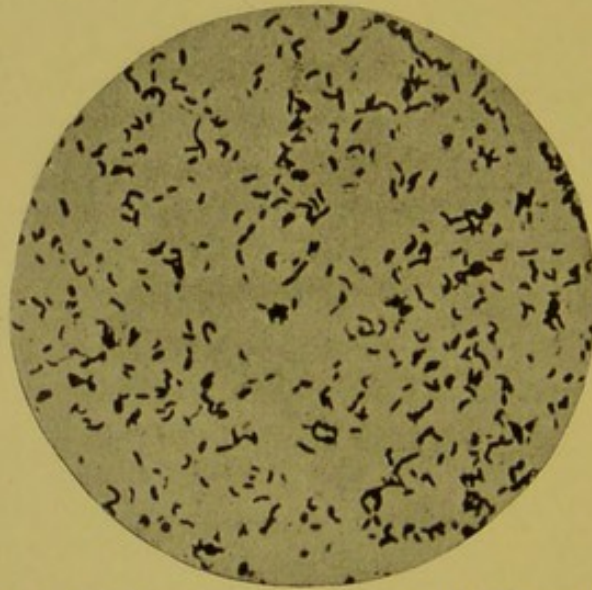


FIG. 92.—*Spirillum Weibeli*, from agar-agar; $\times 1000$ (Itzerott and Niemann).

before been infected by cholera. It is short, rather thick, and distinctly bent, often forming S-shaped figures.

The colonies before liquefaction sets in are described as pale-brown, transparent, circular, and homogeneous. Liquefaction is much more rapid than in cholera, and causes the borders of the colonies to become irregular. In the centre of each colony a little depression is observed.

In gelatin puncture-cultures the growth is rapid, be-

ginning first upon the surface, where a large flat, saucer-shaped liquefaction, extending to the sides of the tube, forms. Scarcely any growth takes place in the puncture, but the superficial liquefaction, separated by a horizontal line from the normal gelatin, descends slowly.

Upon agar-agar a grayish-white layer is formed.

No growth has been obtained upon potato.

In alkaline peptone solution a slow but luxuriant growth takes place.

Spirillum Milleri.—This spirillum (Fig. 93) was found in the mouth by Miller in 1885. It resembles the cholera

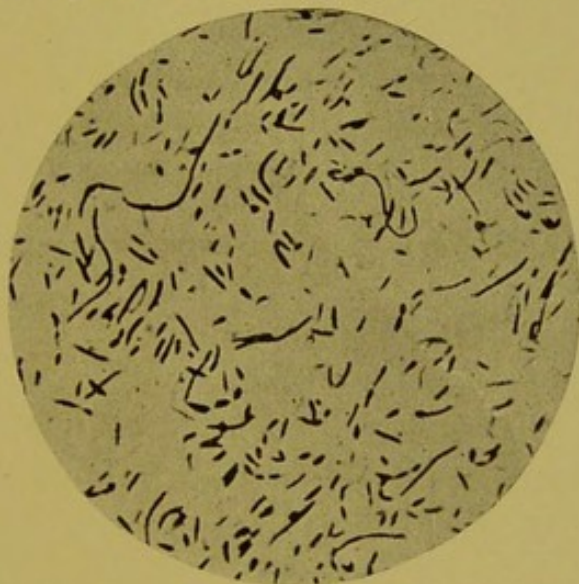


FIG. 93.—*Spirillum Milleri*, from an agar-agar culture; $\times 1000$ (Itzerott and Niemann).

spirillum somewhat, but is much more like the spirillum of Finkler and Prior, with which many bacteriologists think it identical.

Upon gelatin the colonies are small, finely granular, have a narrow border-zone and a pale-brown color. The gelatin is rapidly liquefied.

Upon agar-agar a thick yellowish layer is produced.

The organism seems not to be pathogenic.

Spirillum Aquatilis.—Günther in 1892 found this organism (Fig. 94) in the water of the river Spree. It is similar to the cholera spirillum in shape, has a long terminal flagellum, and is motile.

The colonies which form upon gelatin are circular, have smooth borders, and look very much as if bored out with a tool. They have a brown color and are finely

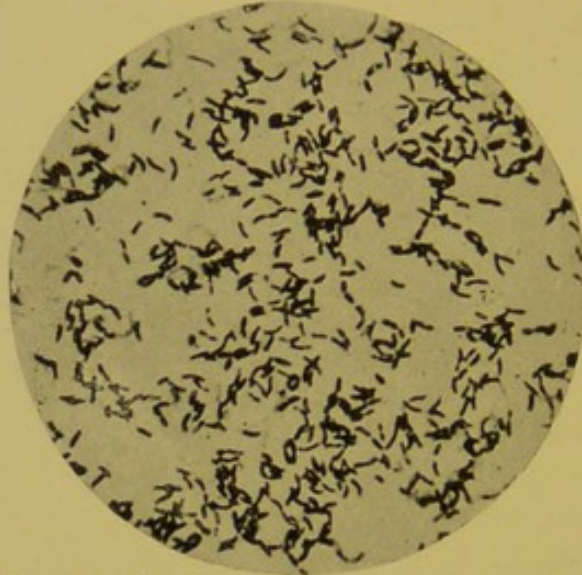


FIG. 94.—*Spirillum aquatilis*, from an agar-agar culture; $\times 1000$ (Itzerott and Niemann).

granular. In gelatin puncture-cultures the growth occurs almost exclusively at the surface.

The agar-agar cultures are similar to those of cholera.

Scarcely any development occurs in bouillon. By the growth of the organism sulphuretted hydrogen gas is produced.

The spirillum does not grow at all upon potato.

Günther did not find the organism to be pathogenic.

Spirillum Terrigenus.—This species, also discovered by Günther, was secured from earth. It generally occurs in a slightly curved form, but sometimes is spiral. It is actively motile and has a terminal flagellum.

The colonies, which appear in twenty-four hours, are small, structureless, and transparent, and later take on a "fat-drop" appearance.

Upon agar-agar a thin white coating is formed. Milk is coagulated by the growth of the organism. No indol is produced.

The organism does not stain by Gram's method, and is said not to be pathogenic for guinea-pigs or for mice.

CHAPTER VIII.

PNEUMONIA.

THE term "pneumonia," while generally understood to refer to the lobar disease particularly designated as croupous pneumonia, is a vague one, really comprehending a variety of inflammatory conditions of the lung quite dissimilar in character. This being true, no one will be surprised to find that a single organism cannot be described as "specific" for them all. Indeed, pneumonia must be considered as a group of diseases, and the various microbes found associated with it must be described successively in connection with the peculiar phase of the disease in which they occur.

I. **Lobar or Croupous Pneumonia.**—The bacterium, which can be demonstrated in at least 75 per cent. of the cases of lobar pneumonia, which is now almost universally accepted as the cause of the disease, and about whose specificity very few doubts can be raised, is the *pneumococcus* of Fränkel and Weichselbaum.

Priority of discovery in the case of the pneumococcus seems to be in favor of Sternberg, who as early as 1880 described an identical organism which he secured from his saliva. Curiously enough, Pasteur seems to have captured the same organism, also from saliva, in the same year. The researches of the observers whose names are attached to the organism were not completed until five years later. It is to Fränkel, Telamon, and particularly to Weichselbaum, however, that we are indebted for the discovery of the relation which the organism bears to pneumonia.

The pneumococcus should rather be called the *pneumobacillus*, for it habitually has an elongated form, and in its most typical form is so distinctly elongate as to be

described as lanceolate. However, popular parlance has now made it almost impossible to introduce *Bacillus pneumoniæ* instead of *Diplococcus pneumoniæ* (Weichselbaum), especially as there is already another organism bearing that name. (See *Bacillus pneumoniæ* of Friedländer.)

The organism (Fig. 95) is variable in its morphology. When grown in bouillon it is oval, has a pronounced dis-

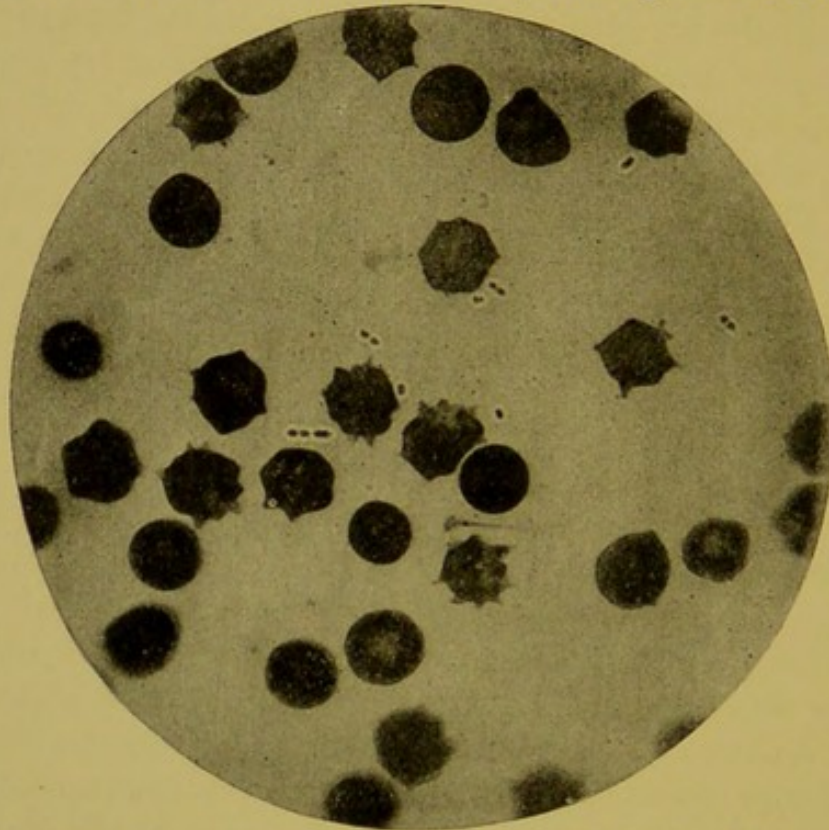


FIG. 95.—*Diplococcus pneumoniæ*, from the heart's blood of a rabbit; $\times 1000$ (Fränkel and Pfeiffer).

position 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 containing them the organisms are arranged in pairs, exhibit a distinct lanceolate shape, the pointed ends generally 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 ordinarily in culture-media, and especially upon solid media, the capsules are absent.

The organism is without motility, has no spores, and does not seem to be able to resist any unfavorable conditions when grown artificially. 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 method. The capsule does not stain.

The bacillus is no stranger to us, but can frequently be found in the saliva of healthy individuals, and the inoculation of human saliva into rabbits generally causes a septicemia in which the bacillus is found abundantly in the blood and tissues. Because of its constant presence in the saliva it was described by Flügge as the *Bacillus septicus sputigenus*.

When desired for purposes of study, it can be obtained by inoculating rabbits with saliva and recovering the organisms from their blood, or it can be secured from the rusty sputum of pneumonia by the method employed by Kitasato for securing tubercle bacilli from sputum. A single mouthful of fresh sputum is secured, washed in several changes of sterile water to free it from bacteria of the mouth and pharynx, carefully separated, and a central portion transferred to an appropriate culture-medium.

The organism grows upon all the culture-media except potato, but only between the temperature extremes of 24° and 42° C.; the best development is at 37° C. The growth is always limited, probably because the formic acid produced serves to check it. The addition of an unusual amount of alkali to the culture-medium favors the growth.

The organisms readily lose their virulence in culture-media, and cease to be pathogenic after a few days. Not only is this true, but they seem to be unable to accommodate themselves to a purely saprophytic life, and unless continually transplanted to new media die in a week or two, sometimes sooner.

