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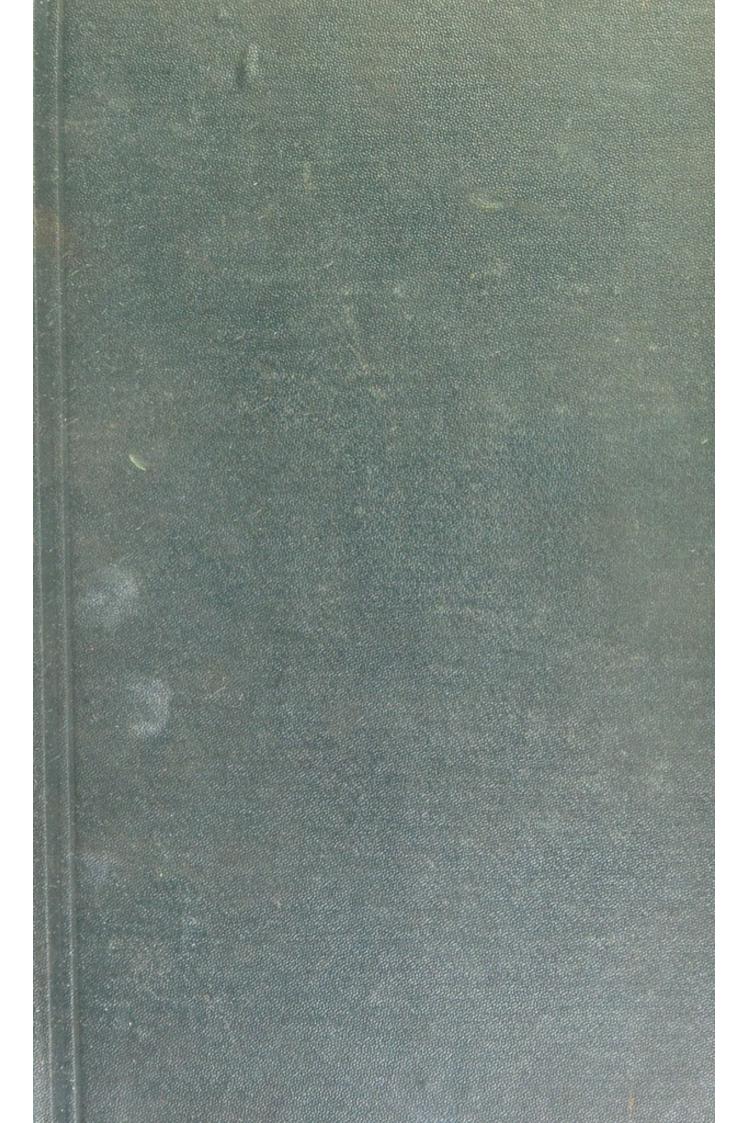Philadelphia : Saunders, 1900.

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ELEMENTS OF

Clinical Bacteriology

FOR

PHYSICIANS AND STUDENTS

BY

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SECOND ENLARGED AND REVISED EDITION

AUTHORIZED TRANSLATION BY

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Philadelphia Hospital ; etc.

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TRANSLATOR'S NOTE.

WORKS on bacteriology, on pathology, and on clinical diagnosis are not wanting in any language, but we know of only one on clinical bacteriology, and as a consideration of the subject from this aspect must appeal to a large body of the profession, it was thought a service might be rendered by a translation of this admirable publication, whose authors are well and favorably known for their original work in both clinical medicine and bacteriology. The general practitioner can scarcely be expected to be a trained and practised bacteriologist, but he must have a working familiarity with the subject of bacteriology in order that he may possess clear conceptions as to the etiology of disease and the nature of the resultant morbid processes, leading to a rational application of measures and methods of prophylaxis and treatment. To these ends it is hoped the present publication will contribute. The work of translation is coupled with especial and personal pleasure from the fact that both junior author and translator participated simultaneously in the work in bacteriology in the Hygienic Institute at Berlin in the spring of 1890.

It has been thought advantageous to add illustrations to the English version. · A. A. E.

PHILADELPHIA, *February, 1900.*

7

PREFACE TO THE SECOND EDITION.

SCIENTIFIC activity in the domain of clinical bacteriology has been not less pronounced than fruitful during the period that has elapsed since the appearance of the first edition of this book. By including the numerous results of recent investigation the size of the present volume has been considerably increased. Chapters have been added on Plague and Botulism, and those on Immunity, Diphtheria, Typhoid Fever, Actinomycosis, Examination of Air and of Water, and others, have been radically revised; and in all other sections numerous changes and additions have been made.

We believe the work adapted to the present position of bacteriologic knowledge, and hope that this edition may have the same friendly reception accorded the first edition.

THE AUTHORS.

PREFACE TO THE FIRST EDITION.

THIS book represents an attempt to group the results of bacteriologic investigation from the clinical point of view. Bacteriology has become more and more an indispensable aid to medical art ; it has enlarged our comprehension of the nature of infectious diseases, and it has established their prophylaxis, diagnosis, and treatment upon a broader and firmer basis. It is hoped that the following exposition of what it has thus far accomplished may help to show how useful to the physician, in his double capacity of counselor of the well and coadjutor of the sick, are bacteriologic thought and action.

THE AUTHORS.

CONTENTS.

PART I.

PART II.

INFLAMMATION AND SUPPURATION.

PART III.

SPECIFIC DISEASES OF BACTERIAL ORIGIN.

PART IV.

I. MYCOSES (INFECTIONS WITH FILAMENTOUS AND BUDDING FUNGI).

II. INFECTIONS WITH THE LOWEST FORMS OF ANIMAL LIFE.

APPENDIX.

I. BACTERIOLOGIC EXAMINATION OF SOIL, AIR, AND WATER.

THE BACTERIA PRINCIPALLY FOUND IN SOIL, AIR, AND WATER.

II. DISINFECTION.

CLINICAL BACTERIOLOGY.

PART I.

I. MORPHOLOGY AND BIOLOGY OF BACTERIA.

THE minute forms of life constituting in largest part the exciting agents of the infectious diseases occupy the lowest level in the vegetable kingdom. They are designated *bacteria* or *fission-fungi*. The latter term is, however, not well chosen. The botanic conception of fungi includes those lower forms of vegetable life that are without the coloring-matter of leaves (chlorophyl) or similar coloring-matters (chromophyls). There are not a few bacteria, however, that are capable of generating such coloring-matters, and, thus, also of decomposing carbon dioxid and its combinations, and utilizing the products of this activity. There is, thus, no justification for the term fission-fungi, and it is, therefore, preferable to designate the minute forms of life under consideration as *bacteria*.

There is as yet no strictly scientific, natural *classification of bacteria*. For the present the adoption of an artificial system, based upon such physical features as the size, the shape, and the arrangement of the bacteria, must suffice (F. Cohn). (See Figs. 1, 2, 3.) Three main divisions are recognized: (1) **Spheric bacteria** (cocci, micrococci, coccaceæ); these are spheric cells. (2) **Rod-shaped bacteria** (bacilli, bacteriaceæ); these are rod-like, cylindric cells. (3) **Spiral bacteria** (vibrios, spirilla, spirillaceæ); these are twisted in both the horizontal and the vertical direction, and are, thus, comparable to the windings of a screw or of a corkscrew.

The *individual bacterial cells* exhibit at times a homogeneous translucent, at other times a granular protoplasmic, structure that, on the whole, possesses the properties of all other protoplasmic structure, but that is not, as in the cells of the higher orders of plants it is, differentiable

Fig. 1.—Various forms of bacteria: 1 and 2, Round and oval micrococci; 3, diplococci; 4, tetracocci, or tetrads; 5, streptococci; 6, bacilli; 7, bacilli in chains, the lower showing spore-formation; 8, bacilli showing spores, forming drumsticks and clostridia; 9 and 10, spirilla; 11, spirochetæ (McFarland).
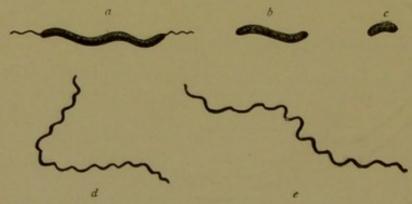
Fig. 2.—Diagram illustrating the morphology of the spirilla: *a, b, c,* Spirilla; *d, e,* spirochetæ (McFarland).
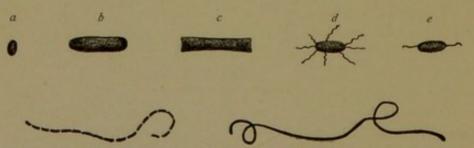
Fig. 3.—Diagram illustrating the morphology of the bacilli: *a, b, c,* Various forms of bacilli; *d, e,* bacilli with flagella; *f,* chain of bacilli, individuals distinct; *g,* chain of bacilli, individuals not separated (McFarland).

into a cell-nucleus and a cell-body. Nevertheless, some observers consider the so-called central body in certain large bacteria as the virtual nucleus, and believe the plasma to have a honeycomb arrangement—a view that has recently been put forth also with regard to the large species of spirilla. According to others, the interior of the bacterium

consists of a lining layer of protoplasm, and a loculated space for the cell-fluid. Not rarely the protoplasm of the bacteria contains granules that stain deeply with aniline dyes. The significance of these so-called *Babes-Ernst bodies* has not been established ; they have been looked upon as the progenitors of spores, as nuclear structures, as degeneration-products, and, finally, as reserve substances.

The **ground-substance** of the bacterial cell is surrounded by a *cell-capsule* or *cell-membrane* constituted of albuminoid material, in rare instances of cellulose and of varying amounts of a carbohydrate that stains blue with iodin. When the outer layers of this membrane possess the property of swelling up greatly and becoming viscid and gelatinous, an appearance is created as if the bacterium possessed a special *capsule*, and under such circumstances it is customary, in view of the physical appearance, to speak of an *encapsulated bacterium*. (Fig. 4.) This
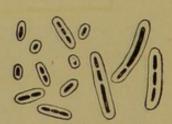
Fig. 4.—Encapsulated bacteria.

mucoid transformation of the capsule of the cell occurs in the case of pathogenic bacteria usually only in the animal body, and but seldom in artificial culture-media.

The length of bacteria varies greatly from one to several micromillimeters (twenty and more), while their thickness is generally less than one micromillimeter.

Bacteria are in part motile, in part nonmotile. When motility is present, it is dependent upon special motor organs—so-called *flagella* (Fig. 5)—which are connected with the body of the cell in varying number and arrangement. These represent protoplasmic structures of extreme attenuation, which arise directly from the cell-membrane, and do not extend into the actual ground-substance. The following varieties of motile bacteria may be distinguished :

1. Monotrocha, with a single flagellum at the pole of the cell.

2. Amphitrocha, with a single flagellum at each pole.

3. Lophotrocha, with a cluster of flagella at one pole.

4. Peritrocha, with a varying number of flagella surrounding the body of the bacterium.

The shape and length of these flagella are extremely variable in the different varieties of motile bacteria. The movement itself is forward, and is always combined with a

certain amount of rotation. It is most marked in quite young cultures, but is in greater degree dependent upon the variety of bacterium, the culture-medium, and the temperature. Many species of bacteria possess spontaneous movement throughout their whole life; others only during certain phases, losing it, for instance, preceding spore-formation.

The **multiplication** of bacteria takes place usually by means of fission in pairs. The time in which this division occurs varies for different varieties. In the case of some species, if all of the extrinsic conditions (culture-medium,
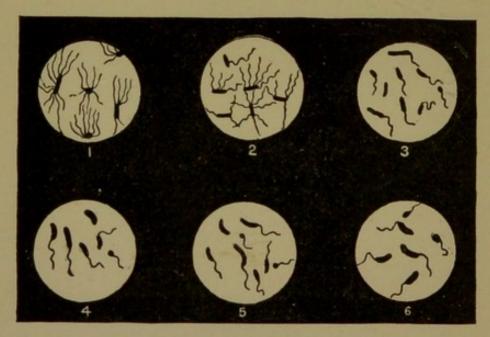
Fig. 5.—Bacteria showing flagella: 1, Bacilli of typhoid fever; 2, bacilli coli communes; 3, spirilla of Finkler and Prior; 4, spirilla of Deneke; 5, vibrios of Metschnikoff; 6, spirilla of Asiatic cholera (Nicolle and Morax).

temperature) are favorable, it is half an hour; in that of others it is somewhat longer—from one to two hours; while in that of still others, as, for instance, the tubercle-bacillus, it is as much as several days. When the division always takes place in the same direction, and the newly formed individuals remain attached to one another, a chain-like formation results. In the case of rods *bacillary filaments* result, or *pseudofilaments ;* the dividing walls between the individual cells of the strand are frequently recognizable with difficulty. In the case of spherules *streptococci* (*chain-cocci*) result. (Fig. 6.) The bacteria may, however, after division be arranged,

not end to end, but side by side in groups. Cocci thus collected are designated *staphylococci* (from σταφυλή, a grape), because viewed microscopically the cocci resemble bunches of grapes. (Fig. 7, *k*.) When the bacteria appear joined in pairs, they are spoken of as *diplococci* (Fig. 7, *b*) or *diplobacilli*. A number of bacteria divide in two or three successively vertical directions. This mode of division has been observed only among cocci, and there thus

Fig. 6.—Streptococci.

result *plate-cocci* (*tetragenus, tetrad arrangement*) and the ball-of-twine-like packets known as *sarcinæ*. (Fig. 7, *i*.)

In the process of cell-division an essential developmental difference is observed between bacilli and cocci. The bacilli become double their ordinary size before they are ready for multiplication ; while the cocci break up, without preliminary increase in size, into two hemispheres (or in the case of tetrad arrangement into four quadrants, and in the case of sarcina-formation into eight octants), and spheric cocci then form from the products of division.
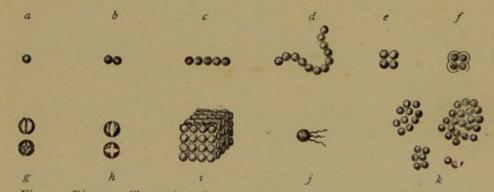
Fig. 7.—Diagram illustrating the morphology of cocci: *a*, Coccus or micrococcus; *b*, diplococcus; *c, d*, streptococci; *e, f*, tetragenococci or merismopedia; *g, h*, modes of division of cocci; *i*, sarcinæ; *j*, coccus with flagella; *k*, staphylococci (McFarland).

Vibrios also form pseudofilaments consisting of numerous spiral cells, and, besides, though seldom, of long spirals consisting each of a single cell. Division occurs among these in much the same manner as among bacilli.

For every bacterium there is a temperature at which it thrives best (*temperature-optimum*), as well as a temperature-limit above, and beyond which it can not survive (*temperature-maximum*), and one below, at which it will just live (*temperature-minimum*). Among pathogenic bacteria the

temperature-optimum is mostly about 37° C. (98.6° F.). There are microorganisms, however, that live at 0° C. (32° F.), and exhibit all of their vital manifestations, such as color-formation, light-development, peptonization of gelatin, etc. Forster was the first to obtain such bacteria in pure culture from street-dirt, garden-earth, sea-water, and from the surface of luminous sea-fish. On the other hand, there are found, widely distributed in the earth, the air, the water, the contents of the intestine, etc., bacteria that under aerobic conditions require for their growth temperatures of from 50° C. (122° F.) to 75° C. (167° F.)—so-called *thermophilic bacteria;* under anaerobic conditions, however, these also thrive only between 34° C. (93.2° F.) and 44° C. (111.2° F.).

The **thermal death-point** for individual mature bacteria at the upper limit, on short exposure, ranges for the different varieties between 55° C. (131° F.), 60° C. (140° F.), and 80° C. (176° F.). The death-point at the lower limit

Fig. 8.—Diagram illustrating sporulation: *a*, Bacillus inclosing a small, oval spore; *b*, drumstick-bacillus, with terminal spore; *c*, clostridium, with central spore; *d*, free spores; *e* and *f*, bacilli escaping from spores (McFarland).

has been determined only for the smallest number of growths; many bacteria withstand a temperature of 0° C. (32° F.)—that is, freezing—without injury. These statements are naturally not applicable to thermophilic and glacial microorganisms.

In addition to dichotomous division some bacteria, especially the bacilli, and only exceptionally spirilla, and perhaps sarcinæ, proliferate through the formation of special *permanent bodies* or *spores.* (Fig. 8.) In the interior of the cell there forms an ovoid, intensely bright, and refractive body, the *spore*, which is enveloped in a firm capsule, the *spore-membrane.* One cell generally contains only one spore, which sometimes is situated in the middle, sometimes at an extremity. In the latter event, the rod appears swollen and club-shaped at the corresponding extremity, and it is designated a *drumstick-bacterium.* (Fig. 8, *b.*) If the swelling of the bacillus takes place at the middle in consequence of

sporulation, a spindle-shaped figure results, which is known as a *clostridium*.

At a later stage the body of the bacillus disintegrates, and the spore is set free. If this finds its way into a suitable nutritive medium of dead or living matter, it germinates, becomes a rod, the rod multiplies by dichotomy, and the whole process again terminates with the formation of new spores. The manner and the method in which the spore becomes transformed into a rod vary among different bacteria. Full details, in so far as they are of importance for clinical bacteriology, will be discussed in the special section of this work. In general the process of development is such that the spore first takes up water, then swells, next doubles in size, and loses its marked refractive property. The spore-membrane ruptures in the center or at one pole ; the young bacillus slips through the rent, retaining for some time the capsule of the permanent body.

Spore-formation and spore-germination take place only within certain temperature-limits, which differ for each individual bacterium. In the mode of sporulation just described the spore develops from the interior of the protoplasmic ground-substance of the rod, and this form of fructification is therefore designated *endogenous spore-formation*, in contradistinction from *arthrogenous spore-formation*, where individual segments of the cell-chain assume spore-qualities without passing through any intermediate stage. In external appearance these particular cells may differ in no respect from other bacteria of the same chain. Sometimes they become somewhat larger and brighter and more highly refractive and surrounded by a firmer capsule.

Attempts have been made, upon the basis of these variations in fructification, to divide bacteria into two great classes—(1) those with endogenous spore-formation (*endosporous bacteria*) and (2) those with arthrogenous spore-formation (*arthrosporous bacteria*). Among the latter class the cocci principally are grouped. However desirable it might be to classify the bacteria according to an intrinsic principle, it must be emphasized that the classification mentioned appears at ·least premature, inasmuch as our knowledge concerning the arthrogenous spores is as yet but slight and uncertain.

The *spores* represent *permanent forms* of the bacteria. Owing to the concentrated nature of their plasma, they are

extremely resistant ; much more so than the mature bacteria themselves. They withstand for years drying and all atmospheric influences. Nature possesses but one agency capable of rendering them harmless—namely, the direct action of the rays of the sun, insolation. In order to destroy the spores of anthrax-bacilli, which are not even among the most resistant, exposure to dry heat of 140° C. (284° F.) for three hours, or to steam of 100° C. (212° F.) for a few minutes, is necessary. Far greater resistance is displayed by the spores of Flügge's peptonizing milk-bacteria, as well as by some members of the group of hay-bacilli and potato-bacilli, which may survive exposure to live steam for five, six, and even for sixteen hours.

The question as to the existence of *pleomorphic bacteria* of varied form has not yet been decided with certainty. Pleomorphism has been conceded by some authorities to the proteus obtained by Hauser from decomposing fluids. It is, however, quite probable that the spheric bodies in the cultures of the proteus result in consequence of the retarded growth in the presence of progressive division. If the actinomyces are included among bacteria, there is no alternative but to admit the occurrence of pleomorphism. The actinomyces-fungus, however, is no longer grouped by most authorities among bacilli. Kruse, in Flügge's " Textbook," makes a special group of *streptothriceæ*, of which the actinomyces may be considered the most important representative. These streptothriceæ stand midway between the filamentous fungi and the true bacteria, and they are characterized by the formation ot filaments and especially by the ramification resulting in consequence of their germination. In old cultures the filaments break up into bodies resembling bacilli, spirilla, and cocci, and there is thus yielded an appearance of pleomorphism. A further peculiarity of the streptothriceæ is the formation of air-hyphæ that give rise to germ-cells (spores) by segmentation. These are probably not analogous to bacterial spores, as they are destroyed by exposure for five minutes to a temperature of 75° C. (167° F.). Nevertheless, they withstand higher degrees of temperature than the filaments, which undergo destruction at 60° C. (140° F.). As the result of personal investigation, we are of the opinion that the actinomyces-group represents a subdivision of the filamentous fungi.

Of late, attention has been directed to ramification, club-formation, and granular disintegration occurring among tubercle-bacilli and diphtheria-bacilli, and strongly suggestive of streptothriceæ. Upon the basis of such observations a number of authorities have concluded that the exciting agents of diphtheria and of tuberculosis, together with the whole actinomyces-group, belong to the hyphomycetæ. Such classification must, however, be considered as premature ; although the presumption is justified that the tubercle-bacillus and the diphtheria-bacillus stand in kindred relationship to the ray-fungus and the group of organisms represented by it.

Involution-changes must not be confounded with *variability in form of growth.* The former take place when the nutritive medium is exhausted and the bacteria begin to die ; the cells then swell, become thick and plump, show deficiencies, and undergo disintegration and other changes of allied nature. Whether all of the resulting peculiar forms are to be attributed to degenerative changes is a question that must, at least, be left open for the present. It is quite possible that the very large, so-called giant-cells result rather in consequence of especially favorable vital conditions. Other involution-manifestations appear to be due to special functions of the bacteria, such as their fermentative activity. In this manner are perhaps to be explained the involution-forms of the acetic bacterium, and especially of numerous microbes that are cultivated in media containing grape-sugar.

Bacteria are *ubiquitous :* they are found everywhere ; only the internal organs of the human and the animal body not in communication with the atmosphere are free from them. Some of the pathogenic germs confine their activities to certain living organisms, so that their area of distribution is, in consequence, a restricted one.

For their *sustenance* the bacteria require preformed organic carbon-compounds, as most of them, by reason of their deficiency in chlorophyl, are capable of utilizing the carbon dioxid of the atmosphere. They require also nitrogen-compounds, which they can obtain from organic substances, as well as from inorganic nitrates and ammonia-compounds. It need scarcely be added that water is necessary for the development of the bacteria. The nutritive material for the bacteria must be feebly alkaline or neutral, as in general they do not develop so well upon acid media.

Light is required only by such species of bacteria as contain chlorophyl. For the far greater number, the action of sunlight and even of diffuse daylight is directly injurious; their growth is inhibited, and after a time the bacteria are entirely destroyed by the action of the light. This sterilizing influence of light is most manifest when the rays of the sun fall vertically upon the surface of the cultures. Not only the growing forms, but also the permanent forms, the spores, succumb to this action of light. The spores of anthrax, for instance, are destroyed in a moist substratum by the action of light in a somewhat shorter time than the mature bacilli. In the dried state, however, the spores prove more resistant to the rays of the sun. The metabolic products of the bacteria also become materially attenuated under the influence of strong light, especially when there is no obstacle to access of oxygen. Nutritive media that have been exposed to light are not, in consequence, unsuitable for the cultivation of microorganisms. Not all rays of the spectrum are bactericidal, but only the blue, the violet, and the ultraviolet.

Of great importance is the *influence of oxygen.* Many bacteria are capable of developing only in the presence of this gas (*aerobic bacteria*); others, on the other hand, thrive only in complete absence of oxygen (*anaerobic bacteria*); a third group occupies an intermediate position: they grow as well in the presence as in the absence of oxygen (*facultative anaerobic bacteria*).

In their growth and multiplication the bacteria generate metabolic products upon and from their culture-medium. Thus the whole range of fermentative processes is an expression of bacterial activity, and the substances that are thereby formed are to be viewed as the metabolic products of fermentative bacteria. The same statement applies to putrefaction and decomposition.

A number of mostly innocuous bacteria are characterized by the formation of *pigment.* These *pigment-bacteria* produce most varied coloring-matters, which appear in all possible shades of color only when access of air is entirely unobstructed. Some bacteria give rise in their culture-media to beautiful fluorescence, others to phosphorescence— that is, they appear luminous in the dark.

The *chemic combinations* to which bacteria give rise in artificial nutritive media are at present of preeminent in-

terest. The ability of various bacteria to effect chemic decomposition may, from the present state of knowledge, be considered as extremely varied. The most profound reductions, and the most far-reaching oxidations, the formation of most complex combinations and their disintegration into atoms, are effected by bacteria. From nitrates are formed nitrites, free nitrogen, and ammonia; on the other hand, bacteria in the earth are capable of forming nitrates from nitrogen and ammonia. The bacteria of earth which are responsible for the process last named—the so-called nitrification-process—are of great biologic interest. They oxidize the ammonia—the final product of the decomposition of nitrogenous substances—into nitrites, and these further into nitrates, which then enter into the formation of new plants. Winogradsky recognized two varieties —the *nitrosobacteria* (nitrosococcus and nitrosomonas), which oxidize ammonia into nitrites, but not further; and the *nitrobacteria*, which are incapable of acting upon the ammonia, but, on the other hand, convert the nitrites into nitrates. Both species are characterized by the remarkable fact that they grow only in culture-media that contain no trace of organic carbon-compounds—that is, they are capable of obtaining the carbon they require from the carbon dioxid of the atmosphere, without the aid of chlorophyl.

Under appropriate nutritive conditions bacteria develop hydrogen sulphid from all sulphur-containing compounds. The so-called sulphur-bacteria (beggiatoa, thiothrix) require a supply of hydrogen sulphid, extracting the sulphur and storing it up in their protoplasm in the form of bright granules. With deficiency of nutritive material the sulphur-granules are oxidized into sulphuric acid.

Urea is decomposed into carbon dioxid and ammonia. There is scarcely an organic combination from which these gases and also free hydrogen may not finally be produced through bacterial activity.

Of especial significance is the bacterial decomposition of substances that serve for the nutrition and for the construction of the body. Starch is dextrinized, saccharified, even subjected to mucoid transformation. Sugar undergoes various forms of fermentation in consequence of bacterial activity; alcohol, carbon dioxid, acetic acid, lactic acid, butyric acid, succinic acid, and others, have been demonstrated

among the products of fermentation. Not only grape-sugar, but also numerous other varieties of sugar (lactose, galactose, arabinose, etc.) may undergo fermentation in consequence of the activity of certain kinds of bacteria. Fats are split up into fatty acids and glycerin. Much study has been devoted to the changes that albuminoid substances undergo as a result of the activity of bacteria, and that, accordingly as more or less fetid gases are generated, are designated putrefaction or decomposition. From the aromatic group of the albumin-molecule there result aromatic amidoacids (tyrosin, amidophenylpropionic acid, etc.) and certain benzol-derivatives, which impart to some bacterial cultures and to the feces their characteristic offensive odor (phenol, indol, skatol, etc.).

From the group of fatty bodies of albumin the bacteria form, in addition to fatty acids, oxyacids, and amidofatty acids (leucin, glycocol, alanin), a large number of organic toxic bases, which are designated *ptomains*, and for our knowledge of which we are indebted to the investigations of Selmi, Gautier, Nencki, and Brieger. The ptomains belong to the group of amins and ammonium-bases—for instance, cadaverin (pentamethylendiamin), putrescin (tetra-methylendiamin), cholin, betain, neurin, and muscarin ; of a portion of these substances only the chemic formula has been determined, although their constitution is as yet unknown—for example, saprin, gadinin, etc.

A knowledge of the metabolic products named is of the highest *diagnostic significance* in bacteriologic investigation. It is important to know whether a bacterium gives rise to the formation of acid or alkali, whether it causes coagulation of milk and liquefies gelatin by peptonization, whether it generates phenol and indol, to what extent it reduces nitrates, whether it forms gases, and which. All of these peculiarities may be of determining significance for the *identification* of a given bacterium.

Of considerable importance are the *chemic activities* displayed by bacteria in the domain of *physiology* and of *pathology*. Bacteria participate in the normal course of the digestive process in the human intestine ; the interesting experiment of Thierfelder and Nuttall, who, with complete exclusion of all bacteria, fed with sterile nourishment a young animal removed sterile by Cesarean section from the uterus of a pregnant mother, demonstrates that this participation

of bacteria in the process of digestion is not indispensable. On the other hand, the far-reaching bacterial decomposition of albumin, the formation of organic acids and of an abundance of gaseous products, not rarely give rise to severe digestive disturbances. The formation of hydrogen sulphid may cause intoxication from the gastro-intestinal tract (hydrothionemia), and in individual cases also hydro-thionuria. Bacterial decomposition of urea is the cause of cystitis ; and the formation of gas in the bladder by bacteria may result in pneumaturia. Cadaverin and putrescin may result from putrefaction in the intestine or in bronchiectatic cavities ; when certain intestinal mycoses exist, these substances appear in the urine (cystinuria).

Of far greater and more comprehensive importance, however, are those *metabolic products* that are produced in the tissues of the body by the exciting agents of disease, the *specific toxins*, which give rise to the toxic constitutional manifestations of the infectious diseases. These poisons are generated also by individual bacteria in artificial cultures. Löffler pointed out that by means of glycerin a chemic poison precipitable by alcohol could be extracted from cultures of diphtheria-bacilli, and with which the disease could be induced in animals in a manner analogous to that in which it is effected by the bacilli themselves. Roux and Yersin demonstrated that this diphtheria-poison is destroyed by a temperature of 100° C. (212° F.). They evaporated the poison-containing bouillon at low temperatures, and obtained a residue that was readily soluble in water, and highly toxic. The alcoholic extract proved innocuous. The poison is thus insoluble in alcohol, and is precipitable by this out of watery solution. On dialysis it passed very slowly through animal membrane. Roux and Yersin demonstrated further that the toxin of the filtered bouillon was most completely carried down with a calcium-chlorid precipitate. Attached to this precipitate and dried with it, the toxin proved much more resistant to heat. The poison proved active only when used subcutaneously or intravenously, while when administered by the mouth, it was harmless. Its action was specific, the palsies characteristic of diphtheria taking place. From the evidence, the French investigators considered the poison a ferment.

In Germany, Brieger and C. Fränkel later studied carefully the diphtheria-toxin, as well as the toxins of numerous

other bacteria, and especially the tetanus-toxin. They prepared the toxic substances either by evaporation of filtered bouillon-cultures in a vacuum at temperatures of from 20° C. (68° F.) to 35° C. (95° F.), and by precipitation of the concentrated bouillon with absolute alcohol; or they supersaturated the bouillon-filtrate with ammonium sulphate or sodium phosphate, and obtained from the precipitate the poison, which proved to be not dialyzable. The poisons thus obtained by alcoholic precipitation or separation by means of salts yielded albuminoid reactions. They could not be included among the globulins, as they were precipitable out of the bouillon-filtrate by the two salts named only, and not by magnesium sulphate. Brieger and C. Fränkel named the toxic substances obtained, and which appeared as amorphous powders, *toxalbumins*. These are destroyed by temperatures of 60° C. (140° F.); according to Buchner, the presence of neutral salts renders them somewhat more resistant. These albuminoid powders certainly contain the *specific bacterial poison*; with a minimal amount of the powder obtained in this manner from tetanus-bouillon, for instance, one can induce typical tetanus in animals. Nevertheless, the bacterial poisons can not with certainty be considered as albuminoid bodies. It is rather more probable that the actual active bacterial poisons are only carried down in the preparation of these albuminoid powders, and that they adhere to the albumin in a purely mechanical manner, as it was possible to obtain without difficulty specifically acting toxins by the growth of pathogenic bacteria in nutritive media free from albumin. (See Uschinsky's Nutritive Medium, p. 87.)

In the course of further investigations of the tetanus-toxin Brieger and Cohn also soon came to the conclusion that this substance represents no true albuminoid body. A notable advance in the knowledge of the nature of bacterial toxins was made through the investigations of Brieger and Boer. These investigators elicited the fact that the poisons of diphtheria and of tetanus are precipitated from their solutions, from filtered bouillon-cultures, by means of heavy metals, in the form of more or less soluble double combinations. A one per cent. solution of zinc chlorid proved most available, being added in double the amount of the toxin-solution. The double zinc-combinations thus resulting no longer exhibit any of the well-known albu-

minoid reactions. They are, however, in no less degree toxic, so that there can be no doubt that they contain the true specific poison. They are insoluble in distilled water, but readily soluble in feebly alkaline water or water containing sodium chlorid; they are most readily destroyed by acids, but, on the other hand, they are unaffected by substances of a feebly alkaline reaction. For the details of the preparation and of the properties of the toxins of diphtheria and of tetanus reference may be made to the proper chapters in the special section.

To be separated from these toxins, formerly designated toxalbumins, and which have, up to the present, been obtained in a manner perfectly free from criticism only in the two toxic-infectious diseases, diphtheria and tetanus, and recently in botulism, are those poisons that are contained within the bodies of the bacteria themselves. R. Pfeiffer first obtained these from cholera-vibrios and typhoid-bacilli, by destroying fresh agar smear-cultures through the action of chloroform-vapor, or through exposure to a temperature of 54° C. (129.2° F.) for an hour. These poisons, in contrast with the virus of diphtheria and of tetanus, are not demonstrable in the filtered cultures. They are, however, like the former, unstable in nature. Exposure to temperatures above 60° C. (140° F.) markedly reduces, without, however, completely abolishing, their activity. According to Pfeiffer's view, there remain secondary toxins that prove to be much more stable, withstanding boiling for several hours, but which are from ten to twenty times less toxic. A still further important difference exists between these intracellular poisons of typhoid fever and of cholera and the toxins of the pure intoxication-diseases—diphtheria and tetanus; while the former manifest their activity in animals immediately after their introduction and at once induce disease-symptoms, the latter only give rise to their toxic manifestations after a well-defined period of incubation.

The physiologic properties of the poisons of typhoid fever and of cholera will be thoroughly considered in the special section.

Finally, those toxic substances must yet be mentioned that are known as *bacterial proteins* (Buchner). These differ from the poisons already mentioned in withstanding a boiling temperature, and they appear, in contrast with the

specific bacterial poisons, to be the same in many or in all bacteria ; at least, their action is not specific. They never give rise to the typical clinical picture that is characteristic of the bacteria from which they are obtained, but they always give rise to fever and leukocytosis only, and, on subcutaneous injection, to local inflammation and suppuration besides. For the white blood-corpuscles they possess especially a most marked attractive influence, being with regard to them positively chemotactic. Administered in large amounts the bacterial proteids cause the death of the animal; but even then the course of the disease presents nothing characteristic, and not more so the postmortem findings. Detailed reference to the latter will be made in the consideration of tuberculin (see Tuberculosis), which, like mallein (see Glanders), is a proteid.

Chemically, these bacterial proteids approach the vegetable caseins. Treated with basic aniline dyes, they lose their toxic activity, and they may be considered as that constituent of the bacterial cell that confers upon it the property of taking up stains. The bacterial proteids are soluble in dilute alkalies, and insoluble, on the other hand, in dilute acids. They are best prepared by adding to a bouillon-culture in as large amount as possible bacterial scrapings from solid culture-media, and boiling the mixture for about two hours, then evaporating down to one-fifth or one-fourth, and filtering through porcelain. From the filtrate the proteid substances are precipitated by the addition of ten times their volume of absolute alcohol. They form an amorphous powder that is readily soluble in water. Besides, the filtrate of unboiled old bouillon-cultures generally contains a certain amount of proteid substances that have gradually found their way out of the bodies of the numerous dead bacteria into the fluid.

E. Buchner has recently devised a procedure for the preparation of unchanged cell-fluid. The cells are rubbed up mechanically and exposed to a pressure of from four to five hundred atmospheres. Buchner obtained in this way from yeast-cells a fluid of yellow color and alkaline reaction that yielded ten per cent. of solid constituents, and an abundance of albuminous bodies precipitable by heat. This so-called yeast-cell expressed fluid no longer contained living germs, although it still was capable of inducing alcoholic fermentation. H. Buchner considers the

substance present in the expressed cell-fluid, which possesses the properties of fermenting sugar, as parablastic, and designates it zymase. This zymase is produced within the cell, and H. Buchner compares the entire process with the production and the action of the toxins of tetanus and diphtheria, which likewise are formed within the bacterial cell.

Some species of bacteria are capable of thriving not only upon dead, but also upon living, matter, in man as well as in animals. Gaining entrance into a living organism, these bacteria multiply at the expense of their host, and, with the aid of their metabolic products, unfold their deleterious activity; the affected individual is made ill. Bacteria capable of inducing disease are designated *pathogenic;* those, on the other hand, that are innocuous and harmless are designated *nonpathogenic.* Those that are capable of undergoing multiplication only within a higher living organism are known as *parasites* (true, strict, obligate parasites). In contrast with these are *saprophytes*, those bacteria that thrive only upon dead material. There is, however, no sharp division between parasites and saprophytes. Many bacteria are adapted to both modes of life : these are *facultative parasites*—that is, they live only temporarily within the animal body, but usually external to it, in the earth or in water. On the other hand, many of the especially pathogenic bacteria are essentially parasitic. By means of our culture-media we have, however, succeeded in cultivating them outside the body, and have thus, by artificial means, made them *facultative saprophytes.*

II. INFECTION.

Those diseases are designated *infectious* that result through the vital activity of vegetable or animal microorganisms. The causative agents of the majority of the diseases included in this group belong to the class of bacteria. The filamentous fungi, as well as the lower forms of animal life (protozoa), have until now played a less important rôle in the etiology of disease. Formerly, the infectious diseases were subdivided into *contagious* and *miasmatic.* With the former, transmission takes place through *contagion*—that is, through the conveyance of the

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materies morbi from the sick to the well. With the latter, the disease-virus, the *miasm*, is taken up only from the air, or, in general, from surrounding nature ; the miasmatic diseases are never transmitted from individual to individual. This division has now lost much of its significance. Most infectious diseases with whose causative agents we are familiar are *contagious-miasmatic*—that is, they are transmitted as well from one person to another as from without, through the intermediation of the air, the water, etc. Even malaria may no longer be considered a strictly miasmatic infection, although under natural conditions it is probably never communicated from one person to another, since Gerhardt has transmitted the disease from a malarial patient to a healthy person by blood-transfusion.

According to the activity of their causative bacteria, infections may be divided into *toxic* and *infectious*.* In the former the manifestations caused directly by the living germ—that is, the local symptoms at the site of infection—are subordinate to the toxic manifestations—those resulting from absorption of the poisonous substances generated by the bacteria. As examples of such toxic infections may be mentioned experimental diphtheria in animals, and in man especially tetanus, the local manifestation of which often consists only in slight suppuration, or is entirely absent, so that the site of infection frequently escapes detection. On the other hand, in the infectious diseases the disease-germs themselves play the most important rôle, acting especially through their enormous multiplication. If this takes place throughout the entire body by way of the blood-stream, the condition is designated *septicæmia*. The type of such a condition is anthrax, in which, no matter in what situation the infection took place, the bacillus may be found present everywhere—in every organ and in every tissue.

Examples of infectious diseases in a restricted sense are, in man, for instance, cholera and pneumonia, in which enormous multiplication of the causative agents takes place within a circumscribed area (intestine or lung), and the local symptoms are very considerable. Just these two in-

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* This nomenclature is awkward and tautologic. The differences intended to be expressed are of degree and not of kind. All of the diseases of this group are infectious, naturally in varying grade, and all give rise to secondary intoxication, also of varying grade.—A. A. E.

stances, however, teach that the distinction between toxic
and infectious diseases is not absolute, but only relative.
Pneumonia is not unattended with constitutional symptoms
(fever, albuminuria, etc.) due to the absorption of the bac-
terial toxins circulating in the blood ; and, also, the pro-
found symptoms of the algid stage of cholera are only
explicable on the assumption of a poison generated by the
vibrios in the intestine, and thence absorbed into the circu-
lation. Even in the true septicemias the disease and death
are ultimately not alone caused mechanically by the indefi-
nite multiplication of the bacteria, but here, also, the chemic
activity of the bacteria—their production of poisons—
comes into play. Upon the other hand, even in the case
of tetanus, the most perfect representative of the toxic in-
fections, it has been demonstrated that the influence of
bacterial multiplication in the course of natural infection is
not entirely wanting. The multiplication of the germs,
however, is quite insignificant and transitory, and the re-
markable activity of the poison dominates the clinical
picture.

The mechanical factor plays a much more important rôle
in so-called pyemia than in the case of septicemia. In
the former condition the bacteria—generally pyogenic
microorganisms or those inducing inflammation—likewise
gain entrance into the blood, by way of the lymphatic chan-
nels, from a local, primary focus of disease. They do not,
however, become generalized, but remain within certain
organs, at times in the serous membranes, at other times in
the joints, at yet other times in the skin, in the liver, the
spleen, the kidneys, the myocardium, etc. The bacterial
masses circulating in the blood cause occlusion in larger or
smaller arterial areas, and there result in this way infarcts,
ischemic softening, abscesses. Pyemia is thus to be consid-
ered essentially a consequence of metastases of the causative
agents of the disease. In each of the metastases, however,
the bacteria again give rise to their toxic products ; which,
in turn, contribute to the further course of the disease.

Perhaps many of the diseases caused by filamentous fungi
(mycoses) depend upon purely mechanical lesions, with-
out constitutional intoxication of the organism ; but in
those diseases caused by bacteria the influence of poison-
production is of importance in every case : every infection
is attended also with intoxication ; only the more marked

prominence or recession of the latter justifies the division into infectious and toxic infections.

The term *infection* indicates, in verbal expression, the entrance of microorganisms into the animal body. With the mere entrance of the disease-germs into the body the infection—that is, the generation of the disease—is, however, by no means completed. The bacteria taken up may again leave the body : may, for instance, pass through the entire digestive tract without causing the slightest injury. Thus, cholera-bacilli may temporarily be found in the intestinal evacuations of healthy individuals, and tetanus-bacilli may be demonstrable in the intestinal contents of healthy animals. The germs taken up may, however, remain latent at the same place at which they later induce disease, without doing this in the first instance. Thus, bacteria, and especially those that we shall further on learn to be the cause of inflammation and suppuration, are present normally not only upon the entire cutaneous surface, but also in the mouth, in the upper air-passages, in the entire digestive tract, in the lower portion of the genito-urinary apparatus, in the external ear, in the eye—in short, wherever the external air has unobstructed access. The uninjured skin, however, as well as the normal mucous membrane, does not permit the bacteria to force their way beneath the surface ; and if they should multiply upon the surface and generate poisons—as, for instance, putrefactive bacteria certainly do in the intestine—these poisons, in the presence of a normal mucous membrane, are either not at all absorbed or not as such. A lesion of the skin or of the mucous membrane is first necessary to permit the entrance of the bacteria into the actual interior of the body (which normally is free from germs) and, thereby, the development of infection. Nor is the entrance of bacteria into the interior of the body by any means synonymous with infection. Even if a lesion has been induced, and if bacteria have gained entrance into the tissues of the body, the disease may not be developed. The bacteria may be taken up by cells of the body and be destroyed (phagocytosis) ; or they may succumb to the bactericidal property often possessed by the blood and the fluids of the tissues. They may, further, remain free and, in a certain degree, inactive until they die ; or, possibly, induce the disease at a later period. Thus, as has been demonstrated by an accidental observation of Vaillard,

infectious tetanus-material may gain entrance into a member, there remain without effect, and when, later, a contusion or some other injury of the member takes place, tetanus may long afterward occur. A similar latent period is probably also responsible for the observation recently made of the presence of tubercle-bacilli in the lymph-glands of apparently healthy individuals. Thus, the entrance of bacteria into the body does not invariably give rise to infection ; but, on the contrary, the conjunction of a whole series of circumstances is necessary in order that infection may take place. Of such circumstances, which are related partly to the infecting material and partly to the infected individual, the following are thus far known :

(a) **The Virulence of the Infectious Agent.**—The virulence of bacteria is a varying one. The degree of toxicity possessed by a bacterial culture obtained from the diseased focus decreases progressively : in the case of some bacteria more rapidly (as, for instance, diphtheria-bacilli, and most rapidly pneumococci) ; in the case of others, more slowly (an anthrax-culture, for instance, will remain toxic for weeks, a tetanus-culture for many months ; a culture of tubercle-bacilli, with suitable transplantation, will still be capable of infection after the lapse of years). The reduction in virulence is frequently accompanied by a corresponding reduction in activity of growth, though not always. Diphtheria-bacilli, for instance, are said to grow more luxuriantly upon artificial culture-media the more their virulence is diminished. The variation in degree of the virulence of bacteria can, therefore, not be dependent upon their varying activity of growth. The virulence must rather be considered to correspond with the capability of toxin-production : the more virulent bacterium generates a more active poison or a greater amount thereof than the less virulent organism. The reduction in virulence in artificial culture is prevented in the case of many bacteria by frequent transplantation upon fresh nutritive media ; it may, therefore, be in some way related to exhaustion of the culture-medium, a want of appropriate nutritive material, an accumulation of inhibiting metabolic products. Such an interpretation is applicable also to the marked reduction in virulence that is often observed in culture-media containing grape-sugar ; the process of fermentation appearing to influence the media in a manner unfavorable for toxin-

production.   Artificial means for reducing the virulence of a culture include heat (p. 40), light, electricity, as well as various chemic influences—for instance, iodin trichlorid with diphtheria and tetanus.

When the virulence of a bacterial culture has once been attenuated, it may again be intensified by passage of the bacteria through the bodies of susceptible animals.   The reverse condition—that is, attenuation of the virulence—may be effected by passing the microbes through relatively insusceptible organisms.   Pasteur in this way attenuated the bacillus of hog-erysipelas, by inoculating rabbits with successive generations of the organism.   In the same way he procured a mitigated virus of hydrophobia by continued inoculation of monkeys.

The more virulent a bacterium, the more readily does it give rise to infection, and the more severe is the course of the latter.   With a small amount of a pneumococcus-culture, prepared a day or two days previously from an infiltrated pneumonic lung, it is possible to destroy rabbits with certainty, in from twenty-four to forty-eight hours after the development of symptoms of septicemia.   With exactly a like amount of the same culture it is possible, two or three days later, to induce only local suppuration, which, after evacuation of the abscess, progresses to recovery ; or very slowly and insidiously, though without septicemia, to death. After a further interval of two or three days, inoculation practised in the same way with the same culture is unattended with result—the virulence is now entirely lost, and infection no longer takes place.   If, under the conditions named, the explanation of the diminution and loss of virulence is to be found in the age of the culture, it remains completely concealed under other circumstances.   Thus, there may be present in the same membrane in a given case of diphtheria, intensely virulent diphtheria-bacilli, and, besides, others free from all virulence, and which, therefore, are designated pseudodiphtheria-bacilli.   Inoculation of the former is capable of causing spread of the diphtheria, while the nonvirulent bacilli are incapable of causing the spread of the disease.

Finally, a further illustration from human pathology may be given to illustrate the relations between virulence and infection.   For the acquisition of pneumonia a predisposing cause frequently is necessary—a lesion of the lungs,

which is usually induced through the action of cold. The disease may be transmitted also from one pneumonic patient directly through the sputum to other individuals. Instances of house-epidemics of pneumonia have been reported, and which are capable of scarcely any other explanation than that the bacteria were present normally in the upper air-passages of the individual first attacked, and whence they have gained entrance into the pulmonary tissue, whose resistance has been diminished in consequence of the action of cold, and here have set up an inflammatory process. The bacteria, however, that are obtained from a focus of disease are more virulent than those that are found upon normal skin and mucous membrane. The virulence of the causative bacteria, which is responsible for the intensity of the disease, is increased in turn with the severity of the disease-process. This is true during the height of the infection for all bacteria. With the decline of the disease, naturally, when the bacteria have remained for a certain time in the organism now acquiring immunity, their virulence lessens, as a rule. Returning to our illustration of pneumonia, the course of events would be that the pneumococci derived from the pneumonic lung of the individual first affected would now be capable, by reason of their increased virulence, of infecting a second and a third individual without the aid of predisposing influences.

A striking illustration of the variation in virulence of microorganisms within the living body is furnished also by the pyogenic streptococci and staphylococci. These are found in association with all possible inflammatory and suppurative processes, from a simple panaris to the most intense septicemia or pyemia. Their morphologic peculiarities remain the same throughout. A difference is apparent only in animal experimentation. The cocci obtained from the benign affections prove slightly, if at all, virulent; while those obtained from severe infections induce the most deleterious consequences in experimental animals.

(b) **The Amount and the Purity of the Infectious Material.**—To induce infection experimentally in animals, a definite amount of the culture is always necessary, and this varies for different bacteria and individual species of animals, although it is almost constant for the same variety of animals and of bacteria. If a smaller amount of bacteria is used, infection will not take place. This relation is

most evident in the case of the purely toxic bacteria. If 0.5 cu. cm. of a tetanus bouillon-culture of determined toxicity are necessary to cause the death of a rabbit, 0.3 cu. cm. may perhaps induce passing rigidity, and the introduction of 0.1 cu. cm. will be unattended with any result; and if 0.00001 cu. cm. of another culture will cause the death of a white mouse, 0.000005 cu. cm. may possibly cause transitory mild tetanus, and 0.000001 cu. cm. no disease whatever. The greater or smaller number of bacteria may in this case play no part in the result, but the larger amount may be fatal because with the larger number of bacteria a larger amount of already prepared toxin also is introduced; the smaller amount of toxin, however, which the smaller number of bacteria carry with them, is readily withstood. With the infectious bacteria, also, a certain amount of the infecting material is necessary to induce infection. For dogs, highly virulent pneumococci injected subcutaneously are in marked degree infectious; they multiply rapidly, giving rise to extensive inflammation in the subcutaneous tissues, and they cause death—without septicemia, however. When the virulence of the culture is sufficiently intense, large dogs may be destroyed by as little as 0.5 cu. cm. of the pneumococcus-culture. There thus results a true infection, and the possibility of a purely toxic action may safely be excluded. From 0.1 to 0.3 cu. cm. of the same culture, on the other hand, fail to induce any disease whatever in the dog. The significance of the amount of the infecting material is more questionable in the case of those bacteria that give rise to septicemia. If pneumococci, which readily cause fatal septicemia in rabbits, are introduced into the circulation of these animals in great dilution, infection does not take place. On the other hand, according to some observers, in the case of the septicemia of white mice and of anthrax of guinea-pigs, a single bacillus, presupposing the virulence of the culture to be sufficiently great, may be adequate to induce infection. This, however, appears questionable, although it has been demonstrated that the individual bacillus also in these two instances does not necessarily cause infection, and that even one or two, or as many as ten, of the most virulent anthrax-bacteria of the guinea-pig may be borne without causing appreciable disease. That the amount of the infecting material is also of significance in the case of these bacteria endowed with especial virulence is

shown by the fact, established experimentally, that the larger the number of bacteria introduced, the more speedily does the death of the animal take place.

With regard to the *purity* of the infecting material, the question especially of *mixed infection*—that is, infection with a mixture of bacteria—arises. In a number of diseases in man several varieties of bacteria may almost always be found in the disease-focus ; thus, for instance, in diphtheria streptococci as well as diphtheria-bacilli, and in advanced tuberculosis pyogenic cocci as well as tubercle-bacilli. The presence of one variety of bacteria may facilitate the entrance and the activity of others, whose virulence is increased by the *symbiosis ;* in other words, infection with the one variety of bacteria is rendered possible through the agency of the other. Attenuated pyogenic cocci may be rendered again virulent by the simultaneous introduction of bacterium coli, of proteus, and even of saprophytes, such as prodigiosus, or hay-bacillus. Streptococci are said to restore the toxicity of attenuated diphtheria-bacilli when injected simultaneously into guinea-pigs. The causative agents of typhoid fever and of cholera regain their infectiousness when they are introduced into animals in association with streptococci, coli commune, or with metabolic products of proteus. Tetanus-bacilli or tetanus-spores alone, without their toxins, do not give rise to disease, as has been demonstrated experimentally by Vaillard ; but if with them are injected other bacteria in themselves indifferent (as takes place in natural infection through earth and splinters of wood), germination takes place, with toxin-production and the development of tetanus. For other anaerobic bacteria similar conditions appear to prevail ; at least, it is possible to favor materially infection with malignant edema and symptomatic anthrax by simultaneous inoculation with pyogenic cocci, proteus, or prodigiosus, or their metabolic products. With such mixed infection, the clinical picture—the infection—may be a mixed one, as the result of the activity of the various bacteria. Thus, in the clinical picture of septic diphtheria the distinctively septic symptoms are to be attributed to streptococci ; the intermittent fever of tuberculous patients, to pyogenic cocci. In other cases, however, the effect of the activity of the specific bacterium only may make itself manifest in the clinical picture (as with tetanus), and the rôle of the second is exhausted with the rendering possible of infection.

Finally, it is also possible, as Nencki has shown, that two microbes may produce an entirely new substance through their influence upon the culture-medium, and which neither of the two bacteria was alone capable of producing. Also, the observation of Nencki's, so characteristic for the significance of mixed infection, is to be borne in mind, that " sterile solutions of grape-sugar, simultaneously injected with two given bacteria, are much more rapidly and more energetically decomposed than by either of the two germs alone."

(c) **The Portals of Infection.**—Natural portals of infection are constituted by all those parts already named that communicate with the external world (p. 36). The most important infectious agents are taken up with the inspired air and with the nourishment or through the skin. The uninjured skin forms an insuperable barrier that can be overcome only by vigorous inunction of bacteria in an ointment-basis. It is possible, in this way, to produce furuncles by the rubbing in of staphylococci, and general infection by the rubbing in of anthrax-bacilli or of glanders-bacilli. If, however, a breach in the continuity of the skin takes place, then the chances for the invasion of bacteria are rendered much more favorable. Superficial cutaneous fissures suffice to permit the bacteria of anthrax and of septicemia to gain entrance into the organism. Deeper subcutaneous wounds are more dangerous, because the lax tissues permit absorption in much greater degree. Contused and lacerated wounds, to which access of the oxygen of the air is not unobstructed, favor the development of anaerobic bacteria, especially that of tetanus. The absence of oxygen appears, further, to constitute a favoring influence for the activity of the ordinary pyogenic cocci. Recent wounds take up microorganisms with remarkable rapidity through the opened blood-vessels. Within as short a time as thirty or forty minutes, bacteria, even saprophytes, placed upon a fresh wound may be found within the internal organs. In the case of old suppurating wounds, on the other hand, absorption of microorganisms takes place only in quite limited degree.

The mucous membranes also, in an uninjured state, prove not especially susceptible to bacterial invasion. If, however, a breach in the continuity of the epithelial covering takes place, then opportunity is afforded for the entrance and the absorption of the germs present. Exceptions to

the rules just cited are furnished by a number of mucous membranes in relation to certain microorganisms. The normal conjunctiva is, for instance, susceptible to invasion by the gonococcus and also by the bacillus of intestinal diphtheria. Upon the mucous membrane of the urethra likewise, only the causative agent of gonorrhea thrives. In the mouth, according to the investigations of Sanarelli, only two microorganisms develop properly : the diplococcus of pneumonia and the bacillus of diphtheria. The tonsils, however, with their markedly irregular surface, and with their richly developed lymphatic structure, do not share in the protection of the remainder of the mucous membrane of the mouth, but, on the contrary, constitute a frequent portal of entry for numerous infections.

The gastric juice, by reason of the hydrochloric acid it contains, is disinfectant and bactericidal, but this gastric-juice hydrochloric-acid barrier has for a long time been greatly overestimated. The permanent forms—the spores —are not at all affected by the gastric juice, and the resistant tubercle-bacilli in no greater degree ; and even less resistant germs pass the pylorus so rapidly, especially after the ingestion of large amounts of fluid, that the gastric juice is not afforded sufficient opportunity to cause the death of the microorganisms. The mucous membrane of the intestine is far more markedly predisposed to infection. The cause for this difference, as in the case of the tonsils, must be looked for in the abundance of glands, of lymphatic elements, and of the absorptive apparatus generally.

Bacteria are, under certain conditions, absorbed from the mucous membrane of the air-passages, and are then intercepted by the bronchial lymphatic glands. Only in this way is to be explained the not uncommon discovery of the presence of tuberculosis of these glands, with complete immunity of the lungs. The uterine mucous membrane is, as may be readily understood, a most suitable surface for the absorption of infectious agents during parturition and also during menstruation.