The colonies which develop at 24° C. upon 15 per cent. gelatin plates are described as small, round, circumscribed, finely granular white points which grow slowly, never attain any considerable size, and do not liquefy the gelatin (Fig. 96).

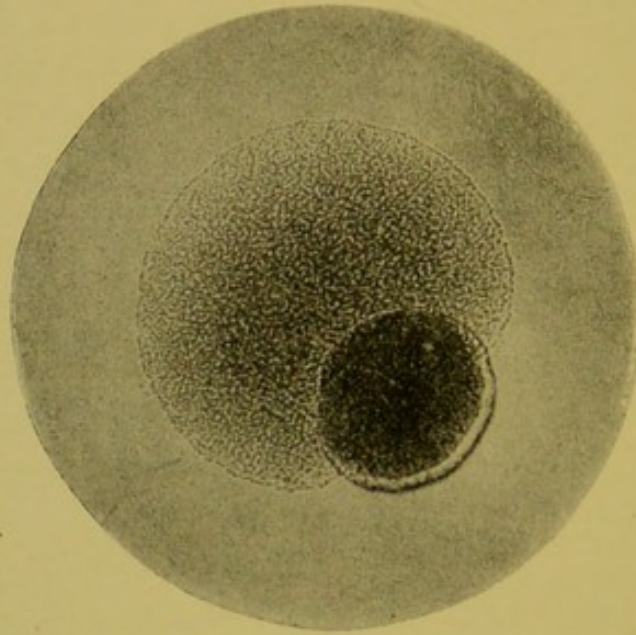


FIG. 96.—*Diplococcus pneumoniae*: colony twenty-four hours old upon gelatin; $\times 100$ (Fränkel and Pfeiffer).

If, instead of gelatin, agar-agar be used and the plates kept at the temperature of the body, the colonies which develop upon the plates appear as transparent, delicate, drop-like accumulations, scarcely visible to the naked eye, but under the microscope distinctly granular, the central darker portion being frequently surrounded by a paler marginal zone.

In gelatin puncture-cultures, made with 15 instead of the usual 10 per cent. of gelatin, the growth takes place along the entire path of the wire in the form of little whitish granules distinctly separated from each other. The growth in gelatin is always very limited.

Upon agar-agar and blood-serum the growth consists of minute, transparent, semi-confluent, colorless, dew-drop-like colonies, which die before attaining a size

which permits of their being seen without careful inspection.

In bouillon the organisms grow well, clouding the medium very slightly.

Milk is quite well adapted as a culture-medium, its casein being coagulated.

No growth can be secured upon potato at any temperature or by any manipulation yet known.

When it is desired to maintain the virulence of a culture, it must be very frequently passed through the body of a rabbit.

If a small quantity of a pure culture of the virulent organism is 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 generally by the introduction of saliva.

The post-mortem shows that an inflammatory change has taken place at the point of inoculation, with a fibrinous exudate resembling somewhat that in diphtheria. At times, and especially in dogs, there may be a little pus formed. The other appearances are those of a general disturbance. The spleen is much enlarged, is firm and red brown. The blood in all the organs contains large numbers of the bacteria, most of which exhibit a distinct lanceolate form and have their capsules very distinct. The disease is a pure septicemia unassociated with pronounced tissue-changes.

In cases of the kind described the lungs show no pneumonic changes. Likewise, if the hypodermic needle used for injection be plunged through the breast-wall into the pulmonary tissue, no pneumonia results. Monti, however, claims to have found that a true characteristic pneumonia results from the injection of cultures into the trachea of susceptible animals. This observation lacks confirmation.

Not all animals are susceptible. Guinea-pigs, mice,

and rabbits are highly sensitive to the operations of the organism; dogs are comparatively immune.

From this brief review of the peculiarities of the pneumococcus it must be obvious that its reputation in pneumonia depends more upon the regularity with which it is found in that disease than upon its capacity to produce a similar affection in the lower animals.

As in numerous other diseases, we are unable to furnish an absolute proof of specificity according to the postulates of Koch.

The disease is peculiar in that recovery from it is followed either by no immunity or by one of such brief duration as to allow of frequent relapses; and it is well known that many cases show a subsequent predisposition to fresh attacks of the disease. This brevity of immunity lessens the probability that in the future we shall discover an antitoxin that shall be powerful in its influence upon the course and termination of the disease.

The experiments of the Klemperers showed that the serum of immunized rabbits protected other animals, and excited our interest a few years ago; they, however, failed when the principle was applied in human medicine, and the treatment of pneumonia by the injection of blood-serum from convalescents has been given up as useless and dangerous.

The pneumococcus is pathogenic in other ways than by the production of croupous pneumonia; thus, Foa, Bordoni-Uffreduzzi, and others have found it in cerebrospinal meningitis; Fränkel, in pleuritis; Weichselbaum, in peritonitis; Banti, in pericarditis; numerous observers have found it in acute abscesses; Gabbi has isolated it from a case of suppurative tonsillitis; and Zaufal, Levy, and Schröder and Netter have been able to demonstrate its presence in the pus of otitis media. It has also been reported as occurring in the joints in arthritis following pneumonia.

The pneumococcus no doubt is habitually present in the mouth of almost every healthy person. Its entrance

into the lung is therefore only a matter of accident, and an unusually long sigh, a deep inspiration before a cough or sneeze, or some unusual respiratory movement, serves to draw it into the bronchioles, which are normally free from bacteria.

In the opinion of most authorities, something more than the simple entrance of the bacterium into the lung is required for the production of the disease, but what that something is, is still a matter of doubt. It would seem to be some systemic depravity, and in support of this view we may point out that pneumonia is very frequent, and almost universally fatal, among drunkards. Whether, however, any vital depression or systemic depravity will predispose to the disease, or whether it depends for its origin upon the presence of a certain leucomaïne, time and further study will be required to tell.

Bacillus Pneumoniæ of Friedländer (Fig. 97).—An un-



FIG. 97.—*Bacillus pneumoniae* of Friedländer, from the expectoration of a pneumonia patient; $\times 1000$ (Fränkel and Pfeiffer).

fortunate accident has applied the name "pneumococcus" to an organism very different from the one just described. It was discovered by Friedländer in 1883 in the exudate from the lung in croupous pneumonia, and, being thought

by its discoverer to be the cause of the disease, very naturally was called the pneumococcus, or, more correctly, the *pneumobacillus*. The grounds upon which the pathogeny of the organism was supposed to depend were very insufficient, and the bacillus of Friedländer—or, as Flügge prefers to call it, the *Bacillus pneumoniae*—has ceased to be regarded as specific, and is now looked upon as an accidental organism whose presence in the lung is, in most cases, unimportant.

As the two organisms are similar in more respects than their names, Friedländer's bacillus requires at least a brief description.

It is distinctly a bacillus, but sometimes, when occurring in pairs, has a close resemblance to the pneumococcus of Fränkel and Weichselbaum. Very frequently it forms chains of four or more elements. It is also commonly surrounded by a transparent capsule. It 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.

Fränkel points out that Friedländer's error in supposing this bacillus to be the chief parasite in pneumonia depended upon the fact that his studies were made by the plate method. If some of the pneumonic exudate be mixed with gelatin and poured upon plates, the bacilli grow into colonies at the end of twenty-four hours, and appear as small white spheres which spread upon the gelatin to form white masses of a considerable size. Under the microscope these colonies are rather irregular in outline and somewhat granular.

The bacillus grows at as low a temperature as 16° C., and, according to Sternberg, has a thermal death-point of 56° C.

When a colony is transferred to a gelatin puncture-culture, quite a massive growth occurs. Upon the surface a somewhat elevated, rounded white mass is formed, and in the track of the wire innumerable little colonies spring up and become confluent, so that a "nail-growth"

results. No liquefaction occurs. When old the cultures sometimes become brown in color.

Upon the surface of agar-agar at ordinary temperatures quite a luxuriant white or brownish-yellow, smeary, circumscribed growth occurs. The growth upon blood-serum is the same.

Upon potato the growth is abundant, quickly covering the entire surface with a thick yellowish-white layer, which sometimes contains bubbles of gas. Gas is also sometimes developed in gelatin cultures.

A most superficial comparison will suffice to show the great difference in vegetation between these two so-called pneumococci.

Friedländer had considerable difficulty in causing any pathogenic changes by the injection of his bacillus into animals. Rabbits and guinea-pigs were immune, and the only actual pathogenic results which Friedländer obtained were in mice, into whose lungs and pleura he injected the cultures. The remarks of Fränkel upon such mouse-operations, which do not add much weight to experiments, have already been quoted.

In the *status præsens* of bacteriologic knowledge the bacillus of Friedländer is regarded as an organism of very feeble pathogenic powers, generally a harmless saprophyte, but which may at times aid in producing inflammatory changes when in the tissues of the human body.

2. **Catarrhal Pneumonia.**—This form of pulmonary inflammation occurs in local areas, generally situated about the distribution of a bronchiole. It cannot be said to have a specific micro-organism, as almost any irritant foreign materials accidentally inhaled can cause it. The majority of the cases, however—and especially those which are distinctly peribronchial—are caused by the presence of the staphylococcus and streptococcus of suppuration. Friedländer's bacillus may also aid in producing local inflammations.

3. **Tubercular Pneumonia.**—At times the process of pulmonary tuberculosis is so rapid, and associated with

the production of so much semi-liquid, semi-necrotic material, that the auto-infection of the lung is greatly favored; the tubercle bacilli are distributed to the entire lung or to large parts of it, and a distinct inflammation occurs. Such a pneumonia may be caused by the tubercle bacillus alone, but more often it is aided by accompanying staphylococci, streptococci, tetragenococci, pneumococci, pneumobacilli, and other organisms apt to be present in a lung in which tuberculosis is in progress and ulceration and cavity-formation are advanced.

4. **Mixed Pneumonias.**—It frequently happens that pneumonia occurs in the course of, or shortly after the convalescence from, influenza. In these cases a mixed infection is present, and there is no difficulty in determining that both the influenza bacillus and the pneumococcus are present. Again, sometimes the pneumococci and staphylococci operate simultaneously, and produce a purulent pneumonia with abscesses as the conspicuous feature. As almost any combination of the described bacteria is possible in the lungs, and as these combinations will all produce varying inflammatory conditions, it must be left for the student to imagine what the particular characters of each may be.

Among these mixed pneumonias may be mentioned those called by Klemperer and Levy "complicating pneumonias," occurring in the course of typhoid, etc.

C. THE SEPTIC DISEASES.

CHAPTER I.

RELAPSING FEVER.

As long ago as 1873, Obermeier discovered that a flexible spiral organism, about 0.1μ in diameter and from $20-40 \mu$ in length, could be observed in the blood of patients suffering from relapsing fever.

Although many of the best bacteriologists of our day have occupied themselves with the study of this spirillum, we really have, at present, very little more knowledge than that given us by Obermeier.

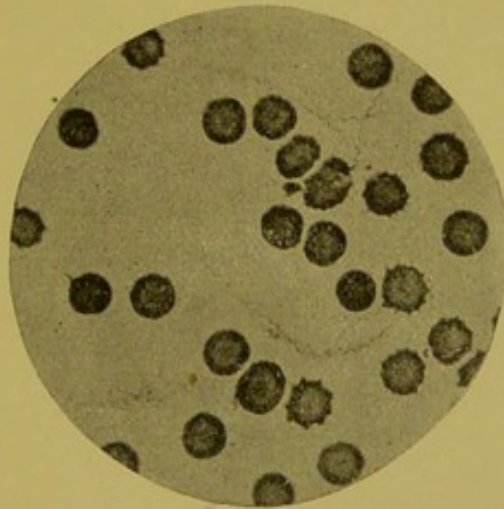


FIG. 98.—*Spirochæta febris recurrentis*; $\times 650$ (Heim).

The spirilla (Fig. 98) are generally very numerous, are long, slender, and flexible (*spirochæta*), and possess a vigorous movement by flagella. The ends are rather pointed.

The spirillum stains well by ordinary methods, but

not by Gram's method. It seems to be a strict parasite, and has never been cultivated artificially.

Of the pathogenesis of the organism there can be no doubt, as it is invariably present in relapsing fever and undergoes a peculiar cycle of changes according to the stage of the disease. During the pyrexia the organisms are found in the blood in active movement, swimming both by rotation on the long axis and by undulation. As soon as the crisis comes on they are found to be without motion, most of them enclosed in leucocytes and seemingly dead. The recurrence of the paroxysm has suggested to many that spores are formed in the spirillum, but no one has been successful in proving that this is the case. Koch, Carter, and Soudakewitch have all succeeded in giving the disease to monkeys, and Münch and Moczutkowsky have gone further and have produced it in men by introducing into them blood from diseased patients.

Soudakewitch finds that the removal of the spleen causes the disease to terminate fatally in monkeys.

CHAPTER II.

INFLUENZA.

NOTWITHSTANDING a large number of bacteriologic examinations conducted for the purpose of determining the cause of influenza, it was not until 1892, after the great epidemic, that there was found simultaneously by Canon and Pfeiffer a bacterium which conformed, at least in large part, to the requirements of specificity.

The observers mentioned found the same organism—one in the blood of influenza patients, the other in the purulent bronchial discharges.

The specific organisms (Fig. 99) are bacilli, very small in size, having about the same diameter as the bacillus.

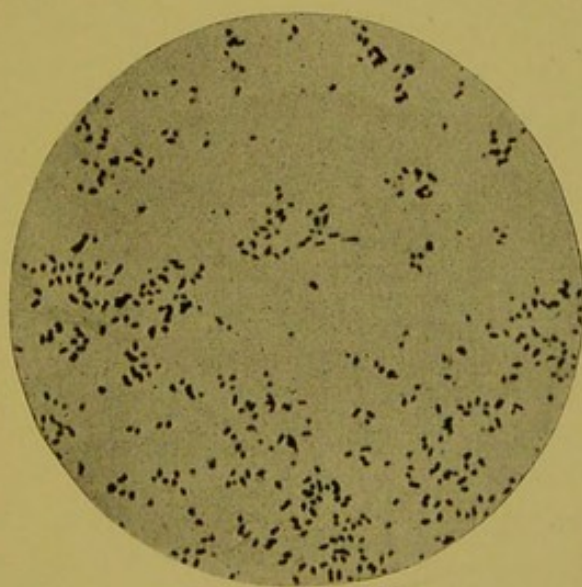


FIG. 99.—*Bacillus influenzae*, from a gelatin culture; $\times 1000$ (Itzerott and Niemann).

of mouse-septicemia, but only about half as long (0.2 by 0.5μ). They are usually solitary, but may be united in chains of three or four elements. They stain rather

poorly, except with such concentrated penetrating stains as carbol-fuchsin and Löffler's alkaline methylene blue, and even with these the bacilli stain more deeply at the ends than in the middle, so that they appear not a little like diplococci.

For the demonstration of the bacilli in the blood Canon recommends a rather complicated method. 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 stain which seems best 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 this solution, and kept in the incubator for three to six hours, after which they are washed in water, dried, and then mounted in Canada balsam. By this method the erythrocytes are stained red, the leucocytes blue, and the bacillus, which is also blue, appears as a short rod or often as a dumb-bell.

Sometimes large numbers of the bacilli are present ; sometimes very few can be found after prolonged search. They are often enclosed within the leucocytes. It really is not necessary to pursue so tedious a staining method for demonstrating the bacilli, for they stain quite well by ordinary methods. They do not stain by Gram's method.

The bacillus is non-motile, and, so far as is known, does not form spores. 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.

The bacillus does not grow in gelatin or upon ordinary agar-agar. Upon glycerin agar-agar, after twenty-four hours in the incubator, minute colorless, transparent,

drop-like cultures may be seen along the line of inoculation. They do not look unlike condensed moisture, and Kitasato makes a special point of the fact that the colonies never become confluent. The colonies may at times be so small as to require a lens for their discovery.

In bouillon a scant development occurs, small whitish particles appearing upon the surface, subsequently sinking to the bottom and causing a "woolly" deposit there. While the growth is so delicate in these ordinary media, the bacillus grows quite well upon culture-media containing hemoglobin or blood, and can be transferred from culture to culture many times before it loses its vitality.

It cannot be positively proven that this bacillus is the cause of influenza, but from the fact that the bacillus can be found only in cases of influenza, that its presence corresponds with the course of the disease in that it is present as long as the purulent secretions last, and then disappears, and that Pfeiffer was able to demonstrate its presence in all cases of uncomplicated influenza, his conclusion that the bacillus is specific is certainly justifiable.

CHAPTER III.

MALIGNANT EDEMA.

THE chief contaminating organism in the preparation of pure cultures of the tetanus bacillus is a large slender bacillus almost as large as that of anthrax, but with rounded ends and an individual motility accomplished by means of flagella attached to its ends and sides (Fig. 100). It is a strictly anaërobic bacterium, and was

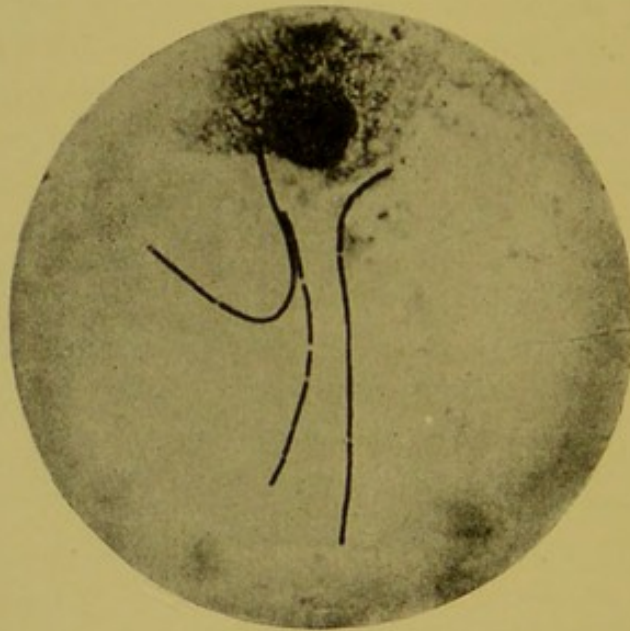


FIG. 100.—Bacillus of malignant edema, from the body-juice of a guinea-pig inoculated with garden-earth; $\times 1000$ (Fränkel and Pfeiffer).

originally described by Pasteur (1875) as the *Vibrion septique*. It grows well at the room-temperature, as well as at the temperature of the incubator, produces oval central spores, and, because of its association with a specific edema in certain animals, is known as the *Bacillus œdema maligni*.

The organism is widely distributed in nature, being almost always present in garden-earth. It is also found in various dusts, in the waste water from houses, and sometimes in the intestinal canals of animals.

When introduced beneath the skin this bacillus proves 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 the disease, because the oxygen which is, of course, abundant there is detrimental to its growth. When an experimental inoculation is performed, a small subcutaneous pocket should be made, and the bacilli introduced into it in such a manner as not to be in contact with the air.

If the inoculated animal be a mouse, guinea-pig, or rabbit, in about forty-eight hours it sickens and dies. The autopsy shows a general subcutaneous edema containing immense numbers of the bacilli. In the blood the bacilli are few or cannot be found, because of the oxygen which it contains. The great majority of them occupy the subcutaneous tissue, where very little oxygen is present and the conditions of growth are therefore good. If the animal is allowed 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 two cases of malignant edema in man. Both cases were typhoid-fever patients injected with musk, and developed the edema in consequence of impurity of the therapeutic agent. No case is reported, however, in which healthy men have been infected with the disease.

Cornevin declares that the passage of the bacillus through the white rat diminishes its virulence, and that the animals of various species that recover from this milder affection are subsequently immune to the virulent organisms.

The bacillus of malignant edema stains well with ordinary cold aqueous solutions of the anilin dyes, but not by Gram's method.

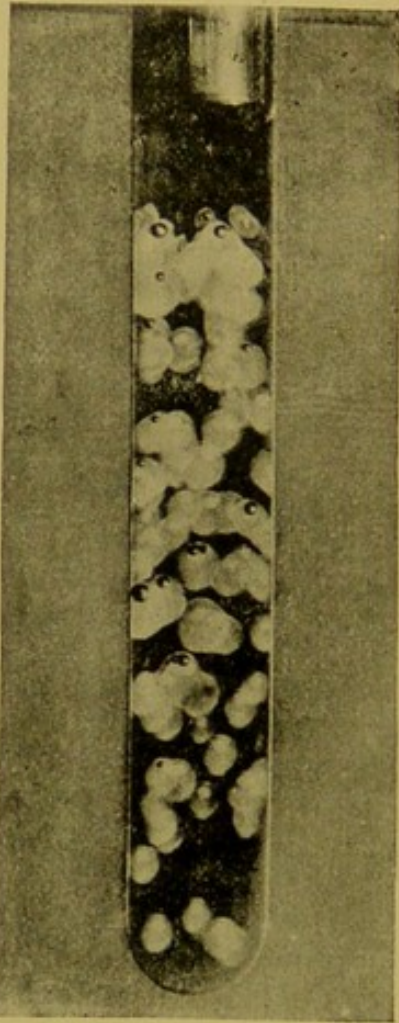


FIG. 101.—Bacillus of malignant edema growing in glucose gelatin (Fränkel and Pfeiffer).

The organism is not a difficult one to secure in pure culture, as has been said, generally contaminating tetanus cultures and being much more easy to secure by itself than its congener. It is most easily obtained from the edematous tissues of guinea-pigs and rabbits inoculated with garden-earth.

The colonies which develop upon the surface of gelatin kept free of oxygen appear to the naked eye as small shining bodies with liquid grayish-white contents. They gradually increase in circumference, but do not change their appearance. Under the microscope they appear filled with a tangled mass of long filaments which under a high power exhibit individual movement. The edges of the

colony have a fringed appearance, much like the hay or potato bacillus.

In gelatin tube-cultures the characteristic growth cannot be observed in a puncture, because of the air which remains in the path of the wire. The best preparation is made by heating the gelatin to expel the air it may contain, inoculating while still liquid, then replacing the air by hydrogen, and sealing the tube. In such a tube the bacilli develop near the bottom. The appearance of the growth is highly typical, as globular circumscribed areas of cloudy liquefaction result (Fig. 101), and may con-

tain a small amount of gas. In gelatin to which a little grape-sugar has been added the gas-production is marked. The gas is partly inflammable, partly CO₂. A distinct odor accompanies the gas-production, and is especially noticeable in agar-agar cultures.

CHAPTER IV.

MEASLES.

IN 1892, Canon and Pielicke, after the investigation of fourteen cases of measles, reported the discovery of a specific bacillus in the blood in that disease.

The organism is quite variable in size, sometimes being quite small and resembling a diplococcus, sometimes larger, and occasionally quite long, so that one bacillus may be as long as the diameter of a red blood-corpusele.