In animal experimentation subcutaneous, intravenous, and intraperitoneal injections are especially employed. Other methods of inoculation, such as the cutaneous, the intraocular, the intracranial (subdural), etc., are employed less commonly, and only for special purposes. In animal experimentation the same amount of a culture exerts differ-

ent effects in the same animal in accordance with the site
of introduction. An amount of pneumococci that, injected
subcutaneously, would cause death in a dog, will, when
given by intraabdominal injection, cause no disturbance.
Conversely, cholera-bacilli act more energetically in guinea-
pigs when introduced into the peritoneal cavity than by
subcutaneous inoculation. Cattle tolerate without ill result
the bacilli of symptomatic anthrax when injected into
a vein, whereas the same material introduced subcuta-
neously invariably gives rise to disease. In the same way
also in human pathology the point of entrance of the bac-
teria into the body is of importance for the occurrence of
an infection. Thus, cholera-infection occurs, as a rule, only
through the intestine, pneumonic infection only through the
upper air-passages—at least, it has been demonstrated that
the subcutaneous injection of not too large amounts of
cholera-bacilli or of pneumococci is without injurious effect
upon human beings.

(d) **The Susceptibility of the Infected Organism (Pre-
disposition).**—The susceptibility of different species of
animals to an infectious disease varies widely. To tetanus,
for instance, the guinea-pig and the white mouse are highly
susceptible, the rabbit far less so, and fowl so little sus-
ceptible that it is only with difficulty that tetanus can be
induced in these animals. To no variety of bacteria are all
animals equally susceptible. Thus, while cattle, mice, and
guinea-pigs are highly susceptible to anthrax, rats, dogs,
and birds are almost entirely insusceptible, and cold-
blooded animals tolerate the pathogenic microorganisms
almost universally without injury.

Also in the same animal species differences in suscepti-
bility exist toward the same bacterium. Thus, field-mice
suffer from glanders, while white mice do not. Older
animals are, in general, less readily infected—that is, they
are less susceptible than young animals. Congenital sus-
ceptibility is designated *natural predisposition*. This pre-
disposition is, however, not constant in degree even in the
same animal. It may be intensified or diminished. Thus,
insusceptible animals may be rendered temporarily sus-
ceptible to certain diseases by protracted hunger, great
muscular exercise, and similar influences. Such a *tempo-
rary predisposition* can, for instance, be induced in frogs to
anthrax by exposure to heat, in fowl to the same disease

by exposure to cold, in pigeons by hunger or long-continued withholding of water, and in white mice to glanders by the production of phloridzin-diabetes. In the same way, intoxication with alcohol or with various substances, especially such as are destructive of the blood-corpuscles, gives rise, temporarily, to especial susceptibility. The predisposition of diabetics to certain infections (suppuration, gangrene, tuberculosis) may also be mentioned. Likewise, a temporary predisposition is established, according to the well-known theory of Pettenkofer, through telluric (ground-water elevation) and temporal influences (summer's heat) when an epidemic of cholera occurs.

In addition to the *general predisposition* a *local predisposition* may be distinguished, depending upon the varying susceptibility of the different tissues of the body. Hermann undertook the establishment of a scale of susceptibility for the staphylococcus. The anterior chamber of the eye proved most susceptible; then followed the circulatory apparatus of the rabbit; next the subcutaneous connective tissue of the dog; then the pleura, the cerebral meninges, the subcutaneous tissue, and the peritoneum again of the rabbit. Little is known with regard to the actual conditions upon which the degree of predisposition or susceptibility of a body for a given species of bacteria is dependent. The word predisposition is only an expression for the sum of resistances that the body offers to infection. What the nature of these resistances is will be fully discussed in the next section in a consideration of the subject of immunity.

A certain measure of resistive influences against infection must be present in every animal tissue; at least, there appears to be no absolute susceptibility. The weapons of the bacteria against these resistances are most probably their toxins; in this way the significance of the virulence and of the amount of the infectious agents introduced is rendered comprehensible. On the other hand, it must be assumed that the devices mentioned that are capable of increasing the susceptibility (inanition, overexertion, overcooling and overheating, anemia, glycemia, etc.) diminish these resistances of the organism.

With regard to the *susceptibility of human beings* to bacterial diseases, this is comparatively slight for most infectious diseases—for the suppurations, pneumonia, cholera,

typhoid fever, and even tuberculosis ; only for influenza, for scarlet fever, and especially for measles, must a greater susceptibility be assumed in the case of man. From the constant contact with infective bacteria to which man is continually exposed, infections would be far more frequent than they really are if the predisposition of human beings to bacterial diseases were not, on the whole, but inconsiderable.

In general, the power of resistance of our tissues against bacteria is so great that, for infection to take place, an additional special contributing cause that diminishes this power of resistance—in other words, a *predisposing influence*—is necessary. Among such etiologic factors exposure to cold, traumatism, emotional disturbances, and overexertion (traumatic pneumonia, traumatic tuberculosis, typhoid fever after grief and worry, etc.) have long been known. Further, the bacilli may gain entrance into the body in especially large numbers, or they may possess increased virulence, as is the case in the event of direct contagion, especially in times of epidemic, when the bacteria possibly have several times completed their passage through the body.

In conclusion, it may be mentioned that the infection itself may act as a predisposing factor in the development of a second subsequent infection. When one variety of bacteria proliferates in a body in which another variety of bacteria is already in activity, the condition is designated a *secondary infection*. Examples are afforded by some varieties of pneumonia in cases of typhoid fever: the typhoid patient is attacked by the pneumonic infection because the resistance of his tissues is impaired as a result of the influence of the typhoid virus. Such secondary infections play a most important rôle in connection with the infectious diseases of man. The oral affections, the otitis, the bronchopneumonia, the cystitis, the suppurative and even the pyemic processes that so frequently complicate the primary disease usually are merely secondary infections ingrafted upon the original disorder. A number of these complications, naturally, do not represent true secondary infections, but only *secondary localizations* of the original disease-causing agent. Thus, pneumonia complicating typhoid fever may be due to typhoid-bacilli, and otitis complicating pneumonia may be due to pneumococci, etc.

Endemic and Epidemic Occurrence of Infectious Diseases.—Most infectious diseases attack human beings with varying frequency. We speak of the *sporadic* occurrence of a disease when only isolated cases appear ; and of *endemic* distribution, when a larger number of cases are constantly present. Measles, scarlet fever, diphtheria, and tuberculosis are, for instance, endemic in middle European populations, while cerebrospinal meningitis, mumps, etc., occur only sporadically. Asiatic cholera prevails endemically in the East Indies.

Under special conditions, any infectious disease may acquire extraordinary distribution, attacking a much larger proportion of the population than before, or extending beyond the borders of its previous area of distribution, and invading neighboring countries. We speak of *epidemics* of typhoid fever and of diphtheria when the usual number of cases occurring on the average within a given district is considerably exceeded. At certain intervals cholera leaves its Indian home to invade in widespread epidemics (*pandemics*) almost the entire inhabited world. The systematic causes that govern the occurrence and the subsidence of such epidemics have been made the subject of investigation, especially by Pettenkofer and by Koch. The epidemic distribution of infectious diseases may be, in large part, explained by the same influences that are responsible for the isolated infection ; but in every epidemic the *special* biologic relations of the causative agents, as well as the varying predispositions of human beings, demand particular study. Thus, pandemics of influenza are readily explicable from the fact that, on the one hand, the bacilli are contained in the sputum of the sick and with this are carried through the air, and, on the other hand, the susceptibility of human beings to this infection is unusually great. For the comprehension of epidemics of cholera it is important to know that the bacilli are contained in the dejections, and that with these they frequently find their way upon linen, clothing, contaminated articles of food, etc. ; that they are conveyed by insects, may be transported with soiled merchandise, etc. In this manner extension of the disease may take place from case to case in a *continuous chain.* *Explosive distribution* of a disease, simultaneous invasion of a large part of a community, occurs through equable distribution of the infective material over an extensive area—as

a whole city. Such dissemination can take place only through the air, the earth, or the water, which are common to all, and are capable of acting upon the entire population of a city at the same time and in the same way. In this connection the demonstration made by Koch in recent epidemics of cholera is of especial significance—namely, that the cholera-dejections gain entrance into the sewage through the first unreported cases and into the public waterways through those living along the streams (sailors), and from both of these sources frequently into the water-supply. An entire community may thus be simultaneously infected with the contaminated drinking-water, and in this way a cholera-explosion takes place.

In addition to these more special causes, social conditions naturally retain their significance as general causes of epidemics. An impaired state of nutrition affecting entire classes in a community, the absence of air and of light in dwellings, the abuse of alcohol, etc., must naturally increase the predisposition to infection in the same way as lack of cleanliness and density of population multiply the possibility of contagion. The disease-germs are, further, the less readily gotten rid of, and they proliferate the more freely the more filth and refuse defile the surroundings of human habitations. In this sense contamination of the soil acts as an important cause, and purification thereof through a proper water-supply and drainage as a fruitful means of prophylaxis, of the infectious diseases. The fact, also, that the warm season of the year favors the growth and the virulence of the causative agents of disease, and, on the other hand, through the numerous digestive derangements resulting in consequence of the increased heat and the thirst, increases the predisposition of the community, may be mentioned in further explanation of the occurrence of epidemics. Thus, a series of factors may be traced that shed light upon the previously mysterious origin of pestilences. On the other hand, it must be emphasized that there is yet much that is obscure in the etiologic relations of epidemics, and that further investigation is necessary to clear up these questions.

The Heredity of Infectious Diseases.—The transmission to the offspring of chronic disease existing in either parent at the time of conception may be considered as *direct infection* of the sperm-cell or the ovum. To what extent this actually takes place will be considered in discussing the

heredity of tuberculosis and of syphilis. A second possibility is that the predisposition to a given infection is inherited by the child from its parents. This aspect of the subject also will be more fully discussed in the chapter on Tuberculosis, with relation to which alone it is of material importance.

Intrauterine infection of the fetus in the course of acute infectious disease in the mother has been observed repeatedly. A number of cases are on record in which children have been born with the eruption of smallpox or with pneumonia. As the result of numerous experimental inquiries upon this subject—through the infection of pregnant animals—it may be accepted that the healthy placenta constitutes a dense filter, which permits the passage only of the toxins, but never of the bacteria, circulating in the blood of the mother. Living disease-germs can pass over to the fetus only when a lesion of the placenta is induced through slight hemorrhages or in some other way. More common than intrauterine infection is *infection during birth*, of which the blennorrhea of the new-born is a conspicuous example.

III. IMMUNITY, IMMUNIZATION, AND CURE.

Immunity is the insusceptibility to an infectious disease, the slighter tendency of an organism, or the complete impossibility, to be attacked by this disease. This property may be congenital in animals and man as *natural immunity*. Our domestic animals are never attacked by the acute exanthemata so widely disseminated among human beings ; and birds under natural conditions never suffer from anthrax, which is not at all uncommon among cattle. In the severest epidemics of cholera a large number of persons escape the disease, including even some living under the most unhygienic conditions and without any special precautionary measures, while those by whom they are surrounded are attacked without exception. Under these and all like conditions a natural protection against the given disease exists : the individuals possess a congenital, natural immunity—that is, the infectious agents that have gained entrance into the organism are incapable of inducing the specific disease-manifestations to which they give rise in other non-immune organisms.

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In the majority of cases the protection that exists is against the living causes of disease themselves, and which are incapable of development within the body of the particular animal. Much more rarely the basis of natural immunity consists in an insusceptibility of the organism to bacterial (p. 29) or similar poisons (snake-venom, ricin, abrin). As examples of this so-called natural immunity to poisons may be mentioned the rather marked immunity of fowl to tetanus-toxin, of rats to diphtheria-toxin, and of swine to snake-venom. Also in the case of the most marked immunity to poisons the importance of destruction of the microorganisms responsible for the poisons is not to be underestimated.

In contradistinction from natural immunity is *acquired immunity*. Human beings are attacked but once by a number of infectious diseases—a manifestation that is most marked in the case of scarlet fever, of measles, and of smallpox, but which is distinctly observable also in the case of cholera, typhoid fever, etc. Recovery from the disease has, under these conditions, conferred immunity—a state of protection against the same disease. All efforts to establish an *artificial immunity* through active intervention are based upon this natural process. The act through which this end is attempted is designated immunization or vaccination. The immunity effected—the disease-protection—is also designated protective inoculation.

The oldest **method of immunization** is represented by vaccination for smallpox. Recovery from mild vaccine-disease protects against severe variolous infection. The causative agent of smallpox is as yet not known, but, from all else that is known concerning immunity and immunization, it may be inferred that the causative agent of cow-pox is identical with that of variola, and represents an attenuated form of the latter—a view that has an experimental basis, especially from the investigations of Fischer (Karlsruhe).

In experimental pathology it is now possible to immunize animals against many, if not most, infectious diseases whose causative agents are known. Progress in this direction dates from the inoculation against anthrax undertaken by Pasteur, who attenuated anthrax-bacilli by exposure to high temperatures, and in this way obtained two vaccines. Vaccine I (exposure for from fifteen to twenty days at a temperature of 42° or 43° C.—107.6° or 109.4° F.) pro-

tected against vaccine II (exposure for from ten to twelve days at 42° or 43° C.—107.6° or 109.4° F.), and preliminary treatment with the latter afforded protection against virulent anthrax. The process here is quite analogous to that of vaccination for smallpox. Vaccine I induces the mildest degree of anthrax, recovery from which confers upon the organism the ability to withstand the moderate infection with vaccine II, and this in turn confers protection against the severest form—against true anthrax. The important and novel feature in this method of immunization consists in the application of heat to attenuate the causative agent of the disease. That which is effected by passage through the body of the cow in the case of cowpox-inoculation, the reduction of the virulence of the disease-virus, is effected similarly by heat.

As the possibility of immunization through heated bacterial cultures has been demonstrated by numerous later experiments for various other infections, there can no longer be any doubt as to the general applicability and the legitimacy of this procedure. The rule in question may be thus expressed : Above the temperature-optimum—that is, that temperature at which a bacterium thrives best and is most virulent—between this optimum and that temperature that causes death of the bacterium, there is for every variety of bacteria a temperature at which, though it still lives, it loses in virulence. This attenuation-temperature is for most bacteria between 40° C. (104° F.) and 70° C. (158° F.). The higher the temperature selected, the closer to the fatal temperature, the more rapidly does the attenuation take place. The property thus newly acquired is, however, generally not constant, the bacteria soon returning in succeeding generations to their original virulence. True vaccines—that is, varieties of bacteria that remain attenuated constantly in all successive daughter-cultures—are obtained only by means of the lowest possible temperature. Thus, anthrax-bacilli are materially attenuated on exposure to a temperature of 55° C. (131° F.) in ten minutes, and at a temperature of 42.6° C. (108.7° F.) in not less than from fourteen to twenty days ; but the latter only are available as vaccines. When, however, all that is desired is the production from a bouillon-culture of an immunizing fluid for temporary use, the attenuation-temperature is made as high as possible ; inasmuch as the cultivation of true vaccines in

the case of the more parasitic varieties, which spontaneously lose their virulence gradually in culture, has not hitherto proved successful. The introduction of a living bacterial culture attenuated by means of such a degree of temperature confers immunity to the corresponding bacterial disease. The act of immunization induces a mild disease, recovery from which gives rise to the immunity. For this reason the advent of immunity is, under these circumstances, never immediate, but it takes place only after the termination of the mild disease induced. According to the degree of attenuation, and the amount of bacteria introduced, as well as the mode of introduction, the time that elapses before immunization is complete varies from three to fourteen days.

The rôle of heat in the attenuation of cultures employed for immunization may be assumed by a number of other factors. Thus, immunization has been practised with cultures through which electric currents have been passed for some time; further, with cultures that have been exposed to sunlight; but most commonly with cultures that have been exposed to the action of chemic substances (antiseptics). Of the large number of these substances and methods of immunization, we shall mention only the attenuation of anthrax-bacilli by the addition of potassium bichromate or of carbolic acid; further, immunization to diphtheria and to tetanus by means of cultures to which iodin trichlorid has been added; and immunization by means of thymus-bouillon-cultures, in which the cellular substances of the thymus gland are supposed to exercise the attenuating influence.

Finally, the living organism also possesses under certain circumstances the capability of diminishing the virulence of microorganisms. Pasteur demonstrated this first for the bacillus of swine-erysipelas, by passing this organism repeatedly through the bodies of rabbits, which are but slightly susceptible. According to this method, two vaccines against swine-erysipelas of different strength are obtained, which are employed largely and successfully in France. Pasteur also obtained a marked reduction in the virulence of the virus of hydrophobia through continued inoculation of monkeys. Cowpox, as has already been stated, represents likewise only an attenuated modification of smallpox, the attenuation being effected by the passage of the disease through cattle.

In place of attenuated causative agents of disease, quantities insufficient to cause infection may be introduced. Immunity has been repeatedly induced by marked *dilution of unaltered living cultures*. The small number of microorganisms introduced causes only slight local disease, in the sequence of which a certain degree of immunity becomes manifest.

An essential difference exists between the methods of immunity hitherto discussed and those in which the immunizing material no longer contains living bacteria. With the germ-free filtrate of a bacterial culture it is possible, in many cases, in an analogous manner to that with the attenuated living bacteria, to confer immunity, and the attenuated virus (through heat, chemic substances, etc.), as well as the unchanged fully virulent filtrate, may be employed ; the latter, naturally, in a dilution and in amount below the lethal. The amount of toxin is gradually increased, a sufficiently long interval being permitted to elapse after the introduction of the next higher dose for the animal to recover completely from the action of the previous inoculation, and to regain its original weight. It may be assumed that here also recovery from the disease coincides with the establishment of immunity, and this seems to point to the fact that not the bacteria themselves, but rather the materials produced by them, contribute to the immunizing effect of the disease.

Such methods of immunization as seek to confer immunity by means of the metabolic products of bacteria, and which succeed in most cases, have been proposed in large number. Besides the germ-free filtrate of bacterial cultures, bacterial poisons of a most varied source have been employed. Immunization to cholera-bacilli and to typhoid-bacilli, for instance, has been attempted by R. Pfeiffer with toxic substances that are contained within the bodies of the bacteria themselves. Similarly, Koch, by mechanical trituration and repeated centrifugation, obtained from the bacilli themselves tuberculin R, with which, according to his own statement, he was able to protect guinea-pigs against highly virulent tubercle-bacilli. Immunization with bacterial metabolic products has acquired the greatest importance, however, in the cases of diphtheria and tetanus. This fact is not surprising, as both of these diseases belong to the toxic infections in which general symptoms

of intoxication especially predominate over those due to the local effects of the bacterial irritants.

We have thus far considered two modes of immunization —(1) the *attenuation-method* (Pasteur), in which attenuated cultures of living bacteria or attenuated toxins are employed ; and (2) the *dilution-method*, in which minimal amounts of virulent cultures or toxic metabolic products are employed.

Immunity to certain microorganisms may further be conferred by introducing them into the organism to be vaccinated through a portal of entry that is different from that through which the same bacteria gain entrance under natural conditions. On introducing the virus of pleuropneumonia of cattle into the extremity of the tail, there results, for instance, insignificant local disease, which is followed by immunity. The subcutaneous injection of cholera-cultures for the purpose of conferring immunity to Asiatic cholera may also be mentioned.

More difficult of comprehension is a smaller number of methods of immunization, in which, apparently, the bacteria themselves take no part. Thus, for instance, injections of hydrogen dioxid are said to afford protection against subsequent infection with diphtheria-bacilli. Under these circumstances it is to be assumed that either the previously injected material remains at the seat of injection, where it encounters the bacilli subsequently introduced, or their poisons, and these are, to a certain degree, thereby attenuated within the body ; or there may result an intensification of those forces of the organism that oppose barriers to the infection—forces that, as has already been said, are present also in the most susceptible organism, at least in a rudimentary degree. But few immunizing methods of this kind are known, and they are of little significance as compared with the large number of methods already mentioned, in which the bacteria themselves, or their metabolic products, participate in the establishment of immunity.

The last method of immunization to be considered is that suggested by Richet and Héricourt, and devised by Behring and Kitasato—namely, *immunization by means of the blood-serum of immunized animals.* Though demonstrated by the investigators named as useful only for staphylococcous septicemia, as well as for diphtheria and for tetanus, this method was subsequently employed against the infections due to other bacteria, and found to be practicable.

The blood-serum of an animal that has been immunized according to one or the other of the methods already mentioned is capable of conferring immunity upon a susceptible animal not previously treated. The same property that belongs to the blood-serum is possessed by all of the tissue-fluids, as well as the milk and the egg-yolk, of highly immunized animals. The most significant feature of this method of immunization is the simplicity and the rapidity with which it confers immunity. The immunity is, apparently, induced immediately as a result of the serum-injection, and no such phenomenon is observed as recovery from a disease marking the development of immunity. On the other hand, the immunity conferred by the injection of serum lasts only a few weeks, and is thus of much shorter duration than that induced more slowly through bacterial activity. Ehrlich has designated as *passive* the immunization that is effected by means of serum and in which the affected organism passes through no disease, in contradistinction from all other *active* methods of immunization in which a more or less severe disease must be passed through. Immunization by means of serum thus is *mediate* (*indirect*), in contrast with *immediate* (*direct*) immunization by means of bacteria and their products. To what extent we have gained a comprehension of the processes that take place in the act of immunization with serum will be discussed later. Here may be mentioned only the one direction in which this method of immunization has attained some degree of importance : it permits, in certain cases, the application of a harmless test as to the existence of immunity. Whereas formerly this could only be determined by inducing infection, at present a small amount of blood is obtained from the individual under examination, and observation is made to determine if the serum is capable of conferring immunity upon a susceptible animal. In this way it has become possible to decide with experimental certainty as to the existence or not of immunity also in human beings. It must not, however, be forgotten that the blood-serum is by no means always an index of the presence or absence of immunity. Behring refers to horses highly protected against tetanus, whose blood-serum exhibited no protective activity. Upon the other hand, there are animals whose serum possesses marked immunizing properties, and which themselves succumb to the mildest infection. Metschnikoff

found in blood-serum from cholera-cadavers, in a number of cases, a protective influence that at times was wanting in the blood of convalescents. We shall return to this variation in the relations between existing immunity and the immunizing power of the blood-serum.

Quantitative Limitations of Immunity.—Immunity is always *relative;* *absolute* immunity is theoretically inconceivable. The susceptibility or the insusceptibility to disease, on the one hand, and the intensity of the disease, upon the other hand, are more or less marked. All processes in this connection stand in regular quantitative dependence upon one another. A mathematically accurate measure of these variations in degree is at present impossible, as the bacterial poisons have thus far eluded chemic analysis, and a unit of measure from which to start out, therefore, does not yet exist. Approximately correct investigations have, however, been made more particularly in the case of tetanus and of diphtheria, whose bacterial cultures contain powerful poisons that pass over into the germ-free filtrate of bouillon-cultures. It has been shown in this connection that a definite amount of the immunizing culture establishes a definite degree of immunity, that with further introduction of the immunizing substances the degree of immunity also increases, and finally that the immunizing power of the blood-serum increases to a certain degree with the degree of the existing immunity. It is obvious that the protection conferred by inoculation is never absolute. A degree of immunity, however high, can protect only against a definite degree of disease ; if the highly immune animal is infected with an amount of bacteria or of toxin representing a still higher degree of disease, it will finally succumb. The highest degree of immunity that is known is probably that possessed by fowl to tetanus, which sufficiently protects against all danger of infection that exists in nature ; there is no natural tetanus among fowl. Even this high degree of immunity may, however, be unable to withstand an excessive degree of intoxication in laboratory-experimentation, and, in fact, tetanus has thus been developed in a typical manner in fowl and pigeons.

Specificity of the Immunity.—Immunity is, in general, specifically limited. The immunity conferred by vaccination protects only against smallpox ; that which follows recovery from an attack of scarlet fever does not protect

against measles. In the same way it has been demonstrated, also experimentally, that the immunity established by the various methods of immunization is specifically limited. Preliminary treatment with pneumococcus-cultures protects only against pneumococcus-infection; inoculation with typhoid-bacilli protects only against infection with these. Also, in the transmission of immunity through blood-serum a specific limitation of the activity of the serum has been demonstrable in all of the cases examined. It is noteworthy that when an animal is protected against several infections through a combination of methods of immunization, its blood-serum likewise is capable of conferring protection against all of these infections. Specificity of immunity and of immunization must be considered the rule, to which, however, there appear to be exceptions. Thus, Roux reported at the International Hygienic Congress at Budapest that tetanus-serum is antitoxic not only to tetanus-toxin, but also to snake-venom, while snake-venom-serum in turn proves active against scorpion-poison.

Heredity of Immunity.—Immunity is hereditary: it passes from mother to child—that is, it is conveyed with the blood from the mother to the child. In addition, as Ehrlich's experiments have shown, immunity is transmitted through the milk of immune mothers, so that the immunity established by nursing fortifies that which is inherited. The immunizing property of milk corresponds with the immunizing power of the egg-yolk of immunized fowl.

Inherited immunity is naturally only transitory, lasting but a few weeks, and ceasing with the elimination from the organism of the infant of the antitoxins conveyed through the blood during fetal life, and through the milk during the period of lactation (p. 70). The conveyance of antitoxins through the milk during the period of lactation has been demonstrated by Ehrlich for ricin, abrin, and tetanus in the case of mice. Vaillard has been able to confirm the accuracy of these observations. He has, however, shown further that they are by no means universally applicable, and that they are not to be applied without qualification to all species of animals.

Investigations into the Causes of Immunity.—Of the older theories as to the causes of immunity only the retention-theory and the exhaustion-theory need be mentioned. According to the first, there exists a substance that the

disease leaves behind in the body, and that prevents re-
peated development of the same disease-causing agents in
the organism. According to the second, recovery from a
disease is attended with the consumption of some substance,
without the presence of which the bacteria are incapable of
existence within the body. Probably neither of these hy-
potheses in its original form has advocates at present. For
a considerable time the scientific world was dominated almost
exclusively by the PHAGOCYTIC THEORY of Metschnikoff.
According to this, the wandering cells, especially the white
blood-corpuscles, of the immune and the immunized organ-
ism, as phagocytes take up the invading bacteria, prevent
their germination, and the production of toxins, and, finally,
cause their destruction. In susceptible organisms, on the
other hand, the bacteria are not attacked, multiply, and gen-
erate their poisons ; and when they gain entrance into
white blood-corpuscles, they prove victorious in the con-
flict that takes place in every instance between the phago-
cytes and the bacteria, and they destroy the leukocytes.
There are thus not only *mobile phagocytes*—as such are
known the wandering cells, the mononuclear and multi-
nuclear leukocytes, with the exception of the small lymph-
ocytes and the mast-cells—but also *fixed phagocytes*, which
are represented by endothelial cells, Kupffer's stellate cells,
the pulp-cells of the spleen and of the bone-marrow, and
connective-tissue cells. Metschnikoff designates the poly-
nuclear leukocytes and wandering cells *microphages ;* the
large mononuclear leukocytes and the fixed phagocytes,
macrophages. The principal rôle, however, is played by
the mobile phagocytes, which, in the sequence of infection,
appear in large numbers upon the scene of conflict. The
hastening of the leukocytes to the threatened area depends
upon the fact that in the immune body the bacteria generate
certain substances that attract the white blood-corpuscles,
and that, since Pfeiffer's investigations, are said to exert
positive chemotaxis. If, however, the leukocytes are re-
pelled, the condition is designated *negative chemotaxis.*

The phagocytic doctrine is based upon an exceedingly
large number of most careful observations. It is undeni-
ably correct that the phagocytic process occurs most con-
spicuously in those cases that terminate favorably—that is,
in the conquest of the microorganisms by the individual
organism—and that, on the other hand, it is less marked

when the bacteria gain dominance. That the leukocytes not only incorporate the bodies of dead bacteria, but also take up living microbes, to destroy them later, can, likewise, no longer be doubted, since Metschnikoff observed in the hanging drop how anthrax-bacilli, already inclosed within phagocytes, still developed, multiplied, and produced virulent cultures. It is possible, also, that a number of methods of immunization, in which relatively simple protective fluids are employed, induce their effects especially through phagocytosis. Thus, for instance, intraperitoneal injection of normal blood-serum, of physiologic salt-solution, of bouillon and other substances, by means of which it is possible to protect guinea-pigs against an otherwise lethal injection of cholera-vibrios, are powerful stimulants of phagocytosis.