The discovery was made by means of a peculiar method of staining, as follows: 'The blood is spread in a very thin, even layer upon perfectly clean cover-glasses, and fixed by five to ten minutes' immersion in absolute alcohol. These glasses are then placed in a stain consisting of

Concentrated aqueous solution of methylene blue,	40;
0.25 per ct. solution of eosin in 70 per ct. alcohol,	20;
Distilled water,	40,

and stood in the incubator at 37° C. for from six to twenty-four hours. The bacilli do not all stain uniformly.

The discoverers of the bacillus claim to have made it grow several times in bouillon, but failed to induce a growth upon other media.

The bacilli do not stain by Gram's method; they seem to have motility; no spores were observed. They were found not only in the blood, but also in the secretions from the nose and eyes. They are said to persist throughout the whole course of the disease, even occasionally being found after the fever subsides.

Czajrowski asserts that the bacillus can be cultivated upon various albuminous media except gelatin and agar. On glycerin agar-agar, especially with the addition of hemotogen, and on blood-serum, they should grow in three or four days with an appearance like that of dew-drops. Under the microscope the colonies are structureless. Mice die of a septicemia after a subcutaneous inoculation.

CHAPTER V.

BUBONIC PLAGUE.

THE bacillus of bubonic plague (Fig. 102) seems to have met an independent discovery at the hands of

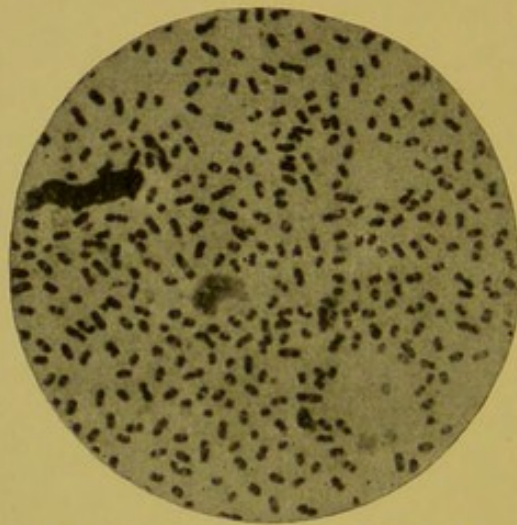


FIG. 102.—Bacillus of bubonic plague (Yersin).

Yersin and Kitasato in the summer of 1894, during the activity of the plague then raging at Hong-Kong. There seems to be not the slightest doubt that the micro-organisms described by the two observers are identical.

The bubonic plague is an extremely fatal infectious disease, whose ravages in the hospital in which Yersin made his observations carried off 95 per cent. of the cases. It affects both men and animals, and is characterized by sudden onset, high fever, prostration, delirium, and the occurrence of lymphatic swellings—buboes—affecting chiefly the inguinal glands, though not infrequently the axillary, and sometimes the cervical, glands. Death comes on in severe cases in forty-eight hours. If the case is of longer duration, the prognosis is said to be

better. Autopsy in fatal cases reveals the enlargement of the lymphatic glands, whose contents are soft and sometimes purulent.

The studies of Kitasato and Yersin showed that in blood drawn from the finger-tips and in the softened contents of the glands a small bacillus was demonstrable. The organisms are small, stain much more distinctly at the ends than in the middle, so that they resemble diplococci, and in fresh specimens seem to be surrounded by a capsule. Kitasato compares the organism to the well-known bacillus of chicken-cholera. It is feebly motile, and does not seem to form spores. Nothing is said about the presence of flagella.

When cultures are made from the softened contents of the buboes, the bacillus can be obtained almost or quite pure, and is found to develop upon artificial culture-media. In bouillon a diffuse cloudiness results from the growth, as observed by Kitasato, though in Yersin's observations the cultures more nearly resembled erysipelas cocci, and contained zoöglea attached to the sides and in the bottom of a tube of nearly clear fluid.

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.

Upon agar-agar—glycerin agar-agar is best—the bacilli grow freely, the colonies being whitish in color, with a bluish tint by reflected light. Under the microscope they appear moist, with rounded, uneven edges. The small colonies are said to resemble little tufts of glass-wool; the larger ones have large round centres. Microscopic examination of the bacilli grown upon agar-agar reveals the presence of long chains resembling streptococci.

Upon blood-serum the growth at the temperature of the incubator is luxuriant. It forms a moist layer of a yellowish-gray color, and is unaccompanied by liquefaction of the serum.

Upon potatoes no growth occurs at ordinary temperatures. When the potato is stood away for a few days in the incubator, a scanty, dry, whitish layer develops.

Kitasato found that mice, rats, guinea-pigs, and rabbits are all susceptible. When blood, lymphatic pulp, or pure cultures are inoculated into them, the animals become ill in from one to two days, according to size. Their eyes become watery, they begin to show disinclination to take food or to make any bodily effort, the temperature rises to 41.5° C., they remain quietly in a corner of the cage, and die with convulsive symptoms in from two to five days.

According to Yersin, an infiltration 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 exudation. The spleen and liver are enlarged, the former often presenting an appearance much like an eruption of miliary tubercles. Sometimes there is universal swelling of the lymphatic glands. Bacilli are found in the blood and in all the internal organs. Very often there are petechial eruptions during life, and upon the inner abdominal walls there are occasional hemorrhages. The intestine is hyperemic, the adrenals congested. There are often sero-sanguinolent effusions into the serous cavities.

Kitasato found that pigeons were not susceptible. Animals fed upon cultures or upon the meat of others dead of the disease became ill and died with typical symptoms. When he inoculated animals with the dust of dwelling-houses in which the disease had occurred, some died of tetanus, one from plague. Many rats and mice in which examination showed the characteristic bacilli died spontaneously in Hong-Kong.

Yersin showed that flies also die of the disease. Macerating and crushing a fly in bouillon, he not only succeeded in obtaining the bacillus from the medium, but infected an animal with it.

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.

The bacillus probably attenuates readily. Kitasato found that it did not seem able to withstand desiccation longer than four days; and Yersin found that although it could be secured from the soil beneath an infected house at a depth of 4-5 c.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.

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.

Kitasato's experiments have shown that it is possible to bring about immunity to the disease, though nothing definite in the way of experiment has as yet been recorded.

CHAPTER VI.

TETRAGENUS.

THERE can sometimes be found in the normal saliva, more commonly in tuberculous sputum, and still more commonly in the cavities of tuberculosis pulmonalis, a large micrococcus grouped in fours and known as the *Micrococcus tetragenus* (Fig. 103). It was discovered by

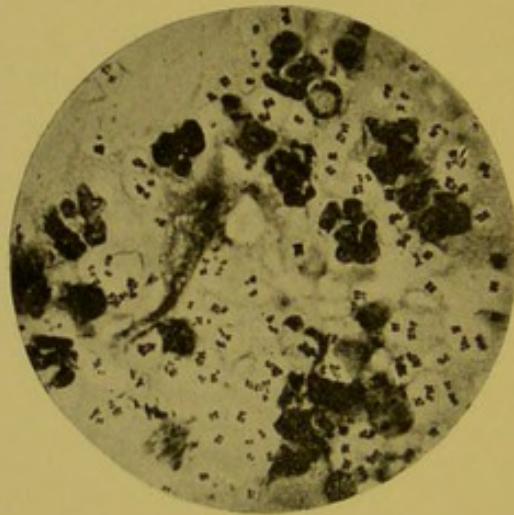


FIG. 103.—*Micrococcus tetragenus* in pus from a white mouse; $\times 615$ (Heim).

Gaffky, and subsequently carefully studied by Koch and Gaffky. It sometimes occurs in the pus of acute abscesses, and may be of importance in connection with the pulmonary abscesses which so often complicate tuberculosis.

The cocci are rather large, measuring about 1μ in diameter. In cultures they show no particular arrangement among themselves, but in the blood and tissues of animals they commonly appear arranged in groups of four surrounded by a transparent gelatinous capsule.

The organism stains well by ordinary methods, and

most beautifully by Gram's method, by which it can be best demonstrated in tissues.

Upon gelatin plates small white colonies are produced in from twenty-four to forty-eight hours. Under the microscope they are found to be spherical or elongate (lemon-shaped), finely granular, and lobulated like a raspberry or a mulberry. When superficial they form white, elevated, rather thick masses 1-2 mm. in diameter (Fig. 104).

In gelatin punctures a large white surface-growth

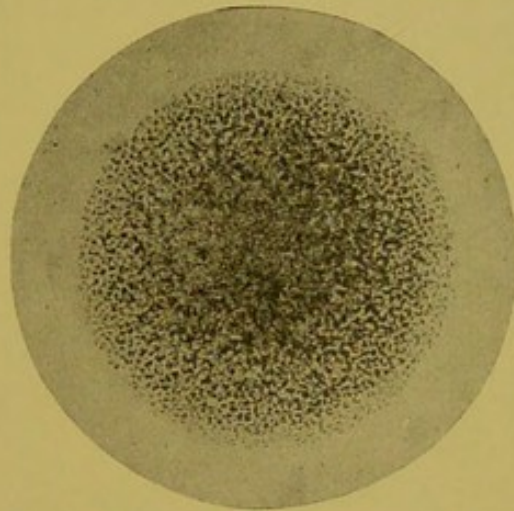


FIG. 104.—*Micrococcus tetragenus*: colony twenty-four hours old upon the surface of an agar-agar plate; $\times 100$ (Heim).

takes place, but very scant development occurs in the puncture, where the small spherical colonies generally remain isolated.

Upon the surface of agar-agar spherical white colonies are produced. They may remain isolated or may become confluent.

Upon potato a luxuriant thick, white growth occurs.

The growth upon blood-serum is also abundant, especially at the temperature of the incubator. It has no distinctive peculiarities.

The introduction of tuberculous sputum or of a most minute quantity of a pure culture of this coccus into white mice generally causes a fatal septicemia.

The organisms are found in small numbers in the heart's blood, but are numerous in the spleen, lungs, liver, and kidneys.

House-mice and field-mice are comparatively immune ; dogs and rabbits are also highly resistant. Guinea-pigs sometimes die from general infection, though sometimes local abscesses may be the only result of subcutaneous inoculation.

The tetragenococci are of no special importance in human pathology, but probably hasten the tissue-necrosis in tuberculosis pulmonalis, and may aid in the formation of abscesses of the lung and contribute to the production of the hectic fever.

CHAPTER VII.

CHICKEN-CHOLERA.

THE barnyards of Europe, and sometimes of America, are occasionally visited by an epidemic disease which affects pigeons, turkeys, chickens, ducks, and geese, and causes almost as much destruction among them as the occasional epidemics of cholera and small-pox produce among men. Rabbit-warrens are also at times seriously affected by the epidemic. When fowls are ill with the disease, they fall into a condition of weakness and apathy which causes them to remain quiet, seemingly almost paralyzed, and ruffle up the feathers. 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 leads to a fatal termination in twenty-four to forty-eight hours. During its course there is profuse diarrhea, the very frequent fluid, slimy, grayish-white discharges containing numerous micro-organisms.

The bacilli which are responsible for this disease were first observed by Perroncito in 1878, and afterward thoroughly studied by Pasteur. They are short, broad bacilli with rounded ends, sometimes united to each other, with the production of moderately long chains (Fig. 105). Pasteur at first regarded them as cocci, because when stained with a penetrating anilin dye the poles stain intensely, but a narrow space between them remains almost uncolored. This peculiarity is very marked, and sharp observation is required to observe the outline of the intermediate substance. The bacillus does not form spores, and does not stain by Gram's method. When examined in the living condition it is found to be motile.

The cultures upon gelatin plates after about two days appear as small white points. The deep colonies reach the surface slowly, and do not attain any considerable size. The gelatin is not liquefied. The microscope

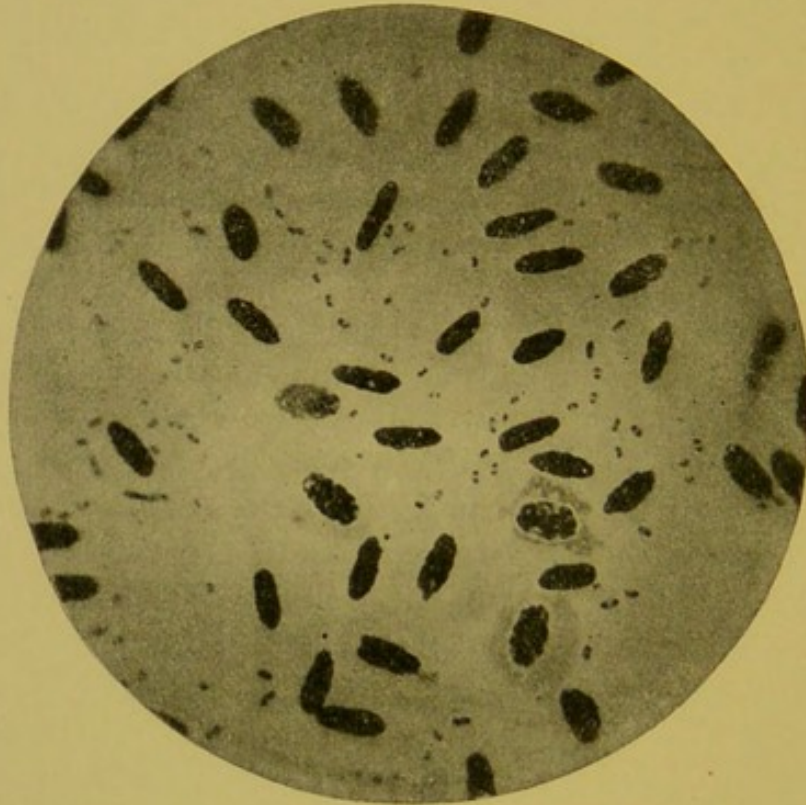


FIG. 105.—Bacillus of chicken-cholera, from the heart's blood of a pigeon; $\times 1000$ (Fränkel and Pfeiffer).

shows the colonies to be irregularly rounded disks with distinct smooth borders. The color is yellowish-brown, and the contents are granular. Sometimes there is a distinct concentric arrangement.

In gelatin puncture-cultures a delicate white line occurs along the entire path of the wire. When viewed through a lens, this line can be seen to consist of aggregated minute colonies. If, instead of a puncture, the inoculation be made 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. This growth is quite similar to that upon agar-agar and blood-serum, which growths are

white, shining, rather luxuriant, and devoid of characteristics.

Upon potato no growth occurs except at the incubation-temperature. It is a very insignificant, yellowish-gray, translucent film.

The introduction of pure cultures of this bacillus into the tissues of chickens, geese, pigeons, sparrows, mice, and rabbits is sufficient to produce the disease. Feeding chickens, pigeons, and rabbits with material infected with the bacillus is also sufficient to produce the disease with pronounced intestinal lesions. Guinea-pigs usually seem immune, though they succumb to very large doses, especially when given intraperitoneally.

The autopsy shows that when the bacilli are introduced subcutaneously a true septicemia results, with the addition 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 intestine shows an intense inflammation with red and swollen mucosa, and occasional ulcers following small hemorrhagic spots. 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 (*sequestræ*) underwent degeneration and appeared anemic, indurated, and of a yellowish color.

The bacillus of chicken-cholera is one whose peculiarities can be made use of for protective vaccination. Pasteur discovered that when cultures are allowed to remain undisturbed for several months, their virulence is greatly lessened, and new cultures planted from these are also attenuated. When chickens are inoculated with such cultures, no other change occurs than a local inflammatory reaction by which the birds are protected against virulent bacilli. From this observation Pasteur worked out a system of protective vaccination in which

fowls can first be inoculated with very weak, then with stronger, and finally with highly virulent cultures, with a resulting protection and immunity. Unfortunately, the method is too complicated to be very practical.

The bacillus of chicken-cholera seems not only to be specific for that disease, but seems able, when properly introduced into various other animals, to produce several different diseases. Indeed, no little confusion has arisen in bacteriology by the description of what is now pretty generally accepted to be this very bacillus under the various names of bacillus of rabbit-septicemia (Koch), *Bacillus cuniculicida* (Flügge), bacillus of swine-plague (Löffler and Schütz), bacillus of "Wildseuche" (Hüppe), bacillus of "Büffelseuche" (Oriste-Armanni), etc.

In 1885, Salmon and Smith wrote upon a bacillus which caused an epidemic disease of hogs in certain parts of the United States, calling it the bacillus of swine-plague, but at first regarding it as different from the disease well known in Europe. This bacillus has, however, now come to be regarded as identical with that of chicken-cholera.

The bacillus of "*hog-cholera*" of Klein, Salmon, and Smith seems to differ from the one described in a few particulars. It is actively motile, is provided with numerous flagella, and produces upon potato a straw color which may turn dark when old. It is said to resemble very closely the *Bacillus coli communis*, and it is thought by Smith to be a close ally of the *Bacillus typhi murium* of Löffler.

CHAPTER VIII.

MOUSE-SEPTICEMIA.

IN 1878, during his investigations upon the infectious traumatic diseases, Koch observed that when a minute amount of putrid blood or of meat-infusion was injected into mice the animals died of a septicemia caused by the multiplication in their blood of a minute bacillus to which he gave the name "Bacillus der Mäusesepticämie" (Fig. 106).

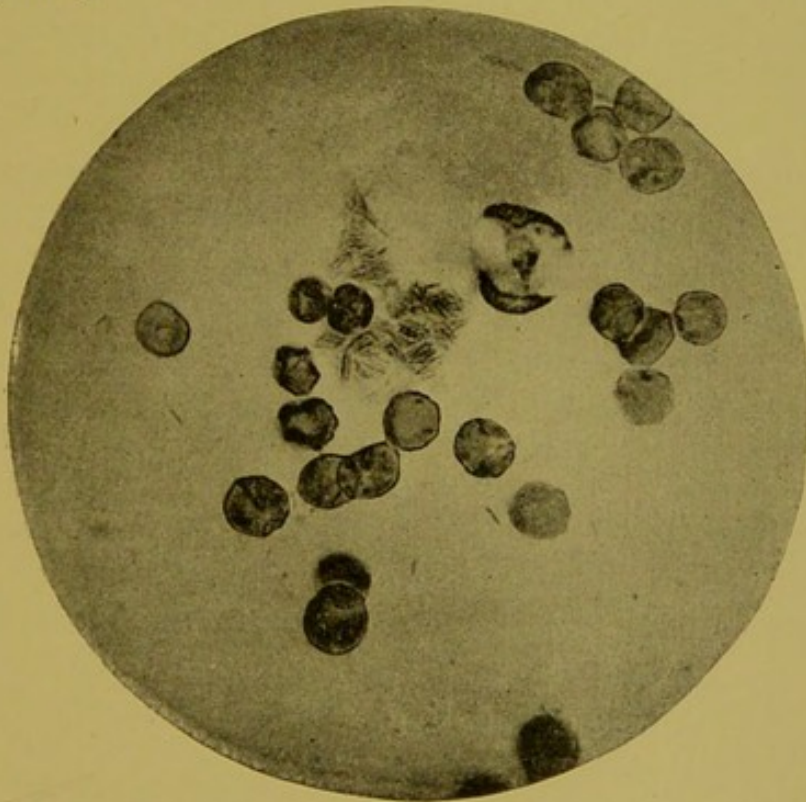


FIG. 106.—Bacillus of mouse-septicemia, from the blood of a mouse; $\times 1000$
(Fränkel and Pfeiffer).

In 1885 the bacillus was again brought into prominence by Löffler and Schütz, who found a very similar,

perhaps identical, organism in the erysipelatos disease which attacks the swine of many parts of Europe.

There seem to be certain slight morphological and developmental differences between these two organisms, but Baumgarten, Günther, Sternberg, and others have regarded them as insufficient for the formation of separate species, and have boldly described the organisms as identical. The described differences are, indeed, so very small that I think it well to follow in the path of the observers mentioned, pointing out in the description such points of difference as may arise.

The bacilli are extremely minute, measuring about $1.0 \times 0.2 \mu$ (Sternberg). Flügge, Fränkel, and Eisenberg find the *Bacillus erysipelas suis* somewhat shorter and stouter than that of mouse-septicemia: there seems to be a division of opinion upon this point.

Sporulation has been described by some observers, but nothing definite seems to be known upon this point.

Motility is ascribed by some (Schottelius and Fränkel) to the *Bacillus erysipelas suis*, and is denied to the bacillus of mouse-septicemia by others. The truth seems to be that the motility of both organisms is a matter of doubt.

No flagella have been demonstrated upon the bacillus. It grows quite well both at the room-temperature and at the temperature of incubation. It can grow well with or without oxygen, but perhaps flourishes a little better without than with it.



FIG. 107.—Colony of the bacillus of mouse-septicemia; $\times 80$ (Flügge).

The colonies upon gelatin plates can first be seen on the second or third day, then appearing as transparent grayish specks with irregular borders, from which many branched processes extend (Fig. 107). Fränkel describes them as resembling in shape the familiar branched cells occupying the lacunæ of bone. When further developed the colonies flow together and give the plate a cloudy gray appearance. The gelatin is not liquefied.

In gelatin puncture-cultures the growth is quite characteristic, and the tendency of the bacillus to grow anærobically is well shown (Fig. 108). The develop-

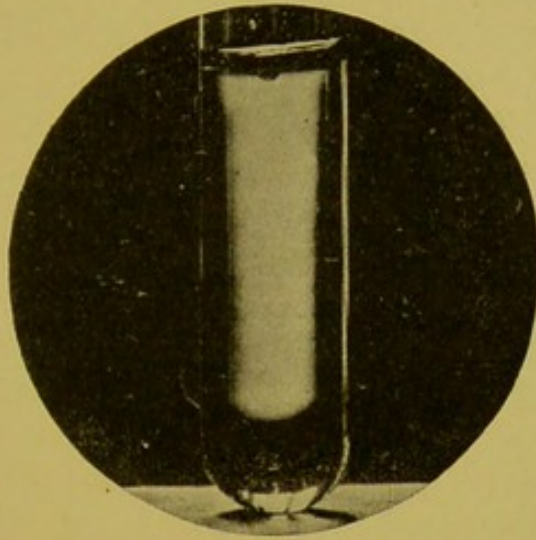


FIG. 108.—Bacillus of mouse-septicemia: gelatin puncture-culture three and a half days old (Günther).

ment takes place all along the line of puncture, but is more marked below than at the surface. The growth takes place in a peculiar form, resembling superimposed disks, each disk separate from its neighbors and consisting of an area of clouded grayish gelatin reaching almost to the walls of the tube. This growth develops slowly, and causes a softening rather than an actual liquefaction of the gelatin.

Upon agar-agar and blood-serum a very delicate, transparent grayish line develops along the path of the needle.

The bacillus grows at the room-temperature, but much better at the temperature of the incubator.

The disease affects quite a variety of animals, notably hogs, rabbits, mice, pigeons, and sparrows. The guinea-pig, which is generally the victim of laboratory experiments, is not susceptible to it.

When mice are inoculated with a pure culture of this bacillus, they soon become ill, lose their appetite, mope in a corner, and are not readily disturbed. As the dis-

ease becomes worse they assume a sitting posture with the back much bent ; the eyelids are glued together by adhesive pus ; and when death comes to their relief, in the course of forty to sixty hours after inoculation, they remain sitting in the same characteristic position.