In the interpretation of his observations Metschnikoff has, however, gone altogether too far. The inflammatory local reaction is, in the case of infections that pursue a favorable course, present, it is true, and prognostic conclusions may even be formulated from its occurrence ; but besides phagocytosis—and in this the opponents of Metschnikoff must be conceded to be correct—other factors enter into play. Among these are the bactericidal or antidotal properties that can be demonstrated to be present in the tissue-fluids, especially in the blood-serum of immune, and, in greater degree still, of immunized animals. These are to be attributed to a number of substances that, although thus far not chemically defined, are distinctly differentiable according to their physiologic activities. These are appropriately grouped together as ANTI-BODIES. They may be divided into (1) bactericidal, (2) lysogenic, (3) agglutinating, and (4) antitoxic substances.

1. BACTERICIDAL SUBSTANCES.—The property of the bodily fluids to destroy bacteria and inhibit their activity was first observed when microorganisms—as a rule anthrax-bacilli—were introduced into immune animals, and the bacteria were, after a time, seen to undergo degeneration at the site of inoculation. To establish the certainty that the living cells did not participate in this process, the anthrax-bacilli were introduced inclosed in paper bags and the like. In the experiments thus conducted various investigators observed degeneration of the inoculated anthrax-bacilli, and in some instances even destruction of their spores.

Metschnikoff and his pupils, however, obtained opposite results, finding that under the conditions named anthrax-spores thrive and produce virulent cultures.

Subsequently, the antibacterial activity of the bodily fluids, especially the blood-serum, was studied in the test-tube. The investigations of Fodor, Nuttall, Flügge, and others demonstrate that the defibrinated blood of various vertebrates destroys anthrax-bacilli in test-tubes, and that this peculiarity of the blood, however, disappears immediately on exposure to a temperature of 55° C. (131° F.). The first work of Behring in this domain tended in the same direction. Behring made the discovery that the blood of the white rat destroys anthrax-bacilli, and he made therefrom the deduction that the immunity of the rat to anthrax is attributable to this bactericidal activity. The most thorough investigations into these relations emanate from Buchner and his pupils. The Munich school assumes that the blood-serum and the bodily fluids derive their bactericidal property from special substances—the *alexins*—whose chemic nature has not yet been definitely determined. They are precipitated out of solution by alcohol, are destroyed by exposure for from half an hour to an hour to temperatures of from 55° C. (131° F.) to 60° C. (140° F.), and are attenuated by brief exposure to a temperature of 37° C. (98.6° F.), and by prolonged exposure to ordinary temperature. In the absence of salts the alexins are absolutely innocuous. For this reason the serum loses its bactericidal activity when it is dialyzed, or when it is diluted with eight or ten times its volume of distilled water. The original bactericidal activity may, however, be at once restored by the addition of sodium chlorid or other salts. Ammonium sulphate has proved most active in this connection, increasing the resistance of the alexins to heat quite ten degrees. The destructive influence of the alexins varies with relation to individual microorganisms. It may be well developed toward one variety of bacteria, and entirely wanting with relation to another. Between these two extremes there are all conceivable gradations. The number of bacteria exposed to the action of the bactericidal serum is, however, always of considerable importance, as even the most active of the bodily fluids are incapable of destroying more than a given number of bacteria.

In spite of these admittedly correct observations, the ex-

istence of immunity is not to be explained upon the basis
of this bactericidal activity, as there is a want of the neces-
sary relation between the degree of bactericidal activity and
that of natural immunity. White rats, to the bactericidal
activity of whose blood reference has already been made,
are not absolutely immune to anthrax, and the blood-serum
of dogs, which possess a considerable degree of immunity
to this disease, constitutes an admirable culture-medium for
anthrax-bacilli. It is, further, questionable whether the
alexins exhibit the same activity in the organism as in the
test-tube. According to Buchner's view this is the case.
Only in the capillaries, where the bacteria accumulate and
are thus washed by but little blood, is the influence of the
alexins not equally manifest.

What has been said of congenital immunity is applicable
also to acquired immunity. Only in exceptional instances
is there a proper relation between the bactericidal activity
of the blood-serum and artificial immunity—as, for instance,
after vaccination of guinea-pigs against the vibrio of
Metschnikoff, when the blood acquires bactericidal activity.

The bactericidal activity of the remaining bodily fluids is,
in general, less than that of the blood-serum. Such activity
has been observed in the aqueous humor of the eye, in all
possible exudates and transudates, and even in the saliva
and nasal mucus. The bactericidal activity of all these
fluids is, however, neither constant nor proportionate to the
degree of immunity present.

The alexins appear to arise from the leukocytes, and to
represent their secretory products. Denys and his pupils
demonstrated experimentally that the bactericidal activity
of the serum increases or diminishes with the larger or
smaller number of leukocytes respectively. Buchner and
Hahn obtained analogous results, and the view of the
Munich school may be expressed as follows : the leuko-
cytes constitute an important factor among the natural pro-
tective forces of the organism through substances in solution
secreted by them. Metschnikoff also assumes as established
the fact that a certain relation exists between the bactericidal
activity of the blood and the number of leukocytes ; and
he adds that with the death of the phagocytes, which takes
place abundantly on abstraction of blood, a portion of these
bactericidal substances is set free, and it is these that repre-
sent a large portion of the alexins of the serum.

2. LYSOGENIC ACTIVITY OF THE IMMUNE SERUM (PFEIF-
FER'S REACTION).—The bactericidal activity of the bodily
fluids in the case of artificial immunity differs from that
described as due to the alexins, as was discovered by R.
Pfeiffer and his pupils. If guinea-pigs are immunized with
carefully destroyed cultures against cholera-vibrios, typhoid-
bacilli, coli commune, and other similar microorganisms,
the animals thus treated acquire the property of dissolving,
after intraperitoneal introduction, the bacteria toward which
they have been immunized. In order to follow this phe-
nomenon, which is now generally known as *Pfeiffer's reac-
tion*, directly under the microscope, a small amount of the
exudate that forms in the abdominal cavity shortly after in-
traabdominal injection of the bacteria is removed, from time
to time, by means of capillary glass tubes. Immediately
after the injection the microscopic field exhibits complete
immobility of the bacteria; several (up to ten) minutes later
these appear swollen, exhibit beginning disintegration into
granules, and after the lapse of ten minutes more the exu-
date contains only fine granules. If, at this time, plates are
inoculated with the fluid obtained, they remain sterile.
Precisely the same results are obtained if, instead of vacci-
nated guinea-pigs, animals are employed that were not pre-
viously treated, and cholera-bacilli or typhoid-bacilli, mixed
with a minimal amount of serum from an animal that has
been immunized against cholera or typhoid fever, are in-
jected into the peritoneum. In this experiment, also, dis-
integration of the bacteria into fine granules, a gradual dis-
solution within the infected body, is observed.

Pfeiffer designates as bactericidal such serum as yields
the reaction described by him. This designation, however,
has, as has already been pointed out, been employed in
another sense—namely, for the germ-destroying action of
blood-serum in the test-tube. It is, therefore, well to
adopt the suggestion of C. Fränkel, who designates such
serum as yields the phenomenon of Pfeiffer as lysogenic, or
solvent, serum.

Lysogenic serum tolerates exposure to 60° C. (140° F.)
for an hour, its bacterial solvent activity being thus scarcely,
if at all, affected.

Pfeiffer himself had recognized that the serum of normal
animals also is capable of inducing the reaction described;
but in this instance it is necessary to inject a much larger

amount of serum than when the serum of immunized animals is employed. From this it appears that the quantitative relations are of significance with regard to the reaction of Pfeiffer. In order to express these relations mathematically, Pfeiffer establishes as a standard or a unit for the serum that amount that is just necessary when injected simultaneously into the peritoneal cavity to destroy ten times the minimal lethal amount of living bacteria. At least 0.05 cu. cm. (of goat-serum, however, only 0.2 cu. cm.) of the serum of a normal animal are necessary for this purpose; whereas only one-tenth of a milligram of the serum of a highly immunized goat, for instance, suffices. With these quantitative limitations the reaction of Pfeiffer may be considered specific. Cholera-serum manifests its lysogenic activity in the peritoneal cavity of guinea-pigs only against cholera-vibrios; typhoid-serum only against typhoid-bacilli, etc. The serum of individuals who have recovered spontaneously from typhoid fever or cholera likewise exhibits the phenomenon of Pfeiffer. The standard equals about 0.01.

The reaction of Pfeiffer may be admirably employed for purposes of differential diagnosis. With its aid it is possible to differentiate true typhoid-bacilli and true cholera-vibrios from the great horde of microorganisms resembling typhoid-bacilli and cholera-bacilli respectively. It need scarcely be emphasized that precisely in this method of differential diagnosis the quantitative relations must be most carefully considered.

3. AGGLUTINATION (GRUBER'S REACTION).—The serum of animals immune to cholera, typhoid fever, coli-infection, etc., or of human beings that have recovered from typhoid fever or cholera, behaves in a peculiar manner when added in small amount to typhoid-bouillon, cholera-bouillon, coli-bouillon, etc. The bacteria lose their motility, collect in masses, and sink to the bottom of the test-tube as a flocculent precipitate; whereas the supernatant fluid remains perfectly clear. General attention was directed to this reaction of immune serum through the labors of Gruber and Durham, and somewhat later of Pfeiffer and his pupils, although a number of investigators, and more particularly Bordet, had described it previously, without, however, considering it of special importance. Gruber introduced the term agglutination to describe the phenomenon. The reaction is ob-

tained either macroscopically or microscopically in one of the three following ways:

1. To fresh, sterile bouillon, in carefully measured amount (p. 65), is added one drop of immune serum, and the mixture is inoculated with cholera-bacilli, typhoid-bacilli, etc., and then placed in the thermostat at 37° C. (98.6° F.). After the lapse of from four to seven hours the first clumps appear in the culture, which after from twelve to twenty-four hours presents a typical appearance. The bacteria are seen deposited at the bottom of the test-tube as small flocculi, in a certain degree precipitated, while the overlying bouillon is perfectly clear. In order that the reaction may not be overlooked, it is advisable to inspect the fluid quite frequently—if possible, from hour to hour.

2. To a twenty-four-hour-old bouillon-culture immune serum is added, as in the previous procedure, and the mixture is placed in the thermostat at 37° C. (98.6° F.) for from one to eight hours. The originally turbid culture soon clears up, and a flocculent precipitate forms.

3. To demonstrate the phenomenon of agglutination microscopically it is best to employ young bouillon-cultures not more than twenty-four hours old. In the employment of older cultures there is danger of mistaking for actual agglutination the clump-formation that not rarely takes place spontaneously, especially on the surface of the fluid. The observer assures himself previously through control-preparations that the bacilli in the bouillon employed are actively motile, and especially that they are distinctly separated one from another. Then to 10, 30, 40, 100 (representing 5 cu. cm.), 200 (about 10 cu. cm.), 1000 (about 50 cu. cm.) or even a larger number of drops of this bouillon in sterile Petri dishes is added one drop of immune serum, and microscopic preparations are made of the various mixtures. If the serum exhibit agglutinating activity, a number of confluent masses or islands of bacteria become visible, as a rule. In these the microbes are absolutely immobile, whereas in the free intervals, a greater or lesser number are at first still in active movement. Should the preparation exhibit molecular movement, it is permitted to stand for from a quarter to half an hour, after which it is again examined. The agglutination is favored by evaporation and by the presence of oxygen. To prevent evaporation, the examination may be made in hanging drop, although this is

somewhat inconvenient. The characteristic islands are then seen to form at the periphery of the drop.

Gruber's reaction can also be obtained with the serum of normal animals and human beings, but in this instance much more serum is required than when the serum of immune animals is employed. In the case of normal serum the proportion in the large majority of cases exceeds 1 : 10—that is, the addition of more than one drop of serum to ten drops of bouillon is required to induce agglutination. Only in rare instances has a proportion of 1 : 30, or even of 1 : 40, been noted in human beings. The serum of rabbits, horses, asses, possesses agglutinating activity that varies between 1 : 30 and 1 : 40. The serum of guinea-pigs, as a rule, exhibits no agglutinating property whatever. It is, therefore, absolutely necessary in studying the agglutinating reaction to consider the quantitative relations in every instance. In applying the test according to either the first or the second of the methods described, several observations are always made. To the bouillon is first added serum in the proportion of 1 : 10, in a second test-tube in the proportion of 1 : 20, then 1 : 30, 1 : 50, 1 : 100, etc.

With an observance of the precautionary measures noted the reaction of Gruber is of great service in the differential diagnosis of bacteria that resemble one another morphologically. It is preferable to the reaction of Pfeiffer, as it is much more easily performed. To identify cholera-bacilli, typhoid-bacilli, etc., the serum of animals immune to cholera, typhoid fever, etc., is tested to determine its power of causing agglutination of bouillon-cultures of the respective organisms. If the reaction is negative when a dilution of 1 : 10 is employed, it may be concluded with certainty that the bacteria under examination are not identical with those to which the animal yielding the serum has been rendered immune. If, however, the reaction prove positive, the result is in favor of the conclusion that the microorganisms are the same. To establish completely the identity of the microorganism it is necessary, however, to determine how far the degree of dilution can be carried. In individual instances this may reach several thousand. The proportion of from 1 : 50 to 1 : 75 is, however, quite sufficient, and such a dilution will be satisfactory if but a small amount of serum is available. The extreme limit of agglutinating activity is best determined with the aid of the

5

microscope. When excessive dilutions are employed, the reaction may escape macroscopic observation; whereas, under the microscope, after a time (from one minute to two hours), agglutination may yet be observed.

In addition to typhoid-cultures, cholera-cultures, and coli-cultures, the reaction of Gruber has been demonstrated with numerous other, and also nonpathogenic, bacteria. The agglutinating property of the serum is, further, not present immediately after introduction of the bacteria into the animal body; at least three and a half, and generally even five, days must elapse before this appears. Besides the blood-serum, the reaction of Gruber may be obtained in an intense degree with the serous contents of blisters induced by vesication, in lesser degree with milk, and in still less degree with urine, dropsical fluid, exudates, bile, tears, and aqueous humor of the respective animals and human beings.

Gruber considered the phenomenon of agglutination as a *reaction of immunity*, and attempted to base upon it a new theory of immunity, to which we shall later refer. A not inconsiderable advance was made in this connection when Widal showed that agglutination, at least in human beings, represents a *reaction of the period of infection*. Widal demonstrated that the serum of typhoid-fever patients at the end of the first or at the beginning of the second week of the disease most distinctly yields the agglutination-phenomenon with typhoid-bacilli (Widal-Gruber reaction). This fact is of the greatest importance from a practical point of view. It renders possible the diagnosis of typhoid fever with the aid of the serum of a suspected patient. The same appears to be true for Asiatic cholera and other diseases. We shall fully discuss the so-called serum-diagnosis in the special section. (See Typhoid Fever.)

The nature of the agglutinating substance has not yet been determined despite numerous investigations, especially on the part of Widal. It appears to be quite resistant, as exposure of the serum to a temperature of 60° C. (140° F.) for an hour fails to abolish the agglutinating phenomenon; this result is obtained only after exposure to a temperature of 80° C. (176° F.). The reaction of Gruber may be obtained also with dead bacilli. The bacteria are best destroyed with formol, and they can then be kept for weeks without losing in the slightest degree their sensitiveness to the action of the serum. From this Widal arrives at the

conclusion that the agglutination is not a manifestation of
the vital activity of the bacilli, but rather that it represents
a passive reaction on the part of the protoplasmic sub-
stance.   According to a more recent statement by Kraus,
a mixture of immune serum with filtered cultures, after
exposure for twenty-four hours to a temperature of 37° C.
(98.6° F.), exhibits a precipitate, and sometimes even the
formation of flocculi.   According to Gruber, the agglutinat-
ing substances are derived from the bodily constituents of the
bacteria.   This statement, however, can not be sustained,
as agglutinating properties may be observed in the serum
of animals after the injection of soluble metabolic products,
of filtered, quite young bouillon-cultures.

We have already stated that, as the result of his investi-
gations of agglutination, Gruber attempted to establish a
new theory of immunity.   This assumes that the aggluti-
nating substances cause the bacterial membrane to swell,
and thus render the bacterial protoplasm accessible to the
alexins of Buchner, in consequence of which death of the
microorganisms is brought about.   This hypothesis of
Gruber, however, is not supported by the facts elicited by
further investigation.   There need be no relation between
agglutination and immunity.   There are cases in which, in
spite of the agglutinating property of the serum, immunity
does not exist, and vice versâ.   Further, the assumed swell-
ing of the bacilli can not be demonstrated microscopically.

4. ANTITOXINS.—The anti-substances of the bodily fluids,
and especially of the blood-serum, thus far described, mani-
fest their activity, on the whole, directly against the bacteria
themselves.   The conditions are quite otherwise with that
class of anti-substances that are the last to be discussed, and
are, probably, the most important.   We have reference to
the substances designated *antitoxins*, which derive their
name from the fact that their energy is directed not so
much against the microorganisms, but rather against the
metabolic products (toxins) generated by them.   Their dis-
covery in the case of diphtheria and of tetanus is the funda-
mental work of Behring, and the stimulus to their further
investigation was given especially by Ehrlich.   The latter
found in ricin and in abrin—two albuminoid vegetable
poisons—substances presenting numerous points of resem-
blance to bacterial poisons, and with which some of the laws
of immunity can be readily studied.   As a result of his

experiments with these substances, Ehrlich recognized that in the process of immunization through the activity of the disease-poisons in the body of the patient, substances are formed that he designates anti-bodies (antitoxins). These are, in a certain sense, antidotes, inasmuch as they neutralize or prevent the toxic action of the disease-poisons. When these antitoxins are present in sufficient amount, immunity exists. In the process of immunization with the aid of bacteria or their poisons (*direct, active, immediate immunization*), the antitoxins are formed either from the bacterial products themselves, or, under their influence, from substances that exist in the body preformed.

The disease continues until an adequate amount of antitoxin has been formed. For this reason the older methods of immunization are effective only after disease of greater or less severity, and after the lapse of a certain time. Immunization by means of serum (*passive, mediate, indirect immunization*) represents the transmission of preformed antitoxins. Therefore, this method, on the one hand, induces no disease, and, on the other hand, it establishes immunity at once ; and this is, for the same reason, also, more transitory, lasting only a few weeks.

Whence the antitoxins are derived has not yet been finally determined. The view that the antitoxin is formed directly from the toxin introduced for purposes of immunization appears gradually to be losing ground. It is more probable that in the course of every toxic disease the antitoxin is produced together with the toxin within the body. The antitoxic activity of the serum is abolished by exposure to a temperature of from 60° (140° F.) to 70° C. (158° F.).

The manner in which antitoxins act upon the bacterial poisons has not yet been clearly determined. Originally, it was assumed that the antitoxins destroy the bacterial poisons. The injection of a mixture of an antitoxin-containing serum and bacterial poison proved innocuous. From this it was concluded that the poison is destroyed by the antitoxin of the serum. It soon transpired, however, that destruction of the poison by the antitoxin does not take place, but that, to use the expression of Ehrlich, in the physiologically neutral mixtures of toxin and antitoxin both sets of constituents are yet present as such. Buchner and Roux assumed an action of the antitoxin upon the cells, as a result of which the latter are rendered immune to the in-

toxication. In contradistinction from this cellular hypothesis is the chemic view of Behring and Ehrlich : that toxin and antitoxin undergo a sort of double combination, which proves innocuous for the tissues. The decision of this question appears finally to have been made in favor of the chemic theory by the more recent investigations of Ehrlich with regard to ricin and antiricin. Ricin has the property of causing the red blood-corpuscles in defibrinated blood to collect together and to be precipitated to the bottom of the vessel—a process from which vital processes may with certainty be excluded. Ehrlich showed, then, that antiricin, which is present in the blood-serum of animals immunized to ricin, abolishes the activity of ricin in the test-tube, and that the peculiar coagulation following the addition of ricin-serum no longer takes place. Ricin and antiricin must, in this instance, have directly influenced each other chemically. Ehrlich was further able to demonstrate that the combination of toxin and antitoxin takes place much more quickly in concentrated than in dilute solutions, that heat hastens and cold retards its occurrence. As similar manifestations are observed in chemistry in the formation of double salts, it would seem probable, according to Ehrlich, that also the neutralization of toxins by antitoxins represents the formation of a double salt.

The immunization of mammals against toxins is always attended with febrile reaction, and it, therefore, appeared that the formation of an antitoxin would not be possible in the absence of fever. Metschnikoff, however, found that of all animals the crocodile produces antitoxin most abundantly and most speedily, in spite of the fact that febrile movement does not take place.

According to the investigations of Behring and his collaborators, the antitoxin distributes itself throughout the organism in man and in animals in such a manner that after absorption of the injected serum, after passive immunization, the blood to a certain extent extracts the antitoxin from the tissues and stores it up. Twenty-four hours after subcutaneous injection of serum, and in a shorter time after intravenous or intraperitoneal injection, the maximum amount of antitoxin in the blood is demonstrable. The antitoxin is absorbed from stomach and bowel only when lesions of the mucous membrane exist. The maximum content of antitoxin in the blood persists for several days.

After this, the amount of antitoxin in the blood gradually diminishes. It now appears in the milk, in the urine, etc., until finally it is wholly swept out of the body. The rapidity with which this elimination of antitoxin takes place is most variable, in accordance with the different conditions present. It is the greater the larger the amount of immunizing serum injected. In the case of diphtheria, the protection in human beings following the usual immunization with 250 antitoxin normal units lasts about four weeks.

Success in the preparation of antitoxic serum may be hoped for only when the poison, the toxin of the respective species of bacteria, is known, and can be prepared of sufficient strength. It has thus far been possible to obtain a high degree of toxin-immunity only in the case of diphtheria, tetanus, botulism, snake-venom, ricin, and abrin.

It has already been mentioned that the lysogenic and the agglutinating substances of the serum are also present in the blood of normal individuals. The same statement applies also to the antitoxins. Attention has been called by a number of observers to a certain neutralizing activity on the part of the normal blood-serum of horses and of human beings against the poison of diphtheria and other similar poisons. The *natural antitoxic power* of the blood-serum is, however, only slight. It by no means attains the high degree of activity exhibited by the serum of animals artificially made poison-proof.

From the fact that immunity can be transmitted by means of serum containing antitoxin, the doctrine that protection against toxins is the cause of immunity has been brought forward. Behring, and also Ehrlich, ascribe the real cause of acquired immunity to the antitoxic property of the blood. However attractive this theory may be, all of the existing facts can not be brought in harmony with it. Reference may be made to the instances of a want of relation between the occurrence of antitoxin in the blood (immunizing capability of the blood-serum) and the presence of immunity (p. 55). It was mentioned that the occurrence of antitoxin in the blood of animals by no means establishes necessarily a condition of immunity in the latter, and that, under certain circumstances, the organism whose blood-serum possesses pronounced immunizing capability, exhibits not only not an increased, but even a diminished, degree of resistance to the bacterial

poisons (Behring's hypersensibility); while, on the other hand, marked immunity may exist without the presence of antitoxins in the blood.

As the result of such observations, a distinction has been made between *tissue-immunity* and *serum-immunity* (*antitoxin-immunity*). The latter is a transitory condition, dependent upon alterations in the blood-mixture from the presence of the circulating toxins; the first is a permanent condition, dependent upon changes in the tissues, upon the activity of the cells, which have become insusceptible to the poisons. Tissue-immunity, or histogenic immunity, is not to be referred to the presence of antitoxins. Fowl, which are highly immune to tetanus, possess little if any antitoxin; but their blood becomes at once antitoxic after injection of tetanus-toxin.

Recently, Behring has returned to his original view, and believes that acquired toxin-immunity, active as well as passive, is always hematogenous—that is, dependent upon the antitoxic activity of the serum. As histogenic he considers only the natural immunity to the bacterial poisons. Finally, the antitoxins alone are as incapable as the other anti-bodies or as phagocytosis of explaining all of the manifestations of immunity.

We have in the foregoing presented, as objectively as possible, the facts that investigation in the domain of immunity have developed in great abundance. They do not permit of the establishment of a single, universally applicable theory of immunity. They rather render it probable that there is no such unity, but that immunity is eventually not a simple and indivisible process, dependent in all cases upon one and the same basis, but, apparently, variable and complex in its nature, dependent in one instance upon this, in another instance upon that, cause, and more frequently due to several in combination.

RELATIONS BETWEEN IMMUNITY AND CURE.—In the case of scarlet fever, measles, and other like diseases, recovery from an attack is attended with immunity. If in the case of other diseases—as, for instance, pneumonia, erysipelas, etc.—recovery from one attack rather predisposes to subsequent attack, this does not exclude the fact that at the time of recovery immunity existed, a so-called temporary immunity, which disappears in the course of a few days or weeks. It is noteworthy in this connection that the dem-

onstration was first made experimentally for pneumonia, later for typhoid fever, diphtheria, and cholera, that the blood of individuals convalescent from these diseases exhibits in many instances transitory immunizing activities with relation to the respective bacteria. It appears from this as if recovery from these diseases also is attended with immunity, but that this—through causes not yet made clear—is a quickly passing one.

It has been demonstrated experimentally with certainty that immunization may also lead to cure. By means of serum-immunization it is possible, if the serum is derived from animals highly enough immunized, to induce curative results, even when the treatment is begun a certain time after infection has taken place. These facts are most conclusively demonstrable experimentally in the case of tetanus. With large amounts of serum from animals immunized to tetanus, it is possible to save mice and guinea-pigs that already exhibit distinct tetanic manifestations. We shall refer more fully to these relations in the special section. (See Diphtheria and Tetanus.)

According to the foregoing, two facts have been demonstrated—in the first place, that immunity exists at the time of recovery in the sequence of toxic diseases in man, and, in the second place, that it is possible experimentally to cure infection by the timely establishment of immunity. From this the conclusion may, with all probability, be drawn that also in human beings the connection between recovery and immunity consists in the bringing about of recovery through the development of immunity; recovery from the given disease immunizes the organism, and cure is effected in consequence of immunization naturally induced, and especially the crisis appears as the expression of cure through the sudden setting in of immunity. Upon this knowledge is based recent therapeutic effort, which is known as *serum-therapy* or *immunization-therapy*. The object to be attained is the immunization of the diseased organism after infection has taken place—that is, the effecting of a cure in the same way as nature brings about recovery in cases of infectious disease pursuing a favorable course. This mode of therapy is naturally *specific*, as immunity also is specific. Much more serum, however, is required to effect a cure than to induce immunity; or, what amounts to the same thing, the serum of much more highly immunized animals.

Recent experiments of Dönitz with tetanus-antitoxin show, further, in a most conclusive manner, that the amount of serum necessary for curative purposes is the greater the longer the period of time that has elapsed between the intoxication and the institution of serum-therapy. Eight minutes after tetanus-intoxication, according to Dönitz, six times as much serum is required in order to save the animal as when the serum is injected immediately after the poison; after an hour the curative dose is twenty-four times the original dose; and so on, until finally a period is reached at which it is entirely impossible to save the animal, even with the largest amount of the most active serum. For this reason it is, above all things, essential in obtaining serum for therapeutic purposes to establish in the animals yielding the blood (generally horses) as high a degree of immunity as possible. The higher the degree of immunization established, the smaller the amount of serum required to effect cure. In making the degree of immunity as high as possible it must be borne in mind that the immunizing process pursues a wave-like course (Brieger and Ehrlich). Immediately after introduction of the next higher toxic dose the immunizing power of the blood-serum diminishes. It remains for a few days at the lower level, gradually rising again until it reaches the maximum. From this point it sinks again, and it finally reaches a level at which it persists for weeks. The most favorable time for injecting new toxin into the animals in order further to fortify their immunity is when the strength of the serum is highest—that is, when the anti-bodies are present in the body in largest number.

The efforts in the domain of curative serum-therapy have already yielded material practical results in the case of diphtheria. In that of tetanus success is as yet doubtful. We shall refer fully in the special section in the discussion of these two diseases to the mode of obtaining and of estimating and to the dosage of the curative serum.

Naturally, immunization is not the only manner in which therapeutic attack upon bacterial diseases is to be made. An infectious disease may be terminated by reason of the death of the bacteria in the body. It would be possible to effect cure in this way if the infectious agent could be destroyed within the body by means of internal disinfection. In spite of the large number of antiseptics at our command,

and in spite of their promptness in destroying bacteria in test-tubes, they are not applicable to the living body, because they either fail or, employed in the necessary strength, destroy not only the bacteria, but also the cells of the body. The possibility, however, that a disinfectant may yet be found that will destroy only the bacteria without affecting the tissues, must remain an open one.

IV. METHODS OF CULTURE AND OF EXAMINATION.

STERILIZATION.

In order to follow the individual varieties of bacteria perfectly in the course of their development, and in order to employ them in animal experimentation, it is necessary to obtain them in *pure culture*. In the making of such pure cultures the most scrupulous care must be observed to exclude the large number of bacteria that are everywhere present. The instruments employed in the various manipulations, the nutrient media, and the vessels that serve as the field of development for the bacteria, must be absolutely germ-free—sterile. To accomplish sterilization of these, ordinary antiseptic measures can not be employed, as the addition of substances capable of destroying the germs or of inhibiting their activity would naturally render the nutrient media unsuitable for culture-purposes. For this reason heat exclusively is employed for the sterilization of all materials used in the culture of bacteria, and both dry heat, as well as moist heat, in the form of live steam.

Dry Heat.—As dry heat penetrates but slowly into the interior of objects, it is employed principally for the sterilization of articles of small volume only; thus, platinum-needles are sterilized directly by exposure to the flame of a spirit-lamp or of a Bunsen burner, and other instruments by being moved to and fro for about a minute immediately above the flame. Articles made of glass and other substances that tolerate high temperatures are placed in a double-walled sheet-iron receptacle covered with asbestos (drying chamber), which is heated to between 150° C. (302° F.) and 170° C. (338° F.) by means of a gas-flame burning beneath it. (Fig. 9.) After exposure for half an hour to air thus heated to from 150° C. (302° F.) to 170° C.

(338° F.), even the most resistant spores are destroyed.   It suffices, further, in this mode of sterilization, to heat the drying chamber in which the instruments to be sterilized are placed, until a thermometer introduced from the top indicates a temperature of 170° C. (338° F.); then the supply of gas is cut off, and after the apparatus has completely cooled, the now sterile contents are removed.
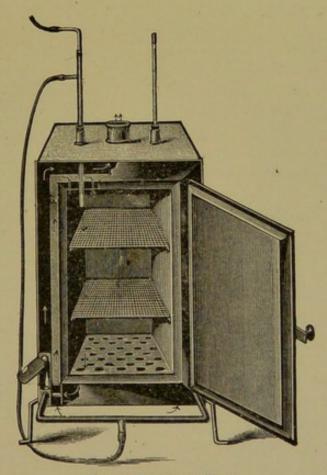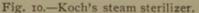
Fig. 9.—Hot-air sterilizer.

Live Steam.—Most substances, however, that are employed in bacteriologic investigations, especially the nutrient media, do not bear sterilization by means of such high degrees of heat as have been mentioned; they are, therefore, rendered germ-free by means of live steam.   For this purpose they are introduced into a cylindric apparatus, made of galvanized iron or of copper, and covered with felt or with asbestos.   (Koch's steam-chamber, Figs. 10, 11.) This vessel is divided by means of a perforated shelf or a wire grating into an upper larger and a lower smaller

space.   The former is intended to receive the substances to
be sterilized, while the latter is intended for the water.   The
bottom of the cylinder is heated, the water is made to boil,
and the steam generated streams through the perforated
partition into the upper sterilizing chamber, which is closed
by means of a loosely applied cover.   Exposure to live
steam for from half an hour to an hour, according to the
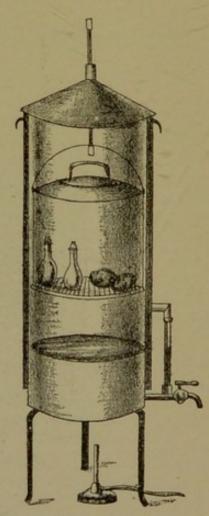
Fig. 10.—Koch's steam sterilizer.   Fig. 11.—Koch's steam-chest.

amount of fluid or the size of the articles to be sterilized,
suffices, as a rule, to free these from germs.

It is useful, when an adequate water-supply is available,
to connect the Koch steam-cylinder with a constant water-
bath.   For sterilization with steam under pressure at a tem-
perature of 110° C. (230° F.) (almost 1½ atmospheres) or
120° C. (248° F.) (two atmospheres of pressure), special
apparatus (digesters, autoclaves) are required, whose con-

struction is rather expensive. Disinfection under increased pressure has the great advantage that it is effected much more rapidly ; with a temperature of 120° C. (248° F.), about fifteen or twenty minutes are required to destroy all germs, even the especially resistant spores of some bacilli of food and of earth which are not destroyed with certainty by free steam even after an exposure of five hours. The objection has been raised to disinfection with steam under pressure that it does not sterilize with certainty because a tempera- ture of 120° C. (248° F.) does not prevail in all parts of the autoclave. This objection is, however, not justifiable. It is only necessary to be certain that no trace of air is left in the apparatus, and to this end the valve is closed after generation of steam has been taking place for five minutes.

The sterilization of certain fluids containing albuminoid substances requires special precautions. Live, or even compressed, steam can not be employed for this purpose, inasmuch as coagulation would take place. Resort must, therefore, be had to so-called *fractional*, or *discontinuous*, *sterilization* (Tyndall). The fluids to be disinfected are ex- posed for four or five hours to a constant temperature of from 56° C. (132.8° F.) to 58° C. (136.4° F.). Exposure for four hours to a temperature of 58° C. (136.4° F.) is sufficient to destroy most developed bacteria. The spores that remain in consequence of their greater resistance are now permitted to germinate, by leaving the fluid undisturbed for twenty-four hours. After the lapse of this interval, the fluid is again exposed for four hours to a temperature of from 56° C. (132.8° F.) to 58° C. (136.4° F.) ; and this mode of procedure is repeated daily for a whole week. At the end of this time all of the spores will have developed into bacteria, and these will in turn have been destroyed. This method is not, however, trustworthy under all condi- tions, as spores may develop after the lapse of a week. It appears better to introduce the albuminoid fluids (blood- serum, etc.) in small amounts into test-tubes or the like (Fig. 12), and to expose these for a considerable length of time (from four to six hours) to a temperature of from 56° C. (132.8° F.) to 58° C. (136.4° F.), and then to place them for two days in the thermostat at a temperature of 37° C. (98.6° F.). The tubes in which contaminations appear are set aside. With care in manipulation the number of turbid tubes will rarely be large. Fractional sterilization

naturally affords no protection against the thermophilic bacteria (p. 22). After sterilization has been effected, the vessels and nutritive media must, as a matter of course, be protected against all subsequent contamination, especially through atmospheric germs. For this purpose the orifices of the tubes are, even before they are filled, closed by means of cotton stoppers; the tubes are then sterilized by exposure to dry heat at a temperature of 170° C. (338° F.), and the fluid is introduced into them. The cotton filters the air, and restrains the entrance of the germs. If molds find their way upon the cotton stopper, they may, under certain circumstances, if the tubes have been kept for a long time, penetrate the cotton with their mycelial filaments. This undesirable occurrence is to be avoided by cutting off the excess of cotton, exposing the free surface in the flame, and
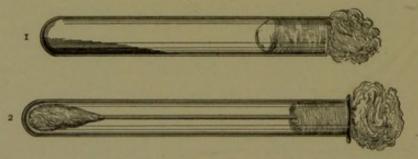
Fig. 12.—1, A tube of blood-serum; 2, a sterilized cotton swab in test-tube.

then applying over it a closely fitting rubber cap that has been previously disinfected in a 1 : 1000 solution of mercuric chlorid.

Preparation of Nutrient Media.—Whereas, previously to the time of Robert Koch, we were restricted almost exclusively to the employment of fluid nutrient media, Koch, through the addition of gelatinous substances—that is, such as become fluid when heated, and solid again when subsequently cooled—introduced the use of solid, and at the same time transparent, culture-media, which made it possible to isolate individual colonies of bacteria and to study their development.

The nutrient media that are most generally employed for the cultivation of pathogenic bacteria are bouillon, gelatin, agar-agar, blood-serum, and potato.

(a) *Preparation of Bouillon* (Löffler).—One pound of chopped meat freed from fat and tendon is macerated for

from twelve to twenty-four hours in a liter of water (at summer-temperature in a refrigerator). The infusion is squeezed through a clean cloth by means of a press, or simply with the hands, until one liter is obtained. If a smaller amount results, sufficient water is added to make a liter. This meat-infusion is the starting-point, not alone for the preparation of Löffler's bouillon, but also for a whole series of other nutritive media. Instead of meat-infusion, a five per cent. solution of Liebig's meat-extract may be employed. To the meat-infusion are added ten grams of peptone (one per cent.) and five grams of sodium chlorid ($\frac{1}{2}$ of one per cent.), and the whole is heated in an enameled vessel upon an open fire, until the peptone and the sodium chlorid have been dissolved. At the same time the coagulable albuminoid substances are precipitated. The coagula floating upon the surface of the fluid are removed with a spoon, and the fluid itself is passed through a folded filter previously moistened with distilled water. The fluid obtained should be perfectly clear and of acid reaction. The solution is now rendered slightly, though distinctly, alkaline by means of sodium hydroxid, and toward the close preferably with sodium carbonate. In order to collect the precipitated earthy phosphates, the fluid is further heated for an hour in the autoclave at a temperature of 110° C. (230° F.), or in the steam-chest for two hours at a temperature of 100° C. (212° F.). Then the still warm solution is filtered, the filtrate, after cooling, again filtered, and made up to a liter by addition of distilled water. The finished bouillon should be amber-yellow in color and transparent, and yield a feebly alkaline reaction. Should the reaction be not alkaline, it must absolutely be made so. The bouillon is now introduced into the test-tubes closed with cotton stoppers, or into Erlenmeyer flasks, which are best sterilized in the dry chamber. Each test-tube will contain from 10 to 15 cu. cm., each flask from 50 to 100 cu. cm., according to size. Finally, the tubes or flasks containing the bouillon are sterilized for an hour in live steam. For the cultivation of special bacteria, additions of certain substances are at times made to the bouillon—as, for instance, grape-sugar, in proportion of two per cent. (grape-sugar bouillon), or glycerin, in proportion of from four to six per cent. (glycerin-bouillon). It is useful to have constantly in readiness a sterile ten per

cent. solution of grape-sugar, of which 20 cu. cm. are added to each liter just before introduction into the test-tubes, in order to limit as far as possible the caramelization of the grape-sugar that occurs on protracted heating. Grape-sugar bouillon is best sterilized by exposure for ten minutes in the steam-chest on three successive days. In order to determine whether a given bacterium generates acid, a few drops of sterile tincture of litmus may be added to the bouillon.

(*b*) *Preparation of Gelatin.*—The same steps are followed as in the preparation of bouillon, except that to every liter of meat-infusion 100 grams (ten per cent.) of gelatin are added besides ; then follow solution by boiling in the steam-chamber, alkalinization, addition of the white of an egg, boiling for an hour, and filtering. In the case of gelatin also the reaction must be tested after boiling. Gelatin thus prepared remains firm up to temperatures of about 24° C. (75.2° F.). If gelatin is desired that remains firm at still higher temperatures (27° C.—80.6° F., 28° C.—82.4° F.), the procedure is modified by reducing the heat applied to the lowest possible minimum. Forster has determined that the solidification-point of gelatin is reduced about 2° for every hour's heating. The most practicable method of preparing gelatin is, therefore, as follows : To one liter of Löffler's nutrient bouillon heated in a vessel over a small flame to about 60° C. (140° F.) are added 100 grams of commercial gelatin cut in strips. With constant stirring complete solution of the gelatin is effected by boiling for seven minutes. Then the fluid, rendered acid by the presence of the gelatin, is carefully alkalinized, and, after addition of the white of an egg, is boiled for fifteen minutes in a Papin dish ; it is next filtered over a water-bath at a temperature of 60° C. (140° F.) into a large flask, and is then distributed among test-tubes (ten cu. cm. to each). The test-tubes are next sterilized by exposure for fifteen minutes in a Papin dish. It is advisable to employ a perforated tin shelf, in the openings in which the tubes may be placed individually, so that each is entirely surrounded by water. To expedite the process of heating, this shelf is at first rotated in the steam-cylinder. The nutrient gelatin thus obtained is completely clear, remains firm at a temperature of 27° C. (80.6° F.) or 28° C. (82.4° F.), and is always sterile. It may be permitted to harden in vertical columns

or in slanting layers, to be employed subsequently for stab-cultures or for streak-cultures. The liquefaction-point of the gelatin may be increased a degree or two if it be permitted to stand for twenty-four hours before being used (Forster).

For special purposes (cultivation of yeast-cells), when gelatin of acid reaction is required, *potato-gelatin* or *malt-gelatin* may be employed. To prepare the former, 500 grams of cleansed, peeled, and grated potatoes and one liter of water are permitted to stand together for three or four hours. The expressed and filtered fluid, the potato-water, is sterilized in the autoclave for an hour at a temperature of 110° C. (230° F.), or for fifteen or twenty minutes at a temperature of 120° C. (248° F.), and then—instead of the bouillon—is used for the preparation of the gelatin. To prepare malt-gelatin, the beer-wort or infusion of malt that can be obtained in any brewery is sterilized, instead of potato-water, and is then further treated in the same way as the bouillon. The only difference, as compared with the preparation of simple gelatin, is observed in the alkalinization, only so much normal sodium hydroxid being added as will restore the original feebly acid reaction of the potato-water or of the malt-infusion respectively.

For the purpose of cultivating typhoid-bacilli directly from the feces, Elsner has devised a modification of potato-gelatin by the addition of one per cent. of potassium iodid. It is best to add, by means of a sterile pipet, ½ cu. cm. of a germ-free twenty per cent. solution of potassium iodid to ten cu. cm. of potato-gelatin directly before use.

(c) *Preparation of Agar-agar.*—Instead of gelatin, from 1.2 to 1.5 per cent. of agar-agar is added to the peptone-sodium-chlorid meat-infusion. Agar-agar is a vegetable gelatin derived from Japanese and East Indian seaweed. It is best to employ for this purpose powdered agar-agar instead of that in strips, as the latter require a longer time for their solution. After the addition of from five to ten grams of gum arabic, to cause the agar to adhere to the surface of the glass, and after solution of the mixture in the steam-chamber for two or three hours, the fluid is rendered alkaline in the usual manner, the white of an egg is added, heat is again applied for one hour, and the solution is filtered. As the agar undergoes coagulation at 39° C. (102.2° F.), the filtering naturally can not be undertaken

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at ordinary room-temperature. It is, therefore, best to place both flask and funnel in the steam-chamber in complete activity. Even under these circumstances the process of filtering occupies several hours. The nutrient agar may, further, be filtered through a warm-water funnel, consisting of a copper vessel closed by a lid and containing a metallic funnel into which a glass funnel can be introduced. The metallic funnel is covered by a brass lid in order to prevent evaporation. By means of a thermo-regulator, the water contained in the outer filter is kept constantly at a temperature of between 60° C. (140° F.) and 70° C. (158° F.). The prepared agar is introduced into test-tubes in exactly the same way as bouillon and gelatin, and it is sterilized by boiling for an hour in the steam-chamber. Then it is placed in a slanting position in order to obtain the largest possible surface for inoculation, or it is permitted to coagulate vertically for the culture of anaerobic bacteria. In the process of solidification the agar expresses a certain amount of water, the so-called *water of condensation.*

Gelatin and agar-agar may, like bouillon, be modified by the addition of all possible substances ; thus, grape-sugar two per cent., glycerin from four to six per cent., etc. Glycerin-agar in particular plays an important part in bacteriologic technic. It is an admirable culture-medium, suitable for many pathogenic bacteria.

For special purposes, as for the cultivation of gonococci and influenza-bacilli, the preparation of blood-agar is to be recommended. By means of a platinum loop several drops of sterile human or pigeon's blood are smeared upon the surface of the agar, the tubes being then placed in the thermostat for one or two days at a temperature of 37° C. (98.6° F.), and the contaminated ones being rejected. Hemoglobin-agar may be prepared in the same manner by smearing a solution of hemoglobin upon the surface of the agar. The solution of hemoglobin is prepared in the following manner : Blood obtained with aseptic precautions is mixed with an excess of physiologic salt-solution, and permitted to stand in the cold for twenty-four hours. The resulting precipitate of red blood-corpuscles, with water in not too large amount, is introduced into a separatory funnel and a like quantity of ether is added. The mixture is well, though not too vigorously, shaken, and the dark-red, watery solution obtained is quickly filtered. A drop of this is smeared

upon the surface of the agar by means of a platinum needle.

(*d*) *The preparation of liquid blood-serum* for purposes of culture requires a rather complex procedure. The blood from a suitable animal, obtained after division of the carotid artery (in slaughtering), is received into tall, sterilized glass cylinders, and is permitted to stand upon ice for two days, in order that the serum may completely separate from the clot. The serum is then distributed among disinfected test-tubes by means of sterilized pipets, and placed for several days in the thermostat at a temperature of 37° C. (98.6° F.). By this means it is rendered certain whether
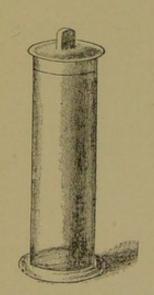
Fig. 13.—Flask to receive blood-serum.
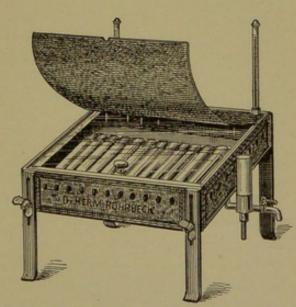
Fig. 14.—Koch's apparatus for coagulating and sterilizing blood-serum.

all of the manipulations have been made without contamination—that is, whether the serum is still germ-free. In this event the serum remains perfectly clear in the thermostat. The tubes that become turbid are rejected. In order to convert the liquid blood-serum into a solid culture-medium, the tubes are placed in an oblique position in the Papin steam-dish, and the serum is permitted rapidly to solidify. After this the tubes are sterilized by exposure for ten minutes on three successive days to a temperature of 100° C. (212° F.).

For the preparation of *serum-plates* the liquid serum is poured into sterilized Petri dishes (Fig. 23), and is then solidified and sterilized in the same way as the serum in

test-tubes. The disturbing water of condensation can be removed from the surface of the plates by placing these in an inverted position for forty-eight hours in the thermostat at a temperature of 37° C. (98.6° F.).

Human blood-serum is obtained either by blood-letting or from placentas. After the umbilical cord has been ligated and divided, the maternal extremity is disinfected by means of mercuric chlorid, which is rinsed off with distilled water. An incision is made into the cord above the point of ligation, and the blood is permitted to escape into steril- ized flasks. The conversion into culture-media is effected in the manner already described. A mixture of three parts of sheep's blood-serum with one part of a one per cent. grape-sugar bouillon (Löffler's blood-serum) may be em- ployed with especial advantage for several purposes (diag- nosis of diphtheria), being solidified and sterilized in tubes or plates in the same way as ordinary blood-serum.

Blood-serum agar (*blood-serum glycerin-agar*), which also is employed with advantage for special purposes, is pre- pared by adding to liquefied agar or glycerin-agar cooled to 40° C. (104° F.) an equal amount or half the quantity of sterile liquid blood-serum heated to 40° C. (104° F.). The mixture is introduced into test-tubes or Petri dishes, and made to solidify rapidly.

(*e*) *The Preparation of Potatoes.*—Potatoes constitute an excellent nutritive medium for many purposes. They may be prepared in various ways. Large potatoes are thor- oughly cleansed with a brush and mercuric chlorid, are carefully freed of their peels and their so-called decayed spots and eyes, and are cut into slices about one cm. thick, which are placed in glass double dishes. The peels, together with the slices of potatoes, are exposed for an hour to steam under pressure at a temperature of 110° C. (230° F.), or for fifteen or twenty minutes at a temperature of 120° C. (248° F.); or suitable oblique pieces are cut out of the potatoes and are introduced into test-tubes with some cotton at the bottom, and these are sterilized in the manner already described. The cotton at the bottom of the tubes is for the purpose of absorbing the fluid that appears in the process of boiling, and with its aid the potato is subsequently kept moist. Roux has devised special tubes for potato-cultures which are narrowed near the bottom. (Fig. 15.) Upon the shoulder or projection

thus formed the bits of potato lie. The lower portion of the tube is filled with sterile salt-solution in order to keep the surface of the potato moist. Instead of salt-solution other fluids are used for special purposes. Thus, bits of potato that just dip into five per cent. glycerin-solution furnish an excellent culture-medium for tubercle-bacilli.

As the cut surface of the potato is more or less changed by protracted ex-posure to the action of steam, it is better to boil the whole potato, and then to divide it. To this end the unpared potatoes are thoroughly cleansed by means of a brush and a solution of mercuric chlorid, the so-called eyes are cut out, and sterilization is practised for two hours in the steam-chamber on each of two successive days, or more simply and better for an hour in the autoclave at a temperature of 110° C. (230° F.), or for twenty minutes at a temperature of 120° C. (248° F.). The potatoes must be sterilized thoroughly because they are often the seat of special bacilli characterized by the extraordinary resistance

Fig. 15.—Roux's test-tube, specially designed for potato-cultures.
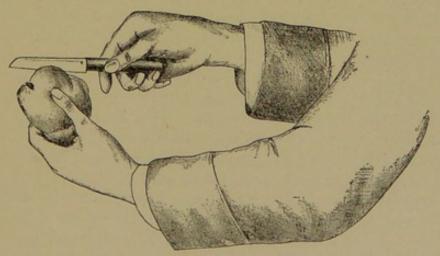
Fig. 16.—Method of slicing potato (after Woodhead and Hare).

of their spores (red potato-bacilli). Then the hands are thoroughly disinfected by means of soap, alcohol, and mercuric chlorid, and the still warm potato is removed with the fingers moist with mercuric chlorid, and it is

divided into two equal parts with a knife sterilized in the flame. The halves, resting upon sterile rubber rings, are preserved in moist culture-chambers, whose individual glass parts have been disinfected with mercuric chlorid.

(*f*) *Peptone-water.*—For the diagnosis of cholera a solution of one per cent. peptone and one per cent. sodium chlorid in distilled water is required. This is rendered alkaline, is sterilized for an hour in the steam-chamber, and is kept in readiness in test-tubes, or, better, in so-called Pasteur flasks. The latter are closed by means of a glass helmet drawn out into a tube, so that evaporation of the water is prevented, and they provide a large surface of fluid for the developing bacteria.

For examinations of water, and also for the cultivation of cholera-vibrios from water, a twenty-five per cent. solution of peptone and of sodium chlorid is kept in readiness, and is preserved in test-tubes, each of which contains four cubic centimeters. If the contents of such a tube be added to 100 cu. cm. of the water to be examined, a one per cent. solution of peptone and of sodium chlorid at once results, which, after being rendered alkaline, is known as a fertilizing solution and can be used for culture-purposes.

(*g*) *Milk* is likewise frequently used as a nutritive medium. Fresh milk is introduced into test-tubes or into Erlenmeyer flasks provided with cotton stoppers and sterilized. In order to exclude bacteria the tubes and their contents are exposed thrice for an hour each time, or once in the autoclave at a temperature of 110° C. (230° F.) or 120° C. (248° F.).

(*h*) To determine the question whether a given variety of bacteria produces acid or alkali in the course of its development, the *whey* of Petruschky may be employed with advantage. One liter of milk is added to a like amount of water, and coagulation is effected by adding to the mixture the smallest possible amount of acid (acetic acid). The fluid is now filtered, and the filtrate neutralized with sodium carbonate and heated to boiling. By this means the acid reaction is, as a rule, restored, and turbidity results, which is removed by filtration. Then the mixture is boiled again, is neutralized with sterile sodium-carbonate solution, and sterile tincture of litmus is added until a faint violet color is produced.

(*i*) Of less common culture-media, employed only for special purposes, may be mentioned *bread-pap* (dry bread

reduced to powder, and introduced into an Erlenmeyer flask with enough distilled water to make a homogeneous soft mass, and sterilized by exposure thrice for an hour each time in the steam-chamber), which is used especially for the cultivation of molds ; also *rice-pap* (Soyka) (boiled rice-milk sterilized in double glass dishes), which is available for permanent cultures ; *infusion of hay, decoction of prunes*, etc., which may be employed as fluid nutrient media, or in combination with gelatin or agar.

(*j*) A special position among culture-media is occupied by those *free from albuminoids*. The constitution of that most frequently employed, and proposed by Uschinsky, is, as modified by Fränkel, as follows :

Potassium biphosphate, ... 2.0
Sodium chlorid, ... 5.0
Ammonium lactate, ... 6.0
Asparagin, ... 4.0.

The mixture is dissolved in a liter of water and is sterilized.

METHODS OF CULTURE.

In nature and in the products of disease individual varieties of bacteria are but rarely found isolated—that is, alone. Mostly, several species are found together in the material subjected to examination.   It is of the utmost importance, in the process of investigation, to separate from one another the different varieties in such a mixture of bacteria—that is, to isolate them in *pure culture*. This has been rendered possible by the bacteriologic methods introduced by Koch. The principle of these consists in inoculating a solid culture-medium that has been liquefied with a trace of the bacterial mixture to be examined, distributing this as well and as evenly as possible, and then spreading the mixture upon a sterile glass plate. The liquid nutrient medium becomes solid again at room-temperature, and covers the plate in a thin layer, in which the bacteria now develop, but not indiscriminately among and next to one another, as in a tube with fluid contents, but separated considerably from one another. At every point where a bacterial germ becomes fixed in the solidifying mass it undergoes isolated development, multiplying and forming for itself a special colony.   By this means it is possible to observe the development and the growth of the individual germs also under the microscope, and to

manipulate further the colonies of a single species of bacteria.

The particulars of *Koch's method of plate-making* are as follows: With the aid of a platinum wire melted into a glass rod (Fig. 17), a small amount of the material to be examined is introduced into a gelatin-tube whose contents
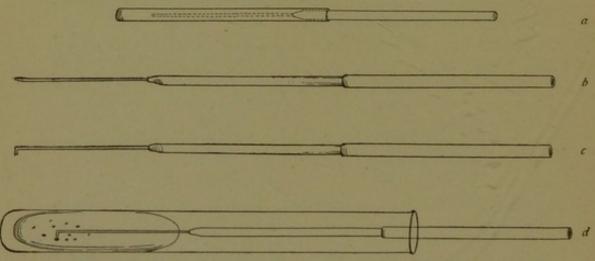
Fig. 17.—Platinum wire swaged into brass wire and reversible for transportation (as devised by Dr. J. H. McCollom): *a*, Closed; *b*, open; *c*, the same with end bent at a right angle, for picking up colonies in test-tube; *d*, the same in operation (Ernst).

have previously been liquefied by immersion in water at a temperature of from 30° C. (86° F.) to 40° C. (104° F.). The platinum wire must, of course, have been heated in the flame of a Bunsen burner or of a spirit-lamp before being used, in order to be freed from germs that may have been attached to it. When the material to be examined contains many bacteria, it is sufficient to insert the extremity of the heated wire, but if the number of bacteria is small, it is
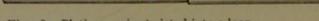
Fig. 18.—Platinum wire twisted into a loop.

better before heating the wire to twist it into a loop in order that it may take up more of the material. (Fig. 18.) If a solid, compact substance is to be examined with regard to the presence of bacteria, it is, by means of a glass rod sterilized in the flame and subsequently cooled, broken up in a watch-glass similarly treated; it is then rubbed up with sterile

bouillon or water, and a small amount of the emulsion is removed with the heated platinum wire.   If the substances to be examined are particularly tough, it is well to render a mortar and pestle germ-free by dry sterilization or by exposure to the flame, and to cover the mortar with sterile paper, in the middle of which is an opening for the handle of the pestle.   The material is now rubbed up with the pestle until a thoroughly homogeneous emulsion is made, while, at the same time, the overlying sheet of paper prevents the entrance of germs from the air.