When the ears of rabbits are inoculated with the bacillus from cases of erysipelas suis, a violent inflammatory edema and distinct redness occurs, much resembling erysipelas. This lesion gradually spreads, involves the head, then the body of the animal, and ultimately causes death.

When swine are affected, they are dull and weak, and have a kind of paralytic weakness of the hind quarters. The temperature is elevated ; red patches appear upon the skin and swell and become tender. Death follows in two or three days.

In all animals the anatomical changes are much alike. The disease proves to be a septicemia, and the bacilli can be found in all the organs, especially the lungs and spleen. They are few in number in the streaming blood.

As the organisms stain well by Gram's method, this stain is of great value for their discovery in the tissues, and can be highly recommended.

Most of the bacilli occupy the capillary blood-vessels ; many of them are enclosed in leucocytes. The organs in such cases do not appear distinctly abnormal, except the spleen, which is considerably enlarged. The mesenteric and other lymphatics are also enlarged, and the gastric and intestinal mucous membranes are usually inflamed and mottled. The bacilli also occupy the intestinal contents, and Kitt, who discovered them in this position, points out that the infection of swine probably takes place by the entrance, along with the food, of the fecal matter of diseased animals into the alimentary apparatus of others.

Pasteur, Chamberland, Roux, and others have worked upon a protective vaccination based upon the attenuation of the virulence of the organism by passing it through

rabbits. Two vaccinations are said to be necessary to produce immunity. The vaccinated animals, however, may be a source of infection to others, and should always be isolated. Klemperer in 1892 found that the blood-serum of immunized rabbits would save infected mice into which it was injected.

Lorenz in 1894 found an antitoxic substance in the blood of rabbits immunized to the disease. The effect of its injection into other animals is, however, only a temporary immunity.

CHAPTER IX.

ANTHRAX.

THE disease of cattle known as anthrax or "splenic fever" is of infrequent occurrence in this country and in England. In France, Germany, Hungary, Russia, Persia, and the East Indian countries it is a dreaded and common malady which robs herdsmen of many of their valuable stock. Siberia perhaps suffers most, the disease being so exceedingly common and malignant as to deserve the name "Siberian pest." Certain local areas, such as the Tyrol and Auvergne, in which it seems to be constantly present, serve as distributing foci from which the disease spreads rapidly in summer, afflicting many animals, and ceasing its depredations only with the advent of winter. It seems to be distinctly a disease of the summer season.

The animals most frequently affected are cows and sheep. Among our laboratory animals white mice, guinea-pigs, and rabbits are highly susceptible; dogs, cats, most birds, and amphibians are almost perfectly immune. White rats are infected with difficulty. Man is only slightly susceptible, the manifestation of the disease as seen in the human species being different from the same disease in the lower animals in that it is usually a local affection—malignant carbuncle—and only at times gives rise to a general infection.

Anthrax was one of the first of the specific diseases proven to be caused by a definite 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 to most unbiased minds

their etiological significance. The further confirmation of Davaine's conclusions and actual proof of the matter rested with Pasteur and Koch, who, observing that the bacilli bore spores, cultivated them successfully outside the body, and then produced the disease by the inoculation of pure cultures.

The anthrax bacilli (Fig. 109) are large rods with a

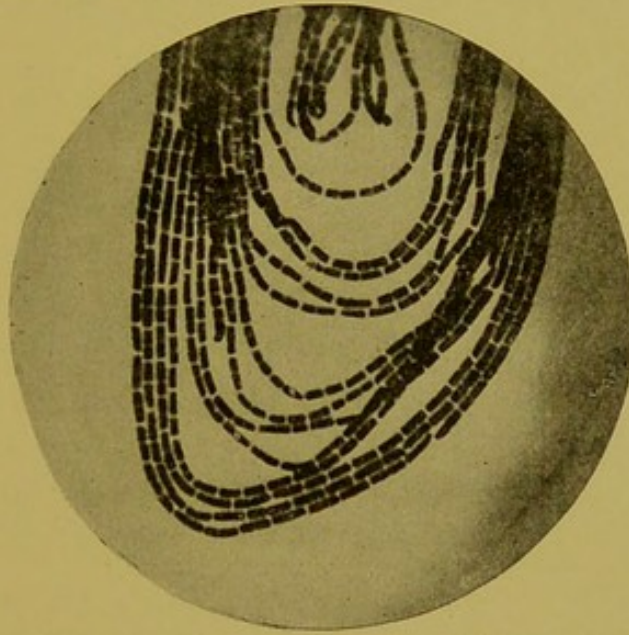


FIG. 109.—*Bacillus anthracis*: colony three days old upon a gelatin plate; adhesive preparation; $\times 1000$ (Fränkel and Pfeiffer).

rectangular form, caused by the very slight rounding of the corners. They measure $5-20 \mu$ in length and are from 1μ to 1.25μ in breadth. The pronounced tendency is toward the formation of long threads, in which, however, the individuals can generally be made out; at times isolated rods occur. In the threads the bacilli seem enlarged a little at the ends, and give somewhat the appearance of a bamboo cane. The formation of spores is prolific: each spore has a distinct oval shape, is transparent, and does not alter the contour of the bacillus in which it occurs. Spores are generally formed in the presence of oxygen upon the surfaces of the culture-media. When a spore is placed under favorable conditions for its development and is carefully watched, it may be observed to

increase in length a trifle, then to undergo a rupture at one end, from which the new bacillus projects. The spores of anthrax (Fig. 110), being large and easily ob-

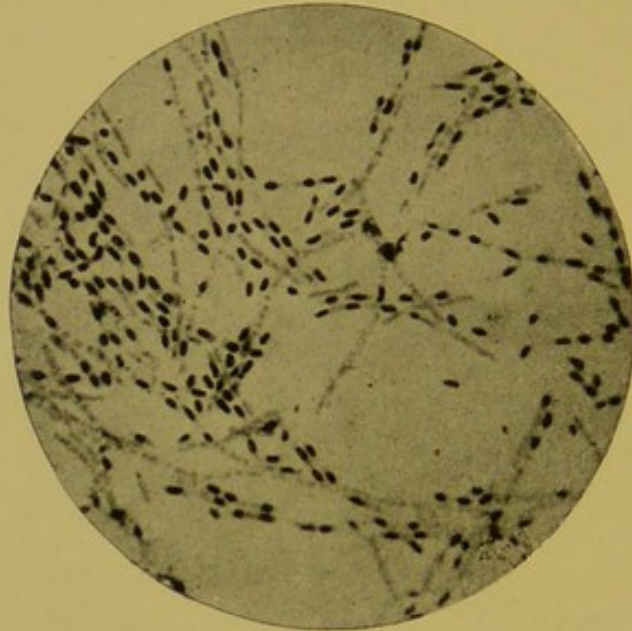


FIG. 110.—*Bacillus anthracis*, stained to show the spores; $\times 1000$ (Fränkel and Pfeiffer).

tainable, are excellent subjects for the study of sporulation, for the action of germicides and antiseptics, and for demonstration by stains. When dried upon threads of silk they will retain their vitality for several years, and are highly resistant to heat and disinfectants.

Spores of anthrax are killed by five minutes' exposure to a temperature of 100° C., and are killed in five minutes in a 5 per cent. solution of carbolic acid, or, at least, are deprived of their vegetative property in relation to culture-media. It is said by some that spores subjected to 5 per cent. carbolic acid can germinate when introduced into susceptible animals. Spores are also killed by simple wetting with 1 : 100,000 bichlorid-of-mercury solution.

The bacilli are not motile and are not provided with flagella. 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 fibrin method.

Picro-carmin, followed by Gram's method, gives a beautiful and clear picture. The spores can be stained with carbol-fuchsin, the bacilli decolorized with a very weak acid, and then counter-stained with a watery solution of methyl blue.

Upon the surface of gelatin plate-cultures the bacillus forms beautiful and highly characteristic colonies (Fig. III). To the naked eye they appear first as minute

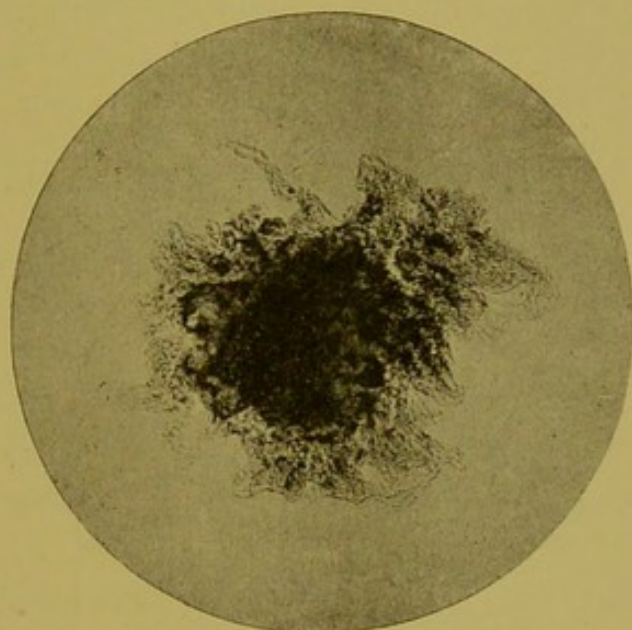


FIG. III.—*Bacillus anthracis*: colony upon a gelatin plate; $\times 100$ (Fränkel and Pfeiffer).

round whitish dots occurring upon the surface, and causing liquefaction of the gelatin as they increase in size. Under the microscope they can be seen in the gelatin as egg-shaped, slightly brownish granular bodies, not attaining their full development except upon the surface, where they spread out into flat, irregular, transparent growths bearing a partial resemblance to tufts of curled wool. From a tangled centre large numbers of curls extend, each made up of parallel threads of bacilli. As soon as the colony attains any considerable size liquefaction begins. These colonies make beautiful adhesive preparations. If a perfectly clean cover-glass be passed once through a flame and laid carefully upon the gelatin, the

colonies can generally be picked up entire when the glass is removed. Such a specimen can be dried, fixed, and stained in the same manner as an ordinary cover-glass preparation.

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, most luxuriantly at the surface, where oxygen is plentiful. As the growth progresses fine filaments like bristles, extend from the puncture into the neighboring gelatin giving the growth somewhat the appearance of an evergreen tree inverted (Fig. 112).

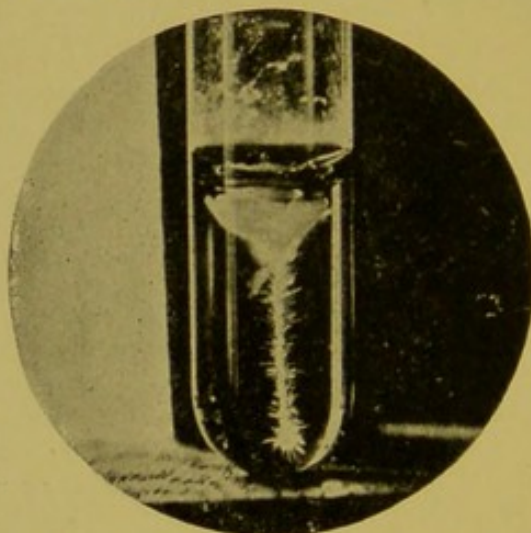


FIG. 112.—*Bacillus anthracis*: gelatin puncture-culture seven days old (Günther).

The more superficial of these threads reach about half-way to the sides of the tube, while the deeper ones are shorter and shorter, until near the apex branches cease. When the projections are pretty well developed a distinct surface-growth will be discerned, and if the tube be tilted, one can observe that the gelatin beneath it has liquefied. As the growth becomes older the liquefaction increases, until ultimately the entire gelatin is fluid and the growth is precipitated.