The tube containing the liquefied gelatin is taken in the left hand with its palm directed upward, and between the thumb
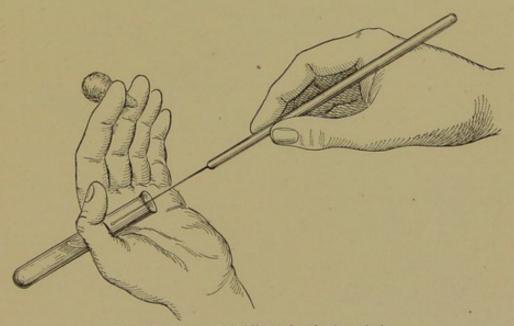
Fig. 19.—Manner of holding tubes for inoculation.

and the index-finger; then, with a slight rotatory movement, the cotton stopper is removed with the right hand, and placed with its free extremity, which always projects from the mouth of the tube, between the index-finger and the middle finger of the left hand. (Fig. 19.) The platinum needle, laden with the well-divided material for examination, is introduced into the nutrient gelatin, great care being taken to avoid bringing the wire into contact with the walls of the tube.   The platinum wire is withdrawn and immediately sterilized in the flame.   The material introduced is admixed with the liquefied gelatin, as intimately and as evenly as possible, by means of repeated shakings, rotation, inclination, and sudden straightening of the tube, now again

closed with a cotton stopper. The culture-medium could now be poured upon a plate for solidification, but this is usually not done at this time, because, as a rule, the first gelatin-tube, or, as it is called, the original tube, contains too large a number of bacteria, whose colonies would therefore develop too closely together upon the plate. For this reason a first and a second dilution are yet prepared. To this end the inoculated original tube is taken between the thumb and the index-finger of the left hand, another gelatin-tube, liquefied at 40° C. (104° F.), is held between the index-finger and the middle finger, and the cotton stoppers
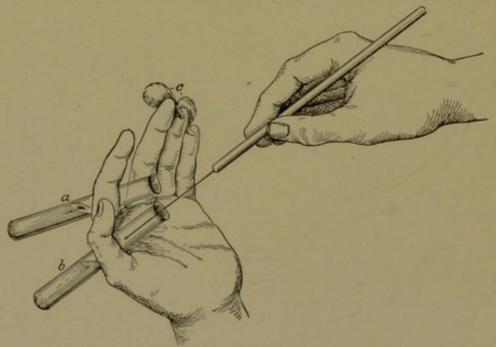
Fig. 20.—Manner of holding tubes during inoculation: *a*, Original tube; *b*, tube to be inoculated; *c*, cotton plugs.

are removed, the first being held between the middle and the ring finger, and the second between the ring and the little finger. Then, with the platinum needle sterilized by heat, three loopfuls of the liquid contents of the original tube are successively taken from the original tube and introduced into the second gelatin-tube (first dilution). (Fig. 20.) After vigorous shaking, in the manner described, of the tubes, now again closed, a third tube of liquefied gelatin is inoculated in an identical manner with three loopfuls of the first dilution (second dilution).

Rectangular plates of glass will meanwhile have been sterilized in sheet-iron boxes (plate-pockets) in the hot-air

chamber, and one of these plates, grasped carefully at its edges with the fingers, or, better, with forceps sterilized in the flame, is placed upon a plate covering a dish filled with ice.   Over this plate, in order to reduce the possibility of aerial infection to a minimum, is placed a bell-glass that
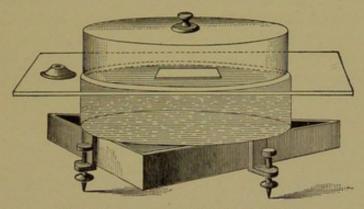
Fig. 21.—Leveling and cooling apparatus.

has been immersed in mercuric-chlorid solution.   The entire apparatus may with advantage be mounted upon a leveling device. (Figs. 21, 22.)   The cotton stopper is now quickly removed from the original tube, the lips of which are passed through the flame and are briefly permitted to cool, and after removal of the bell-glass the contents of the tube are poured upon the center of the rectangular plate, and are distributed evenly with the lips of the tube, care being taken to leave a free space of one centimeter at the borders of the plate.   Some of these glass plates are provided, at a distance of one centimeter from their borders, with a wall of enamel about one millimeter high, for the purpose of preventing overflow of the gelatin.   The
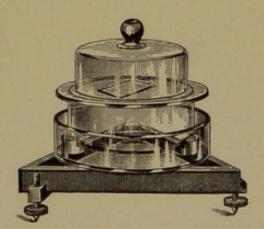
Fig. 22.—Complete leveling apparatus for pouring plate-cultures, as taught by Koch.

gelatin solidifies rapidly upon the ice base after the bell-glass is replaced, and the finished plate is now placed upon a glass shelf or bench in a crystallizing dish, which is converted into a moist chamber by the insertion of bibulous paper moistened with a solution of mercuric chlorid.   The

first and the second dilution are poured upon plates in the same way; a second and a third glass shelf placed upon the first in the moist chamber, and a plate respectively placed upon each of these.

This original method of Koch for pouring plates, which is still used in certain investigations, has been replaced by a simpler and more convenient method : In place of the plates double dishes of glass are used (Petri dishes, Fig. 23). Sterilization, liquefaction, and inoculation of the tubes are effected exactly in the same way. After inoculation, however, the liquefied gelatin of the three tubes is simply poured into three sterile dishes, which are then covered and left to themselves.

In accordance with the bacteria that it is desired to cultivate, and the temperature that prevails in rooms, the finished plates—that is, those that have been solidified—are placed at room-temperature in a dark place, or in the
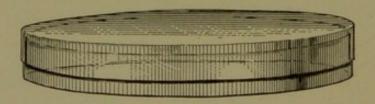
Fig. 23.—Petri dish for making plate-cultures.

thermostat at a temperature at from 24° C. (75.2° F.) to 26° C. (78.8° F.). To protect the plates from the drying influence of the air, which inhibits the growth of the bacteria, it is useful to preserve them in so-called culture-boxes. Each of these consists of a four-cornered box of sheet-iron covered with a glass lid, and which is converted into a moist chamber by the introduction of a dish containing moistened cotton (Forster).

The method described suffices when all that is desired is the determination whether bacteria are present in material submitted for examination, as well as their nature. If, however, the number of bacteria present is to be accurately determined also quantitatively, the following mode of procedure is adopted : Into a gelatin-tube containing exactly ten cubic centimeters, after liquefaction a given amount of the material to be examined is introduced either by means of a sterile pipet (capillary pipet) or with a platinum loop

(spiral or hook), whose capacity has been determined by weighing. Especial importance is to be attached in this connection to an equable distribution of the bacteria by careful admixture. Of the mixture exactly the same carefully measured amount is introduced into ten cubic centimeters of gelatin, and then of this the same amount in a third tube containing ten cubic centimeters, and so on. After three, four, or five dilutions have been made, in accordance with the approximate number of bacteria originally present and the amount of inoculated material, the gelatin is poured into plates, is permitted to solidify, and the number of colonies that develop upon it in the course of the next eight days is counted daily. Generally the colonies are so dense upon the first and perhaps also upon the second plate, that their enumeration,
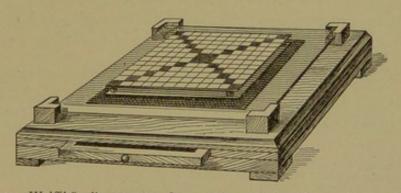
Fig. 24.—Wolffhügel's apparatus for counting colonies of bacteria upon plates.

even with a lens or with a low power of the microscope, is not possible. If the dilutions have been properly made, there will always be one plate upon which the colonies can be counted, and from this the numerical relations of the colonies developed upon the other plates can readily be estimated. The enumeration is greatly facilitated by the use of a dark background divided into square centimeters, and $\frac{1}{4}$ or $\frac{1}{9}$ of a square centimeter. Such an instrument is the apparatus of Wolffhügel (Fig. 24), which consists essentially of a glass plate divided into squares, and provided with a dark, dull background.

In dealing with solid substances, 1 gram, ½ gram, etc., is weighed, rubbed in a sterile mortar with from 5 to 10 cu. cm. of sterile bouillon or with physiologic salt-solution, and the remaining steps are the same as those already described.

If it is desired to make *plates of agar-agar*, special precautions must be observed in consequence of the ready coagulability of this culture-medium (even at 39° C.—102.2° F.). After the agar has been liquefied, best in boiling water (it melts at 90° C.—194° F.), the tubes are placed in a water-bath at a temperature of 40° C. (104° F.). At this temperature the agar just remains liquid, and the bacteria can be inoculated without suffering in vital activity. The pouring of the agar in the double dishes is unattended with difficulty, as is likewise the preparation of dilutions according to the same method; only it is necessary to be expeditious in the execution of the manipulations in order to avoid solidification of the agar in the tubes.

The use of the gelatin-tube itself as a plate is rendered possible by *Esmarch's modification of the Koch procedure:* The liquefied tubes, which are best closed with a plug of nonabsorbent cotton not freed from fat, in order to avoid
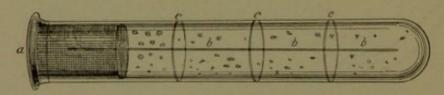
Fig. 25.—Esmarch's roll-culture: *a*, India-rubber cap; *b, b, b*, longitudinal line drawn on the tube; *c, c, c*, transverse lines for counting colonies (Frankland).

saturation with the fluid, are inoculated, closed with a rubber cap, held in ice-water, and rapidly and regularly rotated about its axis. The gelatin is, by this means, distributed in a thin, even layer on the inner surface of the tube, is solidified, and in this way a *roll-plate* (Fig. 25) is prepared, which is then further treated in the same way as ordinary plates.

To prepare so-called *agar streak-plates* for rapid diagnosis, the liquefied agar is poured out before inoculation. When it has solidified, six or seven strokes are made side by side upon an agar-plate with a platinum needle that has been dipped in the fluid to be examined, or with the material to be examined itself (membrane, cotton swab, etc.). Upon the last stroke the bacteria will be so sparsely distributed that individual colonies develop. According to the same principle of dilution, the surface of a number of agar or blood-serum tubes solidified in a slanting position is

rubbed successively with the inoculated platinum needle, or similar body (*fractional streak*).

The finished plates are kept in culture-boxes for from two to four days at room-temperature, or in the thermostat at a temperature of 24° C. (75.2° F.) or 25° C. (77° F.) (gelatin-plates), or they are permitted to remain in the thermostat for from twenty-four to forty-eight hours at a temperature of 37° C. (98.6° F.) (*agar-plates*). After the lapse of this time the plates are examined with the naked eye, with a lens, and also with a low power of the microscope (from 50 to 100 magnifications). It is next determined whether one or several varieties of colonies have developed, and then the peculiarities of these colonies—whether they liquefy the gelatin, whether they possess a sharp or an irregular border, whether they are granular, whether they present a definite arrangement, special tints of color, etc. Variations in the appearance of similar colonies are dependent upon their position in the gelatin. Deeply lying colonies almost always assume a spheric shape, and appear, as a rule, round and dark, whereas the superficial colonies sometimes spread like a membrane upon the surface of the gelatin, and appear bright and transparent. The most important object to be accomplished, however, is to obtain pure cultures from these plates. If the particular colony from which it is intended to make inoculations is not entirely too small, and if it be isolated, the procedure is quite simple : The plate or the dish is placed upon a dark background, the platinum wire is heated in the flame, and with the aid of the naked eye or of a lens the extremity of the wire is introduced into the colony. If, however, the colony is particularly small, there is no alternative but to make the inoculation with the platinum wire with the aid of a low power of the microscope. For this purpose a steady hand and much practice are required. Apparatus have also been devised for this purpose, but they are rather complicated, and scarcely more trustworthy for the experienced manipulator. After the material from the colony has been taken up with the needle, it is smeared upon one of the various culture-media after having convinced oneself, with the aid of the microscope, if necessary, that only one colony has been removed, and in this way a pure culture is made. In inoculating a gelatin-tube the needle is generally introduced from above downward through the middle of the column of

gelatin. In this way a gelatin *stab-culture* (Fig. 26, *a*) is obtained.

Agar, and often also gelatin, is generally solidified so as to yield a slanting surface for inoculation, the point of the needle being passed from below upward upon the surface of the culture-medium (*streak-culture*). (Fig. 26, *b*.) Inoculations upon potatoes are made in exactly the same way.
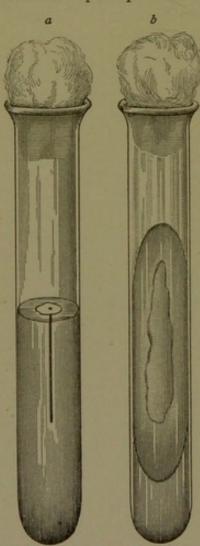
Fig. 26.—Stab-culture (*a*); streak-culture (*b*).

In the preparation of a bouillon-culture or a milk-culture the mass of bacteria is rubbed simply on the inner surface of the tube below the level of the bouillon or the milk, the loop used for the inoculation being then thoroughly shaken.

In order to inoculate one test-tube from another, to continue the pure culture further, the two tubes are grasped between the fingers (Fig. 20) (the first tube between the thumb and the index-finger, and the second tube between the index-finger and the middle finger), their stoppers are removed (the one being held between the third and fourth, and the other between the fourth and fifth fingers), and from the first tube there is taken, with the platinum needle previously sterilized in the flame, or, in the case of fluid culture-media, with the platinum loop, a small amount of the culture, which is then conveyed upon or into the new culture-medium. The reinoculation of pure cultures that it is desired to maintain must be repeated at intervals of four weeks.

The development of gelatin-cultures is permitted to take place at room-temperature, or in the thermostat at a temperature of 24° C. (75.2° F.) or 26° C. (78.8° F.). The remaining cultures are, however, usually kept in the thermostat at a temperature of 37° C. (98.6° F.). A thermo-

stat or incubator consists of a double-walled copper or sheet-iron chamber surrounded with felt or asbestos. The space between the two walls is filled with water, which is kept constantly at an equable temperature by means of a thermo-regulator. The layer of water transmits its heat to the interior of the thermostat, the actual culture-chamber, which constantly should have a temperature of 37° C. (98.6° F.) for agar, or from 24° C. (75.2° F.) to 26° C. (78.8° F.) for gelatin. The culture-tubes, which are to be kept for a considerable length of time in the thermostat, must be provided with rubber caps sterilized with mercuric chlorid, in order to protect them from evaporation.

The growth in a number of these cultures constitutes for many bacteria a definite characteristic, which is of the highest significance in their identification. Least typical, as a rule, is the growth upon agar. Attention must be directed to the thickness, the transparency, and the color of the deposit, etc. In bouillon-cultures
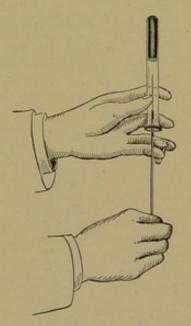
Fig. 27.—Method of inoculating a test-tube containing sterile nutrient jelly.

there may be distinguished bacteria that develop only upon the surface in the form of a thick or a thin membrane, others that render the bouillon more or less homogeneously turbid, and still others that grow only at the bottom as a crumbling or a viscid sediment. Milk is unaltered by many bacteria, while others cause coagulation through the formation of acid. The most important peculiarities are furnished by gelatin-cultures. In the first place the liquefaction that results through the activity of a peptonizing ferment is to be looked for. This occurs in part only superficially, and is in part funnel-shaped or stocking-shaped at a depth. If liquefaction does not take place, varied and often characteristic peculiarities of growth occur (nail-culture, tree-like division, etc.).

CULTIVATION OF ANAEROBIC BACTERIA.

Anaerobic bacteria may be *facultative* or *obligate*. The former may develop also in the presence of oxygen, although but sparingly. The latter require special cultural methods. The cultivation of strictly anaerobic bacteria is undertaken either in a room free from air, or in an atmosphere of indifferent gas—as, for instance, hydrogen—or with the employment of substances that absorb oxygen, or, finally, by means of stab-culture in a high layer. The usual culture-media are employed, but with the addition of
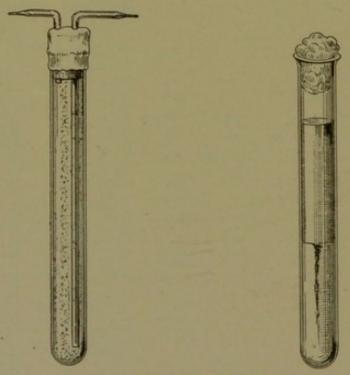
Fig. 28.—Fränkel's method of making anaerobic cultures.   Fig. 29.—Hesse's method of making anaerobic cultures.

two per cent. of grape-sugar, as all of the anaerobic bacteria thus far known form from this substance gas in abundance (carbon dioxid, hydrogen sulphid, methane, mercaptan, etc.), in this way displacing the oxygen of the air.

Plate-cultures.—According to a method devised by R. Koch, a sheet of mica, sterilized by heat, is placed upon the liquid gelatin spread upon the plate. After solidification has taken place the gelatin is thus rendered air-tight, and beneath the mica the anaerobic colonies undergo development. It is more serviceable in the preparation of anaerobic plates to

employ special culture-dishes, which permit the entrance of
hydrogen through two openings in the lid that communicate
with a gutter-like excavation of the dish.   The lid is fas-
tened to the periphery of the dish by means of vaselin, and
it is revolved as soon as the vessel is filled with hydrogen,
in order that the openings in the lid and the gutter are no
longer opposed to one another, and communication with
the outside is cut off (Kamen dish).   The hydrogen is gen-
erated in a Kipp's apparatus (Fig. 30) that is filled with
pure zinc and sulphuric acid, and is freed of hydrogen
sulphid and of oxygen, by means of two wash-bottles con-
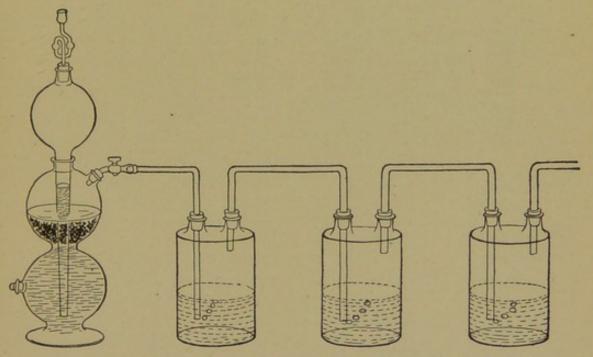
Fig. 30.—Kipp's apparatus for producing hydrogen, with wash-bottles attached
(Ernst).

taining an alkaline lead-solution and an alkaline pyrogallic-
acid solution.   The employment of Kamen's plates is
attended with certain difficulties; especially is it difficult to
drive out all of the oxygen.

In the preparation of anaerobic plates in an atmosphere
of hydrogen Botkin's apparatus (Fig. 31) is employed.   This
consists of a large bell-jar, within which, upon a glass stand,
plates are exposed free, without a cover.   The bell stands
upon a lead cross in a large glass dish.   Between the mar-
gin of the bell and the stand is a space through which
passes a U-shaped rubber tube for the conduction of the

hydrogen gas into the upper portion of the bell.   Perfect closure is effected with the aid of liquid paraffin.   The displaced air escapes at the bottom through a second rubber tube, which is removed after the apparatus has been completely filled. Beneath the glass bell a vessel containing an alkaline solution of pyrogallic acid is placed for further security. A disadvantage of Botkin's apparatus consists in the fact that the plates are but incompletely protected against contamination by the air.

Fig. 31.—Botkin's apparatus for making anaerobic plate-cultures.

Novy's apparatus is to be warmly recommended, both for plate-cultures as well as for test-tube cultures.   This consists of a high glass jar upon the edges of which an air-tight helmet-like cover is placed. The latter is provided above with a revolving, doubly perforated glass stopper, through which the hydrogen gas
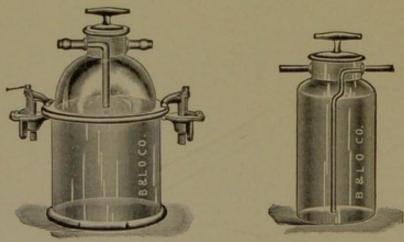
Fig. 32.—Novy's jars for anaerobic cultures.

is introduced and the air is at the same time expelled.   At the close of the operation the stopper is simply rotated, and

the inner space is cut off completely from the outer air. (Fig. 32.) Novy's apparatus may also be connected with an air-pump, and be exhausted of air ; or, finally, it may be freed of oxygen with the aid of an alkaline solution of pyrogallic acid. (See method of Buchner, below.)

Anaerobic pure cultures in test-tubes may be prepared in the following manner :

1. In a High Layer.—Stab-cultures are made in tubes that are filled with nutrient material to a higher level than usual, and that are boiled again shortly before being used. In the lower portions of the culture, free from oxygen, to which the needle has penetrated, development takes place, whereas the upper portions of the medium containing oxygen remain sterile. The anaerobic bacteria thrive more vigorously when reducing substances are added to the nutrient media—*e. g.*, two per cent. of grape-sugar or from 0.3 to 0.5 per cent. of formic acid.

2. In an Atmosphere of Hydrogen.—The test-tubes are closed with sterilized rubber stoppers doubly perforated, through which two glass tubes bent at a right angle lead into the interior. (Fig. 28.) Through the longer tube, which reaches almost to the nutrient medium and is closed with a cotton stopper, hydrogen is passed, while through the shorter tube atmospheric air is expelled. As soon as gas in pure state escapes through the shorter arm, both tubes are sealed by heat, or closed by means of rubber tubes. Inoculation must, of course, have been effected before the introduction of the hydrogen.

3. With Complete Exclusion of Air.—A test-tube with a long-drawn-out tenuous neck is filled with nutritive material and inoculated in the usual manner. The neck is then connected with an air-pump, and when the air has been completely exhausted, the tube is sealed by heat.

4. According to the Method of Buchner.— The culture-tubes, closed by means of a loosely introduced cotton stopper, are in-

Fig. 33.—Buchner's method of making anaerobic cultures.

troduced into a large, hermetically sealed tube, whose floor is covered with alkaline solution of pyrogallic acid (1 gram

of pyrogallic acid, 10 cu. cm. of 1 per cent. potassium hydroxid). (Fig. 33.) Pyrogallic acid has the peculiarity of taking up oxygen, and in this way the space in which the cultures are exposed is quickly freed of oxygen.

Raw eggs also may be employed for anaerobic culture. One extremity of the egg is thoroughly cleansed with mercuric chlorid and sterile water, a puncture is made with a needle sterilized in the flame and still hot, and with the platinum needle a portion of the pure culture is introduced into the interior of the egg. The small opening made is then closed with hot sealing-wax.

MICROSCOPIC EXAMINATION AND STAINING OF BACTERIA.

To examine bacteria in the living state in the hanging drop, a small drop is removed from a fluid culture by means of the platinum loop sterilized in the flame, and it is then placed in the center of a cover-slip carefully cleansed with alcohol. (Fig. 34.) The cavity of a slide that has been
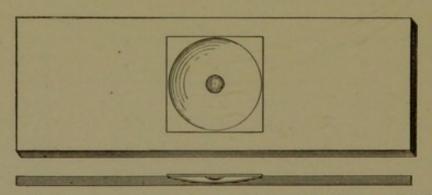
Fig. 34.—The "hanging drop" seen from above and in profile (Mallory and Wright).

excavated at its center is surrounded with vaselin, and the slide is pressed face down upon the cover-slip, so that the drop is suspended exactly in the center of the cavity. If it be desired to prepare a hanging drop from a solid culture, a drop of sterile water or of bouillon is first placed upon the cover-slip, and to this is added a minimal amount of the bacteria to be examined. It is useful in some cases to add to the hanging drop a small amount of a dilute staining solution: *e. g.*, a carbol-fuchsin solution (p. 104) diluted four or five times. The small amount of coloring-matter added does not influence the vital activity of the bacteria. These are stained faintly, but the motile varieties

continue in active movement, and in many of them, as a result of the action of the stain, even the flagella become visible. In making examinations in hanging drop it is advisable first to find the border of the drop with a low power of the microscope, and then to scrutinize it with higher powers. After having observed sufficiently the size and the shape of the bacteria in a thin layer, and while comparatively quiet, the central portions of the drop are brought into the field.

Instead of a simple hanging drop, a culture may be made in hanging drop, and the growth of the individual bacterial cell, the formation of spores and their germination, etc., can be observed directly under the microscope. As a matter of course the cover-slip must previously have been sterilized by being passed through the flame. After the slip has cooled, a drop of sterile bouillon or gelatin is placed at its center and is inoculated. The observation may then be continued, perhaps with the aid of a warm stage, or of a special small incubator in which the entire microscope is introduced.

In order to decide with certainty whether the movement observed in certain bacteria is dependent upon their own motility, upon molecular movement, or upon that due to currents, it is at times necessary to employ liquid gelatin as a diluting fluid instead of sterile water. The degree of temperature necessary for liquefaction is readily obtained by using the warm stage. All such observations are, of course, made through a narrowed diaphragm.

Examination of Stained Preparations.—For staining purposes basic aniline dyes are employed that possess the property of staining nuclei and bacteria. The most important of those in use are gentian-violet, methyl-violet, fuchsin, methylene-blue, vesuvin, and malachite-green. These stains, with the exception of the last two, are kept in readiness in concentrated alcoholic solution, and the two excepted, vesuvin and malachite-green, in about one per cent. watery solution. From the alcoholic stock-solutions the usual watery staining solutions are prepared by dilution with from ten to twenty times the amount of distilled water. A still simpler means consists in permitting a few drops of the stock-solution to pass through a filter into a watch-glass containing distilled water. By the addition of certain substances that, to a certain extent, play the part of mordants,

the staining power of these substances may be increased considerably. Among substances to be mentioned in this connection are :

1. Potassium hydroxid (employed by Löffler in combination with methylene-blue) :

> 30 cu. cm. of concentrated alcoholic solution of methylene-blue.
> 100 cu. cm. of 0.01 per cent. solution of potassium hydroxid (1 : 10,000).

This so-called Löffler's solution stains well, and may be preserved for an almost indefinite time.

2. Aniline water :

Four or 5 cu. cm. of aniline oil, with 100 cu. cm. of distilled water (1 part of aniline oil to about 20 parts of water), are vigorously shaken and passed through a moistened filter. To the clear filtered aniline water (100 cu. cm.) are added 11 cu. cm. of a concentrated alcoholic solution of fuchsin or of gentian-violet or methyl-violet. It is more convenient to filter the aniline water in a watch-glass and to add the solution of fuchsin or gentian-violet or methyl-violet until a metallic, opalescent pellicle appears upon the surface. These solutions of Ehrlich stain well, but they possess the disadvantage that they decompose rapidly, and they must, therefore, be freshly prepared each time that they are used.

3. Carbolic acid :

One gram of fuchsin is dissolved in 10 cu. cm. of 96 per cent. alcohol, and then 90 cu. cm. of 5 per cent. carbolic acid are added.

This solution of Ziehl does not possess quite so intense a staining power as the aniline-water solutions, but it has over these the not inconsiderable advantage of greater durability. In an analogous manner carbolic-acid gentian-violet, and carbolic-acid methylene-blue solutions are prepared, both of which possess excellent staining qualities, and can be preserved for a long time.

Preparation and Staining of Cover-glass Specimens.— In making stained preparations the suspected material is spread in as thin a layer as possible upon a cover-glass thoroughly cleansed with alcohol and dried, a small drop being taken, by means of the platinum loop, directly from liquid cultures not more than one or two days old, or a small amount of the bacterial mass from solid cultures being rubbed up in a drop of sterilized water (or tap-water, which

may be considered sufficiently sterile for this purpose) upon the cover-glass. The preparation is permitted to dry in the air, and then, in order to fix it, it is passed three times, with moderate rapidity, through the flame of a spirit-lamp or of a Bunsen burner. In order to obtain satisfactory preparations from cultures it is useful, before staining, to immerse the cover-glasses for from one-half to one minute in from a one to a four per cent. solution of acetic acid. By this means the preparation is cleared, while the bacteria in no wise suffer. The acetic acid is blown off by means of a glass tube, or the cover-slip is simply dried carefully between filter-paper. By means of a pipet or of a small filter (it is usually well to filter the staining solutions before they are used) several drops of the staining solution are placed upon the smeared surface of the cover-slip (conveniently grasped with Cornet's forceps); the stain is permitted to exert its influence for a short time, and is then washed off
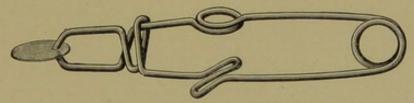
Fig. 35.—Stewart's cover-glass forceps.

in water. If the staining solution has been placed in a watch-glass, the cover-slip is carefully placed upon the surface of the fluid so that it floats thereon with its smeared surface down. As to the length of time that the cover-glasses should be exposed to the influence of the staining solution, no definite statement can be made. This will depend upon the thickness of the layer, the concentration of the stain, and the tingibility of the bacteria. In general, from half a minute to a minute is required for staining. The time of exposure to the action of the staining fluids may be materially shortened by application of heat. For this purpose the cover-glass is held with the forceps directly over the flame until the vapor of steam is given off from the staining solution. The stained specimen is thoroughly washed in water, then carefully dried between filter-paper, and mounted in xylol-Canada balsam. If blood-preparations are to be examined for bacteria, it is likewise well to treat the smeared cover-glasses according to the acetic-acid

method, as by this means the blood coloring-matter and a portion of the plasma are removed.

The specimens prepared in the manner described show the size and the shape of the bacteria, but fail to disclose their mutual positions in the colonies. It this information be desired, so-called contact-preparations or impress-preparations must be made. A carefully cleaned cover-slip is lightly applied or impressed upon an isolated bacterial colony of a gelatin-plate or an agar-plate, and immediately removed. After it has been dried, this preparation is further treated exactly in the manner described.

Staining of Sections of Tissue.—Bits of tissue hardened according to the usual methods and embedded in celloidin are cut with the aid of the microtome. The sections are next placed in distilled water, and from this are transferred to one of the staining solutions mentioned. In this they are permitted to remain in general from two to fifteen minutes. The excess of coloring-matter is removed by means of dilute acetic acid (1 : 1000), and the sections are rinsed in water for the removal of the acid, dehydrated with absolute alcohol, cleared with xylol, and mounted upon a slide in xylol-Canada balsam. It is advisable first to spread the sections upon slides, and to undertake the staining, the decolorization, the dehydration, and the clearing upon these. On the whole, this mode of procedure agrees with that originally described by Weigert. If the tissue has been embedded in paraffin, this must be extracted with xylol before staining, and the xylol be then removed with alcohol.

The methods of staining thus far described have the property of staining all bacteria in the same way. There is, however, a method that stains some of the bacteria specifically—that is, Gram's method.

Gram's Method.—The prepared cover-slips from a fresh culture from twenty-four to forty-eight hours' old are placed in aniline-water gentian-violet solution (cover-glasses for one or two minutes, sections directly out of alcohol for ten or fifteen minutes), and then for half a minute, or for two and a half minutes or three minutes, in an iodin potassium-iodid solution, consisting of iodin 1.0, potassium iodid 2.0, water 300.0. Decolorization is next effected in alcohol, and is continued as long as any color is given off. The preparation, which finally appears colorless (light gray), is then

rinsed in water, dried, and mounted in Canada balsam. Stained in this way certain bacteria assume a bluish-black color, while others yield up the stain completely on decolorization. Gram's method, which is of especial importance in the identification of bacteria, is most difficult of application. The slightest variations in its technic may render the results doubtful. It is well, therefore, to make a control-stain in every instance, examining simultaneously, in addition to the bacteria under investigation, another variety of bacteria that with certainty either stains (staphylococci) or decolorizes (bacterium coli).

Günther's Modification of Gram's Method.—After exposure to the action of the iodin potassium-iodid solution, the specimen is placed for half a minute in alcohol, then for a short time (ten seconds) in three per cent. hydrochloric-acid alcohol, and, finally, for complete decolorization, in pure alcohol. Before mounting, the sections are cleared in oil of cloves or in xylol.

Weigert's Modification of Gram's Method.—After treatment with aniline-water gentian-violet solution, the specimen is rinsed in water, and the section is spread upon a slide, iodin potassium-iodid is added, and then removed with bibulous paper. Next follow differentiation, dehydration, and clearing with aniline oil and xylol, and mounting in Canada balsam.

Double Staining.—To make a contrast between the bacteria and the surrounding tissue, it is advisable, after staining by Gram's method, to stain the sections further with a dilute watery solution of vesuvin, safranin, or carmine, and to rinse in alcohol. In this way, however, the bacteria under some circumstances lose a portion of their stain. For this reason it is preferable to apply the contrast-stain before using Gram's method of staining the bacteria. To this end the sections are washed in water, stained in picrocarmine solution, again washed in water and placed in alcohol, after which the staining, according to the method of Gram, can be undertaken at once or at any convenient time. The staining of the tissues is in no way interfered with by Gram's method. Not all bacteria remain stained after application of Gram's method: a very considerable number can not be demonstrated by this means. Particulars in this regard will be found in the special section.

Staining of Capsules.—Some bacteria are character-
ized by the formation of capsules in the blood and in
other animal substances (anthrax-bacilli, pneumococci, ba-
cillus of Friedländer, etc.). Occasionally, the capsules
appear also in cultures, and their demonstration can be
made in the following method of Johne : The preparations
are stained in a warmed two per cent. solution of gentian-
violet, are rinsed in water, decolorized for from ten to
twenty seconds in two per cent. acetic acid, and are washed
and mounted in water (not Canada balsam, as this causes
the capsules to shrink).

Staining of Spores.—Spores are stained with extraor-
dinary difficulty, because of the dense and impenetrable
membrane by which they are surrounded. The usual
methods of staining are not sufficient for the staining of
spores. This may be effected by exposure of cover-glass
specimens prepared in the usual way with spore-containing
bacteria for an hour in hot carbol-fuchsin solution or aniline-
water fuchsin-solution, which is from time to time brought
to the boiling-point. By addition of solution undue con-
centration is avoided. The specimen is taken directly from
the stain, and is placed in ten per cent. hydrochloric acid
or in three per cent. hydrochloric-acid alcohol, in which it
is washed for about a minute. By this means everything
is decolorized with the exception of the spores, which re-
tain their stain. It is here, also, useful to make a contrast-
stain, by exposing the specimen briefly to the action of a
watery solution of methylene-blue or of malachite-green.
The bacilli then appear blue or green respectively, and the
spores, on the contrary, a bright red.

If it be desired to stain the spores without reference to
the bodies of the bacteria, the specimens may, according to
a suggestion of Buchner, be placed for half a minute in
concentrated sulphuric acid. By this means the vegetative
forms lose their power of staining, whereas the spores,
after thorough rinsing in water, are readily susceptible to the
action of the carbolfuchsin.

Good results are obtained with the aid of the apparently
complicated, but quite reliable, method of staining spores
proposed by Möller. The cover-glass, dried in air, is placed
for two or three minutes in absolute alcohol, is then rinsed
in water, and kept for two minutes in chloroform. After
again rinsing in water it is exposed for one or two minutes

to the action of five per cent. chromic acid, is again rinsed in water, and is stained for two minutes in steaming concentrated carbol-fuchsin solution. The preparation is then treated for a short time with five per cent. sulphuric acid (being passed through once or at most twice), is thoroughly rinsed in water, is counterstained quite deeply with methylene-blue or malachite-green, is again rinsed, and is dried and mounted in Canada balsam. The spores appear deep red, the bodies of the bacteria blue or green.

Staining of Flagella (Löffler).—In staining flagella a mordant is first used : Twenty grams of tannic acid are dissolved in 80 cu. cm. of hot water, and 50 cu. cm. of a saturated watery solution of ferrous sulphate that has stood in the cold for twenty-four hours with an excess of ferric sulphate are added, and finally 10 cu. cm. of a concentrated alcoholic solution of fuchsin. This fuchsin-solution may be advantageously permitted to stand exposed to air for several weeks ; it stains the better the older it is.

The specimens themselves must be prepared with cover-slips most carefully cleansed, and in such a way that an extremely thin layer is spread upon the cover-slip with little rubbing—so much of the material as will adhere to the tip of a platinum needle must yet be largely diluted. Quite young agar-cultures, from fourteen to eighteen hours old, and at the most twenty-four hours, are employed, as the flagella can not be stained in older cultures. After the preparations have been thoroughly dried in the air, the mordant is dropped upon them, and its action is permitted to continue for a minute. It is then entirely washed off, the cover-glass is dried, and upon it is filtered aniline-water gentian-violet or methyl-violet or fuchsin-solution. Heat is now applied carefully by means of a small flame, until vapors of steam arise. Then, after a minute, the preparation is washed in water, is dried, and is mounted. This modification by Günther of Löffler's method of staining flagella yields quite satisfactory results. By another method the preparations, dried in air, are exposed for from six to twelve hours to the action of a two per cent. solution of tannic acid and one-half per cent. hydrochloric acid, are rinsed in water, immersed for an hour in a watery solution of iodin, again washed in water, and then stained for half an hour in aniline-water gentian-violet. The specimen should then be mounted, not in Canada balsam, but in solution of iodin.

DETERMINATION OF THE PATHOGENICITY (OR THE SPE-
CIFICITY) OF BACTERIA BY ANIMAL EXPERIMENTATION.

The possession of pure cultures renders it possible to
undertake experiments upon animals, and thus to deter-
mine the changes that are induced in the animal organism
by a given variety of bacteria.

In order to consider a bacterium as the cause of an in-
fective disease, in order to declare it specific for this disease,
it must comply with three conditions formulated by Koch :
In the first place, it must be present in all cases of the given
disease ; in the second place, it must occur only in this dis-
ease ; and, in the third place, it must be capable of induc-
ing essentially the same disease in experiments on animals.
Animal experimentation, therefore, plays a most important
rôle in bacteriology.

In order to infect animals with bacteria various ways
are open. The natural portals of infection may be em-
ployed, and, besides, new ones may be artificially estab-
lished through which the microbes are introduced into the
organism.

(*a*) *Cutaneous Inoculation.*—Quite superficial wounds of
the skin are made in animals (as in vaccination), and these
are smeared, by means of the platinum wire, with a small
amount of pure culture. In guinea-pigs and in mice, in-
stead of cutaneous inoculation, an incision is made with
scissors through the margin of the ear, and the injured
places are rubbed with the inoculating material.

(*b*) *Subcutaneous Inoculation.*—By means of a scalpel or
an inoculating needle, a pocket is formed in the subcuta-
neous connective tissue, into which the bacterial material is
introduced ; or the bacteria suspended in water or in bouillon
are introduced beneath the skin by means of a hypodermic
syringe.

(*c*) *Intravenous Injection.*—By means of a hypodermic
syringe the infecting fluid is injected directly into a vein
that either lies quite superficially (as the marginal vein
of the rabbit) or is exposed by dissection. Entrance of
air into the vein may cause immediate death through air-
embolism. It is therefore necessary to exclude carefully
all air-bubbles from the system, and, further, to compress
the vein with a small cotton pad, and close the wound
immediately after removal of the needle.

(*d*) *Inoculation of the Anterior Chamber of the Eye.*—With a cataract-needle a small incision is made at the junction of the cornea with the sclerotic, the aqueous humor is permitted to escape, and the infecting material is introduced. The wound closes and heals rapidly.

(*e*) *Inoculation of the Cavities of the Body.*—The needle of the syringe is introduced into the selected cavity (pleural or peritoneal), and the suspension of bacteria is injected. In intraperitoneal inoculation the needle of the syringe, after thorough cleansing of the abdominal wall, is introduced subcutaneously in a horizontal direction ; then the syringe is elevated, and the needle pushed on until the disappearance of resistance indicates that the extremity is free in the abdominal cavity.

(*f*) *Subdural Inoculation.*—A trephine-opening is made to one side of the sagittal suture in order to avoid injury to the longitudinal sinus, and, with the aid of a curved needle, the fluid is introduced beneath the dura.

(*g*) *Inoculation by Inhalation.*—The bacterial mass is minutely subdivided by means of a spray, which is introduced through a tube into a closed space in which the experimental animals are placed.

(*h*) *Inoculation through the Gastro-intestinal Tract.*—The food of the animals is saturated with the bacterial fluid, or this is introduced into the stomach by means of a tube, the jaws of the animal being held apart with the aid of a hollow wooden gag, through which an elastic so-called Nélaton catheter is passed carefully into the stomach.

For special purposes (introduction of bacteria into the liver or into the portal vein or into a loop of intestine) celiotomy becomes necessary. After especially careful sterilization of instruments, hands, and field of operation, the cutaneous incision is made, the muscles are divided layer by layer, and, finally, the peritoneum is divided upon a grooved director. At the conclusion of the inoculation the wound is closed by interrupted peritoneal, muscular, and cutaneous sutures, and it is covered with iodoform-collodion.

All of these various inoculations must naturally be carried out with the most rigorous cleanliness. The skin at the point of inoculation must be shaved and washed with soap, solution of mercuric chlorid, alcohol, and ether. All instruments employed in the inoculation are sterilized by boiling in a one per cent. soda-solution. The disinfection

of the ordinary hypodermic syringe is more difficult, and for this reason quite a number of sterilizable syringes (Fig. 36) have been devised (Roux, Koch, Lewin). The ordinary hypodermic syringe is conveniently and safely disinfected by filling it with, and permitting it to remain for from twelve to twenty-four hours in, a five per cent. solution of carbolic acid, and then removing the carbolic acid by repeated rinsing in sterilized water. In dealing with especially infectious and conspicuously resistant bacteria (anthrax and tetanus), however, the syringe of Roux is employed, and it is boiled for ten minutes in a one per cent. solution of soda. In inoculating the anterior chamber of the eye the greatest care must be taken to secure disinfection of the conjunctival sac. This is cleansed
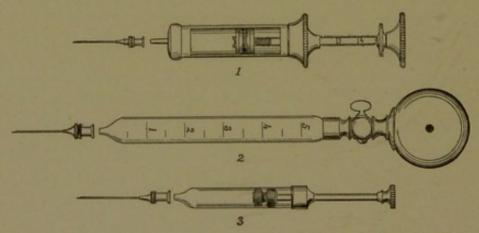
Fig. 36.—1, Roux's bacteriologic syringe; 2, Koch's syringe; 3, Meyer's bacteriologic syringe.

with a 1 : 3000 solution of mercuric chlorid, which is removed by irrigation with sterilized water. Anesthesia is effected by means of boiled cocain-solution.

The inoculated animals are carefully observed, their temperature is taken at regular intervals, the evacuations and the occurrence of convulsions, etc., are noted, and, in short, all manifestations of disease are looked for. Should an inoculated animal die, the autopsy is to be conducted with every precaution. It is usually made as soon as possible after death, in order to avoid the occurrence of putrefaction. The animal, placed upon its back, is stretched upon a board, with somewhat raised borders. The whole surface of the animal is moistened with a solution of mercuric chlorid, in order to prevent the generation of dust. The
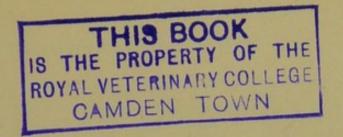
abdominal wall is thoroughly cleansed with the same solution, and then, with instruments sterilized in the flame, it is divided and dissected back on either side sufficiently for the flaps to be fastened to the board with small upholstery-tacks. After renewed irrigation a fold of peritoneum is raised, the abdominal cavity opened with a freshly sterilized knife, and the peritoneum thrown back on either side as far as possible. Pieces of all the organs (liver, spleen, kidney, possibly testicle) are removed, and placed in sterilized glass jars for further investigation. From the tissue-fluids of the organs and from the blood cover-slip preparations are made, and at the same time cultures are prepared and poured into plates. As a matter of course, the macroscopic appearance of the organ also is observed, and every pathologic alteration is carefully noted. If necessary, portions of the various organs are hardened and cut with the microtome, in order that the distribution of the bacteria in the tissues can be studied subsequently in stained sections.

In opening the thoracic cavity the xiphoid process is raised with sterilized forceps, and the ribs on the left side, and then those on the right, are divided with scissors, and, finally, the manubrium sterni above is divided. The heart is now exposed, and is opened with a sterilized and cooled knife; and cultures, plates, and streak-preparations are made from the heart's blood. At the conclusion of the autopsy the instruments and the dissecting table are thoroughly disinfected, and the animal cadaver is incinerated.

Special circumstances justify at times the holding of the autopsy at a later period, or subsequent investigation of individual organs. Thus, typhoid-bacilli are more easily cultivated from the spleen of a patient dead of typhoid fever, if that organ has been kept for some time, than if it is examined fresh. Also, in the blood of rabbits destroyed by pneumonia the bacteria can be more readily demonstrated about twelve hours after death than immediately after. Under these circumstances an increase of the bacteria in the cadaver must obviously take place.

However valuable animal experimentation often proves, it is, nevertheless, not capable, in every instance, of securing the desired information. It fails frequently, and especially in connection with those diseases that occur as infections exclusively in man—as for instance, cholera, typhoid fever,

etc. ; but even in these cases experiments on animals are not useless, for they demonstrate the toxic activity of the bacteria in question, and they lead, above all, to a knowledge of the anti-bodies formed in the blood-serum. Also, the other of Koch's postulates—that a microbe, to be considered as the cause of a disease, must be present only in association therewith—is not fulfilled in certain diseases. Those infections that are due to the bacteria giving rise to inflammation and suppuration are sometimes caused by the one and sometimes by the other of these germs. The clinical picture of these diseases depends less upon the species of infecting bacteria than upon the site at which the infection is localized. This is true of otitis media, of meningitis, of empyema, etc. ; at least, we are as yet not in a position with regard to these diseases to set up different clinical pictures in accordance with the various bacterial findings. A streptococcus-meningitis is clinically not sharply differentiated from a pneumococcus-meningitis or from a meningitis caused by staphylococci, etc. The conception of the specificity of bacteria, which is otherwise fully applicable to the etiology of infectious diseases, must be abandoned with relation to these common excitants of inflammation.

PART II.

INFLAMMATION AND SUPPURATION.

Almost all bacteria exhibit under some circumstances inflammatory and suppurative properties (phlogogenic or pyogenic activity). Inflammation and suppuration may also be induced by chemic substances—as, for instance, ammonia, oil of turpentine, etc.; but, above all, by bacterial metabolic products (ptomains, proteids, etc.) when these are employed apart from the bacteria. For practical purposes, however, this purely chemic mode of origin is of no noteworthy importance, and in almost every suppurative and inflammatory process microorganisms are the responsible agents. The bacteria that are found in the large majority of cases in inflammatory and suppurative areas, and that are known as the common (not specific) causes of inflammation and suppuration, are as follows :

1. The so-called pyogenic cocci (staphylococci, streptococci, pneumococci, etc.).

2. The bacterium coli commune, and the entire group of related bacteria.

3. The far less common pneumonia-bacillus of Friedländer.

4. The bacillus pyocyaneus.

MORPHOLOGY OF THE CAUSATIVE AGENTS OF INFLAMMATION.

Staphylococcus Pyogenes Aureus (Fig. 37).—This appears in the form of spherical nonmotile cells (micrococci), from 0.7 to 1.2 μ in diameter, generally arranged like bunches of grapes; hence the designation staphylococci. They stain readily with all basic aniline dyes, and also according to Gram's method. The temperature-minimum

115

is $+6°$ C. (42.8° F.), the temperature-maximum $+44°$ C.
(111.2° F.), the temperature-optimum from $+34°$ C. (93.2°
F.) to $+38°$ C. (100.4° F.): thus, the temperature of the
body. On gelatin-plates, with low powers of the micro-
scope, they form at first round, coarsely granular colonies,
with sharply limited borders, and of whitish-gray color;
later, they become orange-yellow, and liquefy the gelatin
with moderate rapidity. In gelatin stab-cultures develop-
ment takes place along the line of inoculation, with liquefac-
tion. On agar streak-cultures there forms a moist, shining,
golden-yellow raised column, and also upon potatoes.
Bouillon is rendered densely turbid, and presents a yellow
sediment. Milk is coagulated. In milk and bouillon, prin-
cipally lactic acid, also propionic acid, valerianic acid, and
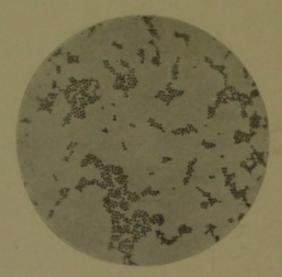
Fig. 37.—Staphylococcus pyogenes aureus, from an agar-agar culture (Günther).

isobutyric acid, are formed. Facultative anaerobic, the
staphylococcus aureus generates its yellow pigment only in
the presence of oxygen. Cultures retain their vitality for
more than a year. They are destroyed by brief exposure
to the action of live steam, and in pus dried on silk threads
by exposure to the action of two or three per cent. carbolic
acid for five minutes.

Staphylococcus pyogenes albus (Fig. 38) is absolutely
identical with the foregoing except that it does not give
rise to pigment-formation.

Staphylococcus pyogenes citreus generates a citron-
yellow ferment, but in its other properties it is identical
with the staphylococcus aureus.

Both *staphylococcus cereus albus* and *staphylococcus cereus flavus*, which are uncommon, are characterized by not liquefying gelatin. The one possesses a wax-like white color, while the other generates a wax-like yellow pigment.
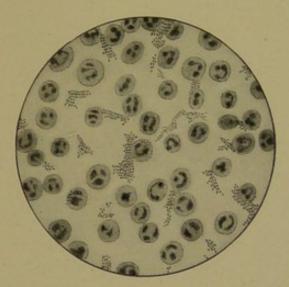
Fig. 38.—Staphylococcus pyogenes albus (Jakob).

Streptococcus Pyogenes (Erysipelatis).—This appears in the form of nonmotile micrococci arranged in chains of greater or lesser length. (Fig. 39.) They vary in size
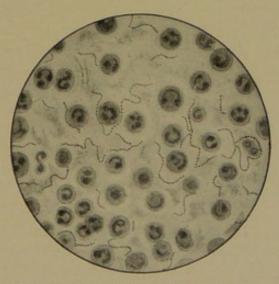
Fig. 39.—Streptococcus pyogenes (Jakob).

between 0.3 μ and 1 μ. They stain readily, and by Gram's method. The temperature-optimum is between 30° C. (86° F.) and 37° C. (98.6° F.); they develop also at

room-temperature, which, however, must not be too low. Upon gelatin-plates the streptococcus grows in the form of white, small, granular colonies that do not cause liquefaction. With high powers of the microscope the chains can distinctly be seen projecting beyond the margin of the plate. In gelatin stab-culture there is no confluent growth ; the line of inoculation can be seen distinctly to be made up of individual colonies separated from one another. The stab-culture on agar presents the same appearance. Bouillon, which constitutes an admirable culture-medium for streptococci, is usually not rendered turbid, but it presents a flocculent crumbling sediment. Upon potatoes only slight growth takes place. In milk the streptococcus induces coagulation. It exhibits facultative anaerobiosis. In cultures the streptococcus dies much more quickly than the staphylococcus—within as short a time as four months. Accordingly as the streptococci develop in bouillon into long or short chains, a distinction has been made between *streptococcus longus* and *streptococcus brevis*. Besides, a *streptococcus conglomeratus* has been distinguished that resembles superficially the staphylococcus in the interlacing and adhesion of the individual chains. This differentiation, however, has had to be abandoned. In order to maintain the virulence of streptococci, Petruschky recommends their renewal in gelatin stab-cultures every five days, and their preservation in the refrigerator.

Diplococcus Pneumoniæ Fränkel (*Streptococcus Lanceolatus Pasteur*).—This organism is, as a rule, a nonmotile coccus, arranged in pairs, with lancet-shaped extremities, and frequently joined together in small chains. (Figs. 40, 41.) They possess a capsule, which, however, is only clearly visible in the products of disease, but which is usually absent in cultures. They stain readily, and also by Gram's method. The capsule can be demonstrated by placing the cover-glass preparation for a minute in 1 per cent. acetic acid, drying, and then staining in aniline-water gentian-violet. Johne's method of staining capsules may also be employed (p. 108). The temperature-minimum is 22° C. (71.6° F.) ; the temperature-maximum 39.5° C. (103.1° F.) for cultures upon solid media, 42.5° C. (108.5° F.) for cultures in fluid media ; the temperature-optimum from 35° C. (95° F.) to 37° C. (98.6° F.). Upon gelatin the pneumococcus appears at temperatures above 25° C. (77° F.) in

the form of small, delicate colonies.   Upon solidified agar in slants, when only feebly alkaline, upon agar-plates and upon blood-serum it develops in the form of small, finely
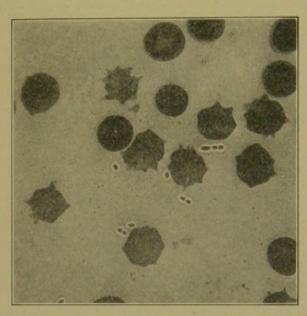
Fig. 40.—Diplococcus pneumoniæ in the blood (Fränkel and Pfeiffer).

granular colonies, resembling dewdrops.   Development in bouillon presents nothing characteristic.   Some varieties induce coagulation in milk.   The cultures die with extraordinary rapidity, usually in the course of a few days.   The cause of death has been attributed to the formation of lactic acid and formic acid, which can always be demonstrated in cultures several days old.   If the cultures are neutralized with calcium carbonate, they may retain their vitality for several months.   The diplococcus of Fränkel thrives best upon culture-media that contain considerable blood.   This is added to solid media in a thick layer, or it is mixed in considerable amounts with fluid media.   The diplococcus grows well in the
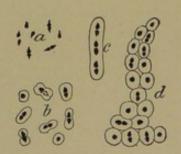
Fig. 41.—Diplococcus pneumoniæ: *a*, Cocci without capsules ; *b*, single and paired cocci with capsules ; *c*, chainform; *d*, colony of cocci (Ziegler).

absence of oxygen and thus retains longer its vitality and its virulence.

Diplobacillus Pneumoniæ Friedländer.—Much larger than the preceding (minimum, 1 μ), the bacterium of Fried-

länder is rod-shaped. It is arranged in pairs and in chains.
(Fig. 42.) It also possesses a capsule, which is distinct,
except in cultures. It does not stain by Gram's method.
The bacillus grows vigorously as well at room-tempera-
ture as at the temperature of the
body. Upon gelatin-plates it forms
small, porcelain-like points. The gela-
tin is not liquefied, but in the course
of time is discolored brown. A stab-
culture assumes a typical nail-shape ;
an agar streak-culture, a thick succu-
lent layer. In an agar stab-culture
gas is generated. Potato exhibits a
yellowish coating and gas-formation. Grape-sugar nutrient
solutions undergo fermentation, with the formation of car-
bon dioxid, hydrogen, ethylic alcohol, and acetic acid.
Milk is not coagulated. Friedländer's bacillus retains its
power of development for a long time.
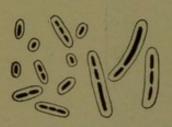
Fig. 42.—Bacillus pneu-
moniæ of Friedländer.

Bacillus Pyocyaneus.—The bacilli of green or blue
pus are small, slender, exceedingly active motile rods (with
a single flagellum) that in culture sometimes arrange them-
selves in small chains. They do not stain by Gram's
method. They grow almost as well at room-temperature
as in the thermostat. They exhibit facultative anaerobiosis,
but no spore-formation. On gelatin-plates flat, irregularly
circumscribed colonies form, with a radiate arrangement,
about which a zone of liquefaction soon forms. In gelatin
stab-culture liquefaction takes place rapidly. Upon agar
and potatoes there is vigorous development. Bouillon is
rendered densely turbid. Milk is coagulated and pepton-
ized. All cultures, especially, however, those containing
grape-sugar, soon assume a green or a greenish-blue color
that is imparted to the entire nutrient medium. The bacil-
lus pyocyaneus generates various pigments, according to
the constitution of the culture-medium, but only with free
access of oxygen. The best known of these is pyocyanin,
a crystallizable aromatic combination, related to anthracene
(Ledderhose), and a fluorescent green pigment.