Upon agar-agar the characteristics are few. The growth takes place all along the line of inoculation as

a slightly translucent, slightly wrinkled layer with irregular edges, from which sufficient bacillary threads project to give it a ciliated appearance to the naked eye. When the culture is old the agar-agar turns a distinct brown. Spore-formation is luxuriant upon agar-agar.

On potato the growth is white, creamy, sometimes rather dry in appearance. Sporulation is marked.

Blood-serum cultures lack peculiarities; the culture-medium is slowly liquefied.

The bacillus only grows between the extremes of 20° and 45° C., best at 37° C. The exposure of the organism to the temperature of 42-43° C. for twenty-four hours is sufficient to destroy its virulence.

The culture-media should always be faintly alkaline, as anthrax bacilli will not grow in the presence of any free acid.

The micro-organism under consideration is a parasitic microbe, yet is one which, because of its spores, can, in a latent form, exist without the animal organism until appropriate conditions for its natural development are presented.

Ordinarily, the infection takes place either through the *respiratory tract* or through the *alimentary canal*.

Buchner has shown that when animals are allowed to inhale anthrax spores they die of typical anthrax. The spores establish themselves in the alveoli of the lung, penetrate the epithelium, enter the vascular system, and soon give rise to typical lesions. Strange to say, the appearance caused by the inhalation of the bacilli in their perfect form is entirely different, for a rapid multiplication occurs without sporulation, and causes a violent irritative pneumonia with serous or sero-fibrinous exudate in which large numbers of the bacilli occur. In these cases there may be no general infection.

When the bacilli are taken into the stomach in food they meet with a rapid death because of the acidity of the gastric juice. Should spores, however, be ingested, they are able to endure the gastric juice, to pass into the

intestine, and, as soon as proper conditions of alkalinity are encountered, to develop into bacilli. They develop rather rapidly, surround the villi with thick networks of bacillary threads, separate the epithelial cells, enter the lymphatics, and thus find the appropriate environment for the production of a general infection.

Sometimes the bacillus enters the body through a wound, cut, scratch, or fly-bite. This is especially the case with men who come in contact with diseased cattle. As has already been pointed out, a malignant pustule is apt to follow, and may cause death. Men whose occupations bring them in contact with skins and hair from animals dead of anthrax are not only liable to wound-infection, but are sometimes the subjects of a pulmonary form of the disease—"wool-sorter's disease"—caused by the inspiration of the spores attached to the wool.

The disease as we see it in the laboratory is accompanied by few but marked lesions. The ordinary method of inoculation is to cut away a little of the hair from the abdomen of a guinea-pig or rabbit or the root of a mouse's tail, make a little subcutaneous pocket with a snip of a pair of sterile scissors, and introduce the spores or bacilli from a pure culture upon a rather heavy platinum wire, the end of which is flattened, pointed, and perforated. An animal inoculated in this way generally dies, according to the species, in from twenty-four hours to three days. The symptoms are weakness, fever, loss of appetite, and sometimes a bloody discharge from nose and bowels. There is much subcutaneous edema. At the autopsy very little change is observed at the seat of inoculation. The subcutaneous tissue beneath it for a considerable distance around is occupied by a peculiar colorless gelatinous edema which contains the bacilli. The abdominal cavity shows injection and congestion of its viscera. The spleen is considerably enlarged, is dark in color, and of mushy consistence. The liver is somewhat enlarged. When the thorax is opened, the

lungs may be slightly congested, but otherwise no changes are to be found.

When the various organs, which present no appreciable changes to the naked eye, are subjected to a microscopic examination, the appropriate staining methods bring out a most remarkable and beautiful change. The capillary system is almost universally occupied by bacilli, which extend throughout its meshworks in long threads. Most beautiful bundles of these bacillary threads can, at times, 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, the bacteria are relatively few, so that the burden of bacillary obstruction is borne by the minute vessels. The condition is thus seen to be one of pure septicemia, and bacilli can be secured in pure cultures from the blood and tissues.

The susceptibility of the anthrax bacillus to the influence of heat, cold, antiseptics, etc. not only permitted Buchner, Behring, and others to produce biological curiosities in the form of bacilli unable to bear spores and robbed of their pathogenic powers, but also suggested to Pasteur the important practical measure of protective vaccination. Pasteur found that the inoculation of non-virulent bacilli into cows and sheep, and their reinoculation with slightly virulent bacilli, gave them the ability to withstand the action of highly virulent organisms. Löffler, Koch, and Gaffky, however, found that these immunized animals were not absolutely protected from intestinal anthrax.

The methods of diminishing the virulence of the anthrax bacilli are numerous. Toussaint, who was certainly the first to produce immunity in animals by injecting them with sterile cultures of the bacillus, 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 it removed 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 found that the inoculation of the bacilli into immune animals, such as the frog, and their subsequent recovery from its blood, diminishes the virulence markedly.

Protection can be afforded in still other ways. The simultaneous inoculation of bacteria not at all related to anthrax will sometimes recover the animal, as Hüppe found. Hankin found in the cultures chemical substances, especially an albuminose, which exerted a protective influence. Chamberland has shown that protective inoculation by Pasteur's method has diminished the death-rate from 10 per cent. for sheep and 5 per cent. for cattle to about 0.94 per cent. for sheep and 0.34 per cent. for cattle, so that the utility of the method is scarcely questionable. In 1890, Agata and Jasuhara showed that in the convalescents from anthrax among their experimental animals an antitoxic substance was present in the blood in such quantities that 1 : 800 parts per body-weight of dog's serum containing the antitoxin would protect a mouse. Similar results have been attained by Marchoux.

Experiments of interest have been performed to show that the natural immunity enjoyed by many animals can be destroyed. Behring found that if the alkalinity of the blood of rats was diminished, they could become affected with anthrax, and numerous observers have shown that when anthrax bacilli and unrelated organisms, such as the erysipelas cocci, *Bacillus prodigiosus*, and *Bacillus pyocyaneus*, are simultaneously introduced into immune animals, the immunity is destroyed and the animals succumb to the disease. Frogs have been made to succumb to the disease by exposure to a temperature of 37° C. after inoculation. Pasteur destroyed the immunity of fowls by a cold bath after inoculation.

In the natural order of events anthrax in cattle is

probably the result of the inhalation or ingestion of the spores of the bacilli from the pasture. At one time much discussion arose concerning the infection of the pasture. It was argued that, the bacilli being enclosed in the tissues of the diseased animals, the infection of the pasture must be due to the distribution of the germs from the buried cadaver to all parts of the field, either through the activity of earth-worms, which ate of the earth surrounding the corpse and then deposited the spores in their excrement at remote areas (Pasteur), or to currents of moisture in the soil. Koch seems, however, to have demonstrated the fallacy of the theories by showing that the conditions under which the bacilli find themselves in buried cadavers are exactly opposed to those favorable to fructification or sporulation, and that in all probability the majority of bacteria suffer the same fate as the animal cells, 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 in all probability it is the live, and not the dead, animals that are to be blamed as sources of infection.

As every animal affected with anthrax is a source of danger to the community in which it lives, to the men who handle it as well as the animals who browse beside it, such animals, as soon as the diagnosis is made, should be killed, and, together with the hair and skin, be burned. When this is impracticable, Fränkel recommends that they be buried to a depth of at least $1\frac{1}{2}$ -2 meters, so that the sporulation of the bacilli is impossible. The dejecta should also be carefully disinfected with 5 per cent. carbolic-acid solution.

Of course, animals can be infected through wounds. This mode of infection is, however, more common among men, who suffer from the local disease manifested as the malignant carbuncle, than among animals.

CHAPTER X.

TYPHUS MURIUM.

THE *Bacillus typhi murium* (Fig. 113), which created havoc among the mice in his laboratory, causing most of them to die, was discovered by Löffler in 1889. It is a short organism, somewhat resembling the bacillus of chicken-cholera. It is rather variable in its dimensions, and often grows into long, flexible filaments. No

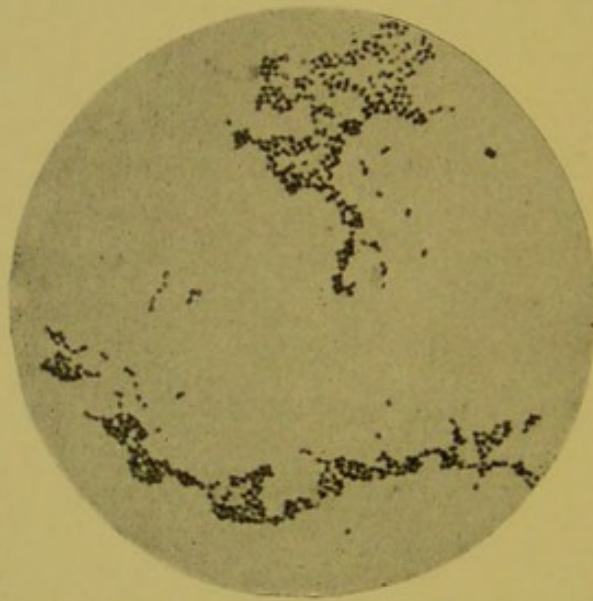


FIG. 113.—*Bacillus typhi murium*, from agar-agar; $\times 1000$ (Itzerott and Niemann).

sporulation has been observed. It is a motile organism, with numerous flagella, like those of the typhoid-fever bacillus. It stains well with the ordinary dyes, but rather better with Löffler's alkaline methylene blue.

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. In

gelatin punctures there is no liquefaction. The growth takes place upon the surface principally, where a grayish-white mass slowly forms.

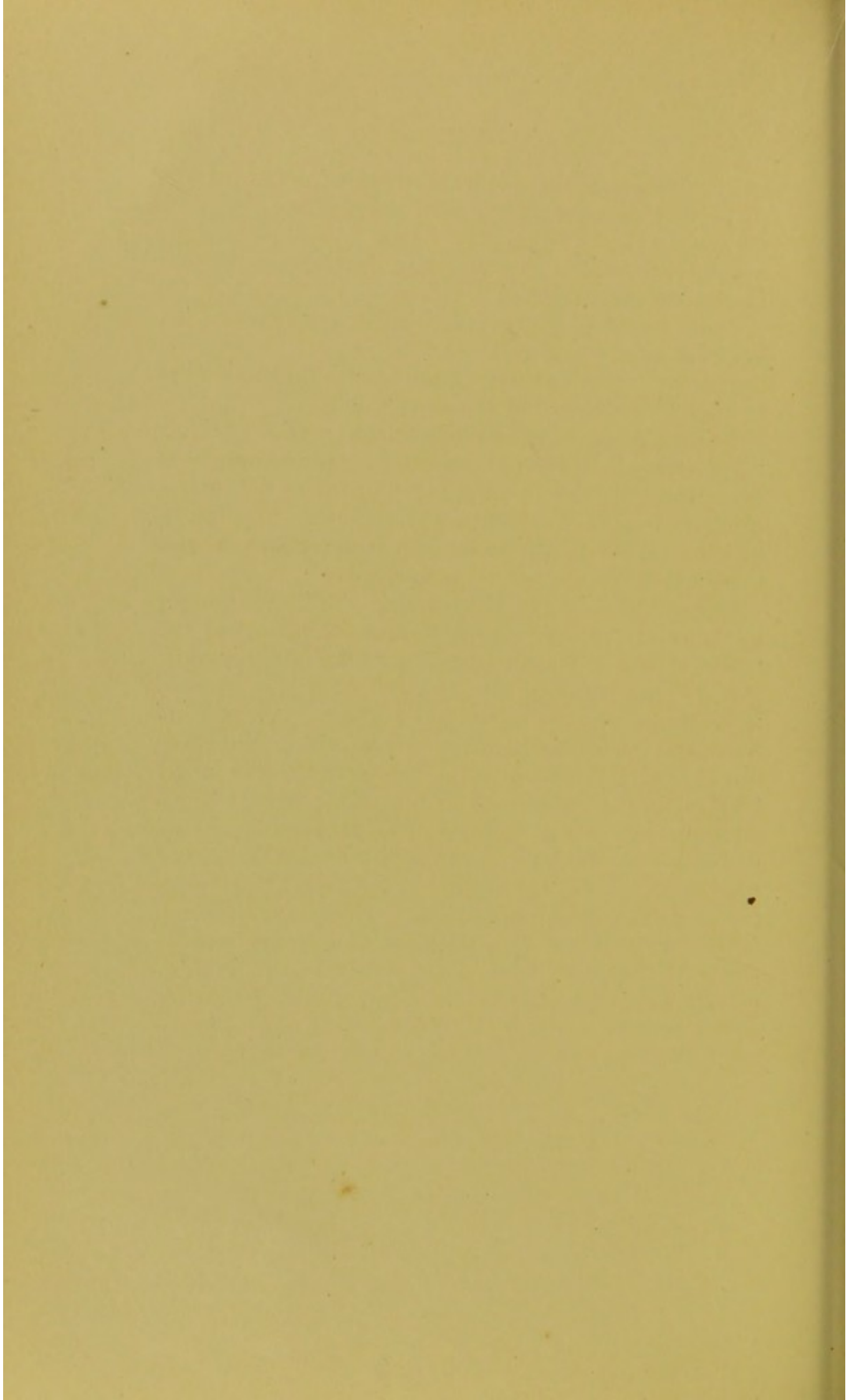
Upon agar-agar a grayish-white development devoid of peculiarities occurs.

Upon potato a rather thin whitish growth may be observed after a few days.

The bacillus grows well in milk, with the production of an acid reaction, but without coagulation.

The organism is pathogenic for mice of all kinds, which succumb in from one to two days when inoculated subcutaneously, and in eight to ten or twelve days when fed upon material containing the bacillus. The bacilli multiply rapidly in the blood- and lymph-channels, and cause death from a general septicemia.

Löffler expressed the opinion that this bacillus might be of use in ridding infested premises of mice, and the results of its use for this purpose have been highly satisfactory. He has succeeded in ridding a field so infested as to be useless for agricultural purposes by saturating some bread with bouillon cultures of the bacillus and distributing it near the holes inhabited by the mice. The bacilli that were eaten by the mice not only killed them, but also infected others which ate the dead bodies of the first victims, and so the extermination progressed until scarcely a mouse remained in the field. The bacilli are not pathogenic for the 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.



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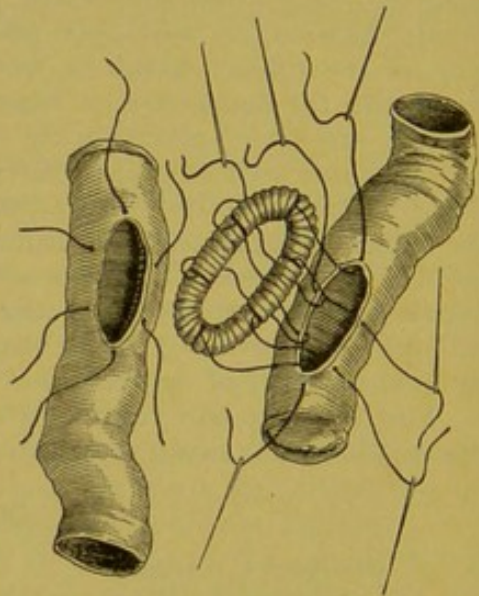
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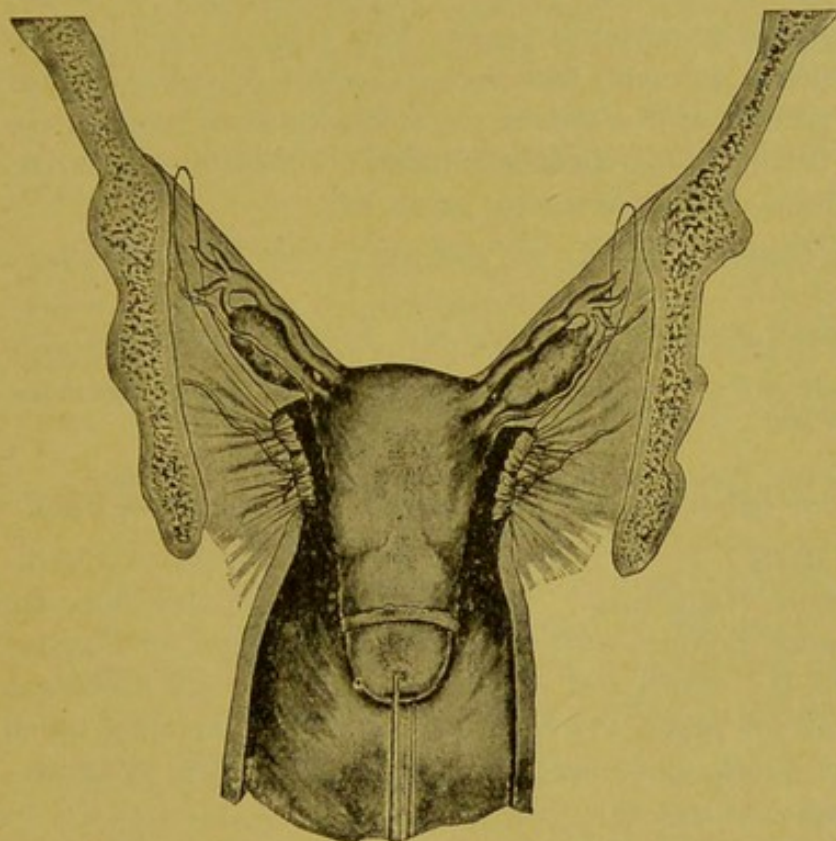
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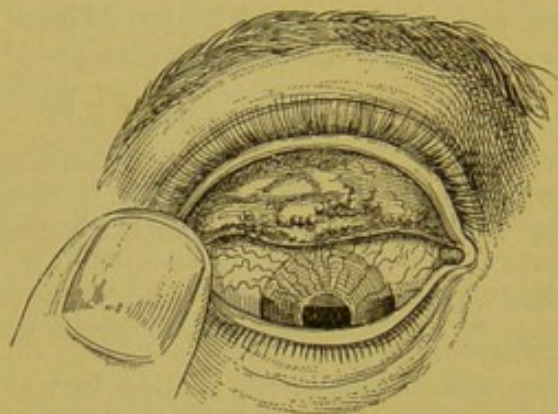
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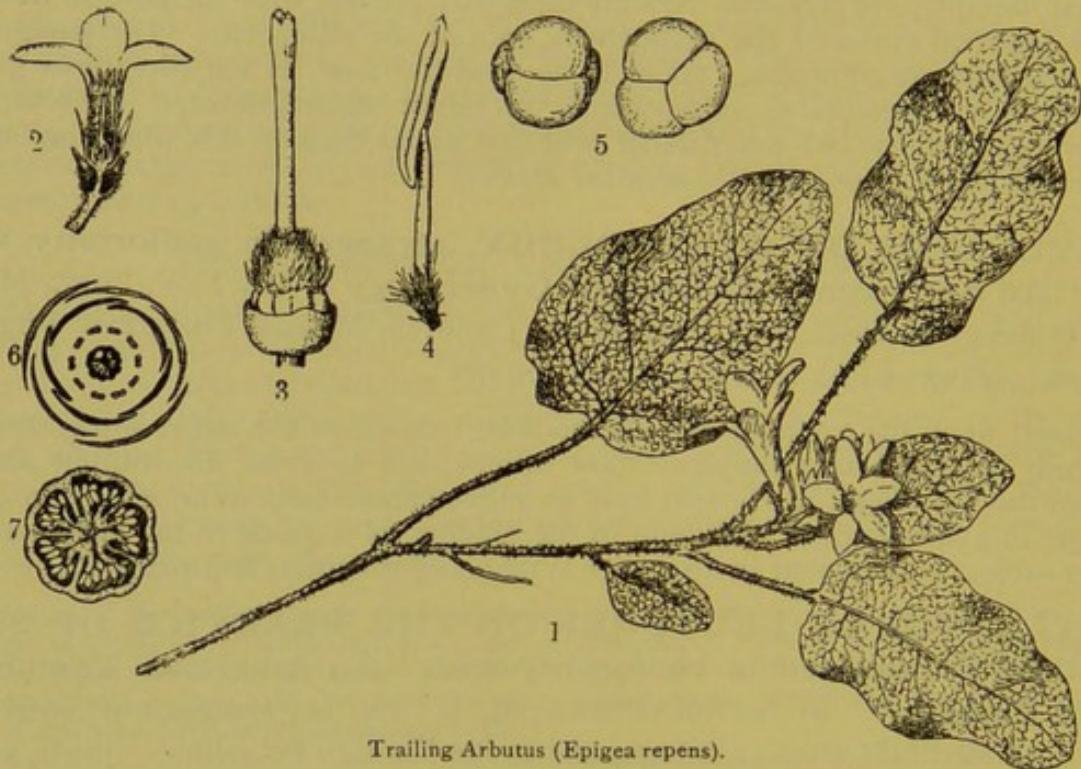
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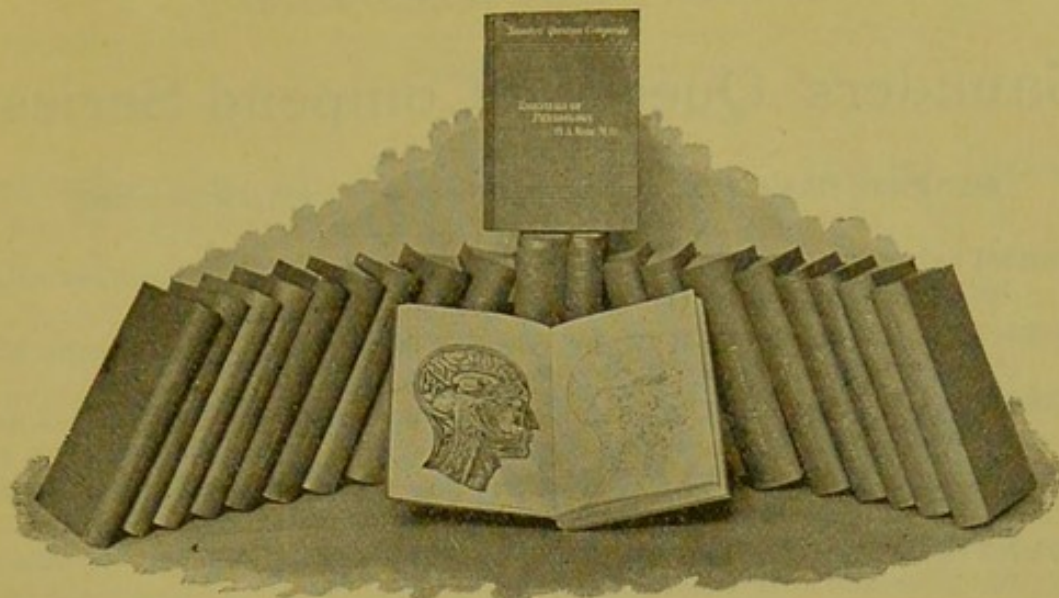
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
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