Bacterium Coli Commune.—This appears as short,
narrow rods, frequently with vacuoles, twice as long as
wide, arranged in pairs. (Fig. 43.) Besides this simple form,
there is, however, marked pleomorphism, with long rods,
coccus-like bodies, and filaments. Motility is generally quite

active, although it may at times be wanting completely. The motile varieties possess flagella. For staining, Löffler's methylene-blue solution or carbolfuchsin is most available. The bacteria do not stain by Gram's method. They thrive
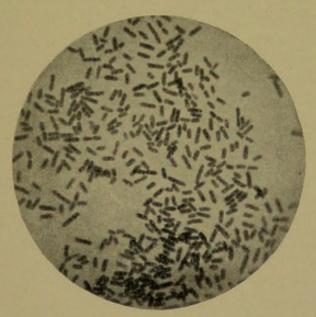
Fig. 43.—Bacillus coli communis, from an agar-agar culture; × 1000 (Itzerott and Niemann).
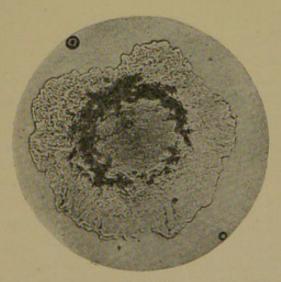
Fig. 44.—Bacillus coli communis: superficial colony two days old upon a gelatin-plate; × 21 (Heim).

best at body-temperature, although they grow well, also, at room-temperature. Upon gelatin-plates (Fig. 44) there form deeply situated, yellow, punctate colonies. The superficial colonies either spread upon the surface, possess

a dense center, and are surrounded by a thin, serrated, leaf-like, bluish, iridescent coating, which, with high powers of the microscope, exhibits a delicate linear network-arrangement; or they present a sharp boundary, and appear as porcelain-white points half the size of a pinhead. In gelatin stab-cultures a chain of small, white, spherical colonies forms along the line of inoculation, while upon the surface in individual cases a roset appears, and in other instances a hemisphere. The gelatin is never liquefied, but soon exhibits a dense turbidity and distinct iridescence. In all gelatin-cultures ammonia and crystals of ammonio-magnesium phosphate (coffin-lid shape) are formed. In agar streak-cultures a dense yellowish deposit forms, and upon potatoes a brownish membrane. Bouillon is rendered turbid, and in the case of the actively motile varieties a coating is often formed. Distinct gas-formation takes place in growths upon all of the nutrient media mentioned. It is favored by the presence of reducing substances, and by anaerobic growth. Addition of potassium nitrite and sulphuric acid to peptone-bouillon cultures (1 cu. cm. of a 0.02 per cent. potassium-nitrite solution and several drops of sulphuric acid to 10 cu. cm. of bouillon) causes the development of a red tint (nitroso-indol reaction). Coagulation takes place in milk. Grape-sugar, cane-sugar, and glycerin undergo fermentation. Acid urine is not altered by the growth of the bacterium coli, while alkaline urine at times undergoes ammoniacal decomposition. The bacterium coli commune retains its vitality for a long time.

Bacillus Aerogenes.—This organism is closely related to the preceding. It appears as short, nonmotile rods, forming filaments, sometimes with capsules, and without spores. They do not stain by Gram's method. Upon gelatin-plates they form round, porcelain-white, prominent colonies. In gelatin stab-cultures they form a nail-like growth, and gas-formation takes place. In agar streak-cultures a white, slimy deposit forms. Upon potato a yellowish deposit takes place. Bouillon is rendered turbid and a coating forms. Milk is coagulated. Growth takes place in urine without decomposition of urea. In all nutrient media energetic gas-formation takes place, and in those that contain sugar acetic and formic acids are generated. The bacillus aerogenes is probably identical with the organism described by the Guyon school as bacillus

pyogenes, which plays so important a rôle in the pathology of the genito-urinary tract.

THE PATHOGENIC PROPERTIES OF THE CAUSATIVE AGENTS OF INFLAMMATION WITH RELATION TO ANIMALS.

The pathogenic activity of the exciting agents of inflammation and suppuration with regard to animals is, on the whole, much the same for all members of the group. Introduced subcutaneously, they cause *local inflammation*, which manifests itself in simple swelling and slight febrile movement. If the virulence of the bacteria be somewhat greater, this inflammation leads to suppuration, and there results a *local abscess*, which may rupture outward, and undergo cure. Still greater virulence of the microorganisms may cause the local imflammation and suppuration to be complicated by *sepsis*—that is, by intoxication with bacterial poisons generated at the site of inflammation and thence absorbed ; or it may lead to *pyemia*—that is, to dissemination of the bacteria through the lymph-channels and the blood-vessels ; in the latter instance probably through emboli containing bacteria—and which give rise to the formation of *multiple purulent foci*.

The introduction of the causative agents of inflammation into the serous cavities leads to the development of serous or purulent pleuritis or peritonitis. Under all of these conditions both the clinical course of the disease and the postmortem findings exhibit completely the conditions sufficiently well known from human pathology.

Septicemia is exceedingly common in animal pathology, while it occurs but seldom in human beings. This may be considered as the expression of especially virulent activity on the part of the causative factors of inflammation. It occurs after intravenous introduction of the bacteria ; in some cases, however, also in connection with every other variety of inoculation — and especially the infection of rabbits with pneumococci. Characteristic of septicemia is the absence of any localization of the disease. The clinical picture has, therefore, no distinctive features. The animal is severely ill, as may be recognized from the appearance of the skin, of the eyes, and the general attitude. It does not eat, and it often suffers from diarrhea, especially after

infection with bacterium coli or with another member of this group. At the same time there is always fever, which gradually increases ; and usually also dyspnea. After the disease has existed for a shorter or a longer period of time, death results, with decline of temperature, and not rarely with convulsions. Upon postmortem examination the individual organs exhibit parenchymatous cloudiness, the spleen is enlarged, there is often nephritis, but there are otherwise no special alterations. On microscopic examination and cultural investigation, however, the bacteria introduced can be seen in large numbers everywhere : in every tissue, in the secretions, in the blood. They have multiplied in the blood, and fill all of the vessels down to the smallest capillaries, which, by reason of their numbers, they often actually plug. The bacteria are demonstrable in the blood already during life, after the disease has existed for some time, but generally not in the numbers in which they are found after death. Their greatest multiplication appears to take place just before death, and in the cadaver.

The pathogenic activity of the individual causative agents of inflammation may be stated as follows :

Staphylococci : Cutaneous inoculation is unattended with ill results ; subcutaneous injection induces a local abscess in mice, guinea-pigs, rabbits ; and intravenous injection, at times, pyemia in rabbits.

Streptococci : If the material is rubbed upon small cutaneous wounds of the rabbit's ear, erysipelatous inflammation follows. Subcutaneous injections in mice and guinea-pigs are followed by septicemia, with or without local abscess ; and intravenous injection induces septicemia.

Diplococcus lanceolatus Fränkel is pathogenic for rabbits, mice, and guinea-pigs. Death from septicemia occurs with certainty in rabbits after subcutaneous injection of even small amounts. Mice are somewhat less susceptible, but they die mostly of pneumococcus-septicemia ; and guinea-pigs, only after the introduction of considerable amounts. After intraperitoneal injection guinea-pigs frequently die in consequence of fibrinous peritonitis. In dogs the inflammation caused by the diplococcus remains local, the bacteria not entering the blood ; the dogs, however, die as a result of the poisoning (sepsis), and most readily after subcutaneous inoculation.

Diplobacillus pneumoniæ Friedländer readily destroys mice and guinea-pigs on subcutaneous and intravenous injection, and dogs and rabbits less readily. Postmortem examination discloses here, also, septicemia.

Bacillus pyocyaneus, when employed in considerable amounts, is pathogenic for guinea-pigs and rabbits, inducing local suppuration, diarrhea, and sepsis.

Bacterium coli commune : Mice, guinea-pigs, rabbits, and dogs are susceptible to subcutaneous, intraperitoneal or intravenous injection. There result local abscesses, hemorrhagic diarrhea, and septic manifestations ; postmortem examination disclosing marked hyperemia of all the abdominal organs, and often septicemia.

In conclusion, it may be again especially emphasized that the results obtained in experiments on animals with all of these common causative agents of inflammation are by no means constant. They vary quite extraordinarily, in accordance with the virulence of the material employed, and the inoculations frequently enough pursue a completely negative course. The virulence of the exciting agents of inflammation varies, however, according to their source. The bacteria, for instance, cultivated from a fatal pyemia, are much more virulent than the relatively harmless bacteria derived from a mild suppuration of the skin, etc. It is to be emphasized, further, that all of these exciting agents of suppuration and inflammation are not exclusively pyogenic, but they may also induce purely serous processes : as, in the main, the three principal varieties of inflammation—the serous, the fibrinous, and the purulent—differ from one another only quantitatively.

THE OCCURRENCE OF THE CAUSATIVE AGENTS OF INFLAMMATION AND SUPPURATION IN HEALTHY PERSONS AND OUTSIDE THE BODY.

Staphylococci have been found in dust, upon the surface of the earth, in the air, in household wash-water ; further, almost constantly upon the surface of the skin, in the accumulations beneath the finger-nails, in the saliva, in the mucus of the pharynx, in the nasal secretion, in the intestinal contents, in the vagina, and in the urethra.

Streptococci have been cultivated from the air of hospi-

tal-wards and dissecting rooms, from the mouth, the nose, the pharynx, and the skin ; further, from the intestine, and—though seldom—from the vagina of healthy women.

The **diplococcus pneumoniæ Fränkel** is an exceedingly frequent inhabitant of the buccal and nasal cavities, and especially of the entire upper portion of the respiratory apparatus ; at times it is present in the intestine.

The **diplobacillus Friedländer** is found normally in the same situations as the diplococcus Fränkel, although its occurrence is much more seldom.

The **bacillus pyocyaneus** has been found upon the skin, especially in the axillary cavity ; further, in the external auditory canal and in intestinal mucus ; it is not rarely encountered also in the air and in water.

The **bacterium coli commune** and **bacterium lactis aerogenes** are found throughout the entire digestive tract, especially in the intestine (the aerogenes more commonly in the feces of infants) ; further, in the vulva and the vagina and on the prepuce, upon the skin, in the air, in water, and in milk.

The common causative agents of inflammation and suppuration are thus found in healthy persons in the main upon the skin and the so-called open cavities of the body, the pyogenic cocci more commonly upon the surface of the skin and in the upper portion of the digestive and respiratory tracts, and the pyogenic bacterium coli more commonly in the intestine. It is, therefore, to be anticipated that the inflammatory and suppurative processes that take place upon the skin and in the neighborhood of the buccal, nasal, and pharyngeal cavities are excited especially by cocci, while those of the intestinal tract and of the genitourinary tract and their entire neighborhood are due more commonly to the bacterium coli or the bacterium lactis aerogenes.

THE OCCURRENCE OF THE CAUSATIVE AGENTS OF INFLAMMATION IN DISEASE.

CUTANEOUS SUPPURATION.

Furuncle and carbuncle are almost always attended with the presence of staphylococci (staphylococcus aureus and albus). That staphylococci are, in fact, the cause of

carbuncles has been demonstrated by Garré, in an experiment upon himself. He rubbed a staphylococcus-culture into the intact skin of his left forearm. Four days later a characteristic carbuncle developed, and around it several isolated furuncles. The pus from all of the lesions contained the same staphylococcus that had been employed for the inoculation. This experiment renders it at the same time probable that furuncles and carbuncles are probably due to infection of the excretory ducts of the glands of the skin, into which the pyogenic material is in some way forced. Practically, furuncles are often seen to develop in places subjected to pressure or friction : as, for instance, by parts of the clothing.

Panaris.—In the pus from panaris staphylococci, as well as streptococci, and in rare cases also the bacterium coli commune have been found.

Abscess and Phlegmon.—These are due to staphylococci and streptococci, and in some cases also to Fränkel's pneumococci, especially in childhood and in the course of genuine croupous pneumonia. In abscesses in those sick with and convalescent from *typhoid fever*, staphylococci and streptococci are found frequently, and not at all seldom also typhoid-bacilli and bacterium coli. *Urinary phlegmons*, so frequently observed in the sequence of urinary infiltration, are usually due to the bacterium lactis aerogenes or the bacterium coli commune, which play the most important rôle in the suppurative processes of the genito-urinary apparatus.

So-called *cold abscesses* are generally found to be sterile. They may be considered as the product of tubercle-bacilli, and actually it has frequently been possible in experiments on animals to induce tuberculosis with the pus from such abscesses. Microscopically, however, it has been possible, only in the rarest of instances, to demonstrate in the pus the tubercle-bacilli that are present in small number. Also, in the larger, nontuberculous abscesses bacteria are sometimes not found at the center of the area of suppuration, and the pus, therefore, appears sterile. It is only necessary in such cases to examine material from the periphery, the so-called abscess-membrane, in order to find the pyogenic agents without difficulty.

Gas-abscesses, like ordinary abscesses, do not possess a uniform etiology. From them there have thus far been cultivated : (1) The bacterium coli commune and the bac-

terium lactis aerogenes, principally from the gas-phlegmons in the neighborhood of the intestinal canal ; (2) a special bacillus—the bacillus emphysematosus. (Fig. 45.) This appears in the form of nonmotile, plump rods, forming filaments. These are anaerobic, and they stain by Gram's method. They grow slightly in gelatin, without causing liquefaction. In bouillon, and also in agar, they give rise to the formation of fetid gas. This bacillus is but seldom found alone in gas-abscesses, but almost always in association with the ordinary pyogenic cocci. In experiments on animals a severe nonsuppurative inflammation, with gas-formation, and which at times causes death, is induced in guinea-pigs by sub-

Fig. 45.—Bacillus aerogenes capsulatus (from photograph by Prof. Simon Flexner).

cutaneous injection of this bacterium. That the bacterium coli and the bacterium lactis aerogenes may at times cause gas-phlegmons is not surprising, as it is known that both are active gas-producers, especially in the absence of oxygen.

In the *bacteriologic investigation* of a gas-abscess it is always necessary to be prepared for the presence of anaerobic microorganisms, and the choice of a culture-method must be governed with reference thereto.

Impetigo.—This is a peculiar anatomic form of suppuration within the layers of the epidermis that leads to the formation of pustules. From the contents of the pustules the staphylococcus pyogenes albus and aureus and the

cereus albus are obtained. These observations with regard to the common exciting agents of inflammation explain the clinical fact that the most variable suppurative processes—furunculosis, etc.—sometimes precede and sometimes follow the actual attack of impetigo.

Ecthyma.—This also is a form of cutaneous suppuration in which the pyogenic cocci are found. From the pus staphylococci and streptococci have been cultivated.

Herpes.—*Herpes zoster* has been designated by Pfeiffer (Weimar) an infectious disease whose causative agent belongs to the class of protozoa. The cells believed by Pfeiffer to be protozoa have, however, not received recognition, and, above all things, it has as yet been impossible to cultivate them. In the vesicles that become turbid staphylococci and streptococci are always found, while the contents of the clear vesicles are often sterile.

In cases of *herpes labialis* the vesicles contain the exciting agents of inflammation from the beginning, and more frequently streptococci and Fränkel's diplococci than staphylococci. According to Pfeiffer, protozoa are absent in this form of herpes. As soon as the contents of the vesicles are turbid, staphylococci will be found present therein, and partly in association with streptococci, partly alone. These observations suggest that herpes labialis is not a true zoster. In general it occurs only as a complication of such infectious diseases as themselves stand in etiologic relation with the common exciting agents of inflammation (pneumonia, meningitis, etc.); it may, perhaps, be viewed as a secondary localization of the causative agent of the primary process.

In *herpes of the pharynx* (angina herpetica) *and of the larynx* the same conditions seem to prevail as in herpes labialis.

ERYSIPELAS.

Erysipelas is excited by the streptococcus. The old discussion whether or not the streptococcus erysipelatis (Fehleisen) is distinct from the streptococcus pyogenes, the exciting agent of suppuration, can now be considered as finally decided. The two microorganisms are without doubt identical. This is demonstrated as well by their complete agreement in morphologic and cultural peculiarities, as also by the results of experiments on animals and man.

9

Microscopic Arrangement of the Streptococci in the Invaded Skin.—As suggested by Fehleisen, three zones may be distinguished : (1) A central, in which the process is in retrogression ; (2) the elevated erysipelatous margin ; and (3) a peripheral zone surrounding this deeply red erysipelatous zone, which, microscopically, is apparently still completely normal.   Streptococci are found in each of these three zones, but in much the largest number in the erysipelatous margin and in smaller number in the central and in the peripheral zone.   The organisms lie in the

Fig. 46—Streptococcus erysipelatis, seen in a section through human skin ; × 500 (Fränkel and Pfeiffer).

lymph-spaces and the lymph-vessels of the skin and the subcutaneous fatty tissue ; the lymph-spaces are almost completely choked up by them.   Sometimes they collect also about the lymph-vessels and the blood-vessels.   In the vesicles in cases of *erysipelas bullosum* streptococci are not constantly present.   On the other hand, they are never absent from the purulent products of *phlegmonous erysipelas*.   As a rule, in cases of erysipelas pursuing a favorable course the streptococci do not gain entrance into the blood.   Metastases are, therefore, rare in the symptom-

complex of erysipelas. Cases are, however, on record in which it was possible to cultivate streptococci from the blood of patients ; general infection, therefore, having existed.

Immunity.—Recovery from one attack of erysipelas confers no immunity to subsequent attack. Erysipelas is one of the diseases attended with numerous recurrences. The blood-serum of persons just recovered from an attack of erysipelas does, however, at times possess immunizing properties ; and large amounts thereof may, according to some reports, immunize animals (guinea-pigs and mice) to streptococci.

Experimental Evidence of the Etiologic Significance of the Streptococcus for the Development of Erysipelas. —The introduction of streptococcus-material into shallow, superficial wounds of rabbits' ears is followed by the development of erysipelatous inflammation. Much more important are the observations that have been made in human beings. Proceeding from the clinical experience that in individuals with advanced lupus or with inoperable tumors an attack of intercurrent erysipelas may lead to the cessation, or even the cure, of the original disease-process, it was ventured in such cases to undertake cutaneous inoculations with streptococcus-cultures derived from cases of erysipelas. These experiments yielded positive results : typical erysipelas pursuing a characteristic course occurring almost constantly. A similar result was attained by Koch and Petruschky by means of a streptococcus derived from a purely suppurative process. There is no doubt that a therapeutic influence is exerted by several streptococcus-infections upon the course of carcinoma, but the strength of the patient suffers so much in consequence of the inoculations with erysipelas that a practical application of this method is entirely out of consideration.

The Occurrence of Other Microorganisms in Erysipelas.—In two cases of true erysipelas (the second infected by the first) Jordan cultivated the staphylococcus pyogenes aureus. From this it may be concluded that the other causative agents of inflammation are capable, under certain circumstances, of inducing erysipelas. The usual cause, however, is without question the streptococcus.

The **bacteriologic diagnosis** of erysipelas is only most rarely necessary. In such an event a small scarification is made with a sterilized lancet at the border of the inflamma-

tory area, and plates are prepared from the fluid that escapes ; or four or five obliquely solidified gelatin-tubes or agar-tubes are smeared therewith. In this way, in most cases, individual colonies of streptococci will be obtained probably in the first tube, and with certainty in the remaining tubes.

Specific Treatment of Erysipelas and of Streptococcus-diseases Generally.—Marmorek increased the virulence of streptococci, which are readily attenuated in artificial culture, by cultivating them in bouillon to which sterile horse-serum had been added. With progressively increasing doses of the highly virulent cultures thus obtained he immunized horses, whose serum was then reported to exhibit immunizing and curative properties with relation to all streptococcus-infections also in human beings. Petruschky, who repeated the experiments of Marmorek, was, however, unable to confirm his conclusions. The serum of Marmorek obtained from the Pasteur Institute possessed neither immunizing nor therapeutic activity, also in observations made upon human beings. The streptococcus of Marmorek was most highly virulent for rabbits, but not for human beings.

PHLEBITIS AND LYMPHANGITIS.

Lymphangitis, which arises from some peripheral inflammation or suppuration—in some cases not demonstrable—is attended with the presence of the exciting agents just described, the pyogenic cocci, and at times with that of the bacterium coli. Also, the infectious form of **phlebitis,** which occurs as an associated manifestation of numerous infectious diseases, may be caused by the same microorganisms as give rise to the original disease. Thus, for instance, tubercle-bacilli have been found in the phlebitides of tuberculous individuals, and the streptococcus pyogenes in the phlegmasia alba dolens of puerperal women. More frequently, however, the inflammation of the vein is the expression of a secondary infection, and, therefore, its causes are, as a rule, found to be the common pyogenic cocci. The bacterial inflammation of the vein or the lymphatics leads to the formation of a bacteria-containing thrombus. This may be conceived as originating from adhesion of the microorganisms circulating in the vessel to a projecting point of its wall, or of a valve, or from the penetration by the bacteria of the wall of the vessel from without through the interme-

diation of the vasa vasorum. In the bacteriologic examination of the thrombus it is important to know that the bacteria are to be found only in the oldest, first-formed portion thereof, and on the corresponding portion of the wall of the vessel. The central and peripheral portions of the thrombus that subsequently form mechanically are often sterile.

INFLAMMATIONS OF THE NOSE AND THROAT.

In the secretions in cases of **acute rhinitis, pharyngitis, and laryngitis,** staphylococci and streptococci have been found, and also pneumococci and pneumobacilli, the last most frequently in the nose.

In cases of *fibrinous rhinitis* diphtheria-bacilli have been found repeatedly. In other cases their demonstration was not successful, so that it is not certain whether all cases of croupous rhinitis are to be considered as instances of diphtheria.

Ozena.—From the nasal crusts in cases of ozena a microorganism has been cultivated by various observers (Löwenberg, Abel, Paulsen), which presents so close a resemblance to the bacillus pneumoniæ Friedländer that a detailed description will not be necessary. The cultures are slimy and viscid, form comparatively little gas, and do not cause coagulation in milk. That this so-called ozena-bacillus is the cause of the disease is scarcely probable. The characteristic odor of ozena is wanting in all of the cultures. Further, the bacillus is never found in the diseased structures of the nose, but only in the secretions whose peculiar putrid decomposition it may possibly cause. For the present, the ozena-bacillus may be considered a variety of Friedländer's bacillus, slightly modified in consequence of the peculiar conditions related to the ozenous nose.

Rhinoscleroma.—Also the bacilli found in rhinoscleromatous tumors resemble Friedländer's bacilli. They induce fermentation of sugar and of milk, though in slighter degree than the pneumobacillus. On potatoes an often invisible, at times brown, gas-forming coating occurs. In the tissues the bacilli are found almost exclusively in the cells, in which they displace the nucleus and the protoplasm to one side, themselves almost entirely filling the whole cell (Mikulicz).

Noma.—Schimmelbusch described special noma-bacilli.

In a case of noma studied personally the bacterium coli commune was found. Concerning these observations the same criticism may be made that has been suggested with regard to the presence of Friedländer's bacillus in cases of ozena and rhinoscleroma.

ANGINA.

In the inflamed mucous membrane of anginas pursuing their course without the development of membrane staphylococci have, as a rule, been found, as well as in the purulent plugs of follicular (or lacunar) angina. The anginas characterized by the presence of a membranous deposit are frequently associated with streptococci, and often besides also with staphylococci, but at times with the latter alone. Finally, a considerable number of cases have been reported in which only pneumococci were present.

An attempt has been made on the basis of these bacteriologic observations to distinguish a *staphylococcus-angina*, a *streptococcus-angina*, and a *pneumococcus-angina*, each of which is held to be etiologically a distinct variety and to possess its special characteristics both clinically and from the diagnostic and prognostic point of view. Accordingly, staphylococcus-angina would be a relatively harmless affection, scarcely ever leading to complicating disorders. Streptococcus-angina would be severer, exhibiting more pronounced manifestations of intoxication (fever, glandular enlargement), and possibly being followed by nephritis and even general sepsis. Pneumococcus-angina, finally, would be characterized by the resemblance of its clinical course to that of pneumonia, by its stormy onset, possibly with a chill, and by high fever, with critical defervescence.

It must, however, be emphasized that the clinical picture of these anginas is by no means a constant one, and that the severity of the disease depends more upon the virulence than upon the species of the bacteria present. There occur staphylococcus-anginas (*phlegmonous anginas*), as well as anginas associated with the presence of streptococci alone, that pursue as mild a course as those with which the staphylococcus alone is associated. On the other hand, severe *pseudo-membranous anginas*, which clinically are indistinguishable from true diphtheria, and in association with which only streptococci are demonstrable, are not rare.

Pneumococcus-angina, further, pursues frequently the characteristic course outlined, though by no means always. It is to be added that isolated cases of tonsillitis have been reported in which the bacterium coli commune exclusively has been found.

Above all, however, in a not inconsiderable number of cases of angina, there is *mixed infection* with several of the micrococci named. Then, most commonly the two varieties of bacteria are found together from the beginning. At times, however, only streptococci can be demonstrated at first, and after some days also staphylococci, or even the latter alone. Under these conditions it must be concluded that the one coccus has associated itself with or overgrown the other. The frequency of these mixed infections, which clinically are indistinguishable from the pure infection, alone renders impossible a rigid separation of the anginas according to their causative agents.

Scarlatinal angina is usually associated with the presence of streptococci.

The **bacteriologic diagnosis** can, according to what has been said, be made only with a certain degree of probability from the clinical picture and the course of the disease. A positive diagnosis is possible only on microscopic and cultural investigation. The latter is made simply by rubbing a sterile swab of cotton that has been applied to the tonsil, or a bit of secretion or membrane obtained by means of a sterilized platinum loop, upon from three to five glycerin-agar tubes, or, better—in the differential diagnosis of diphtheria—upon from three to five tubes of Löffler's serum, or upon a serum-plate. The bacterial decision will frequently be questionable on account of the impossibility of excluding diphtheria with certainty by other means. Detailed reference will be made to this point in the section on Diphtheria.

From what has been said, it will be clear that the demonstration of staphylococci, streptococci, or pneumococci, is available for purposes of *prognosis* only with great caution.

OTITIS MEDIA.

In cases of serous inflammation of the middle ear, as well as in cases of suppurative or hemorrhagic type, there have been found the diplococcus pneumoniæ Fränkel, the

streptococcus pyogenes, the staphylococcus pyogenes, the diplobacillus pneumoniæ Friedländer, the bacillus pyocyaneus, alone or in mixed infection. All of these organisms are more or less common inhabitants of the buccal cavity, and migrate through the Eustachian tube into the middle ear under special circumstances. Influenza-otitis is associated with the presence of influenza-bacilli; tuberculous otitis, constantly with that of the tubercle-bacillus.

Bacteriologic Diagnosis.—The auditory canal is cleansed carefully with mercuric-chlorid solution, paracentesis is practised with a suitable needle or knife sterilized in the flame, the escaping pus is taken up with a platinum wire bent at an angle, and from it plates are cast (agar, on account of the presence of pneumococci).

After the occurrence spontaneously of perforation, the examination is of little value, as the secretion will have become contaminated by the numerous microorganisms of the external auditory canal. If tuberculosis is suspected in a case of chronic otitis media, the pus should be examined microscopically for tubercle-bacilli, and possibly animals should be inoculated with it.

MENINGITIS.

Inflammation of the cerebral meninges occurs as a *primary* disorder in the form of epidemic cerebrospinal meningitis, and as a *secondary* (metastatic) condition in connection with pyemia and numerous infectious diseases, especially in the sequence of otitis media, inflammation of the accessory cavities of the nose, and croupous pneumonia. A special position is occupied by tuberculous meningitis, which preferably involves the base of the brain. From the exudate in cases of secondary meningitis there have been cultivated the diplococcus lanceolatus Fränkel, the streptococcus pyogenes, the staphylococcus pyogenes, the bacterium coli, and the diplobacillus pneumoniæ Friedländer. In cases of tuberculous meningitis the pus and sections of tissue contain tubercle-bacilli, partly alone and partly in association with phlogogenic cocci. These bacteria gain access to the cerebral or spinal membranes from the nasopharyngeal space (lamina cribrosa), from the middle ear, or from the original focus of suppuration : in the last instance through the intermediation of the blood-stream.

The portal of entry for the bacteria in cases of epidemic meningitis has not yet been determined with certainty. The principal causative agent of this disease was, until recently, believed to be the diplococcus lanceolatus Fränkel, which was found in numerous cases in pure culture. A special organism is, however, to be taken into consideration in connection with the etiology of epidemic cerebrospinal meningitis—namely, the diplococcus intracellularis meningitidis (Weichselbaum, Jäger). This is a biscuit-shaped organism, arranged in pairs, which is almost always contained within the cells, and which is strongly suggestive of the gonococcus. It is readily stained in the exudate, but with more difficulty in sections. The most suitable stain is Löffler's methylene-blue. The organism does not stain by Gram's method, although Jäger maintains that it does. It grows best at 37° C. (98.6° F.). On agar-plates there develop superficial gray colonies that with low powers of the microscope appear dirty-yellow at the center and become lighter toward the periphery. The deep colonies are quite small, and marked by fine granulation and a slightly indented border. Upon glycerin-agar there develop small gray colonies that at times coalesce to form a thin coating. Upon blood-serum a slight deposit forms. In order to continue the culture of meningococci for a protracted period they must be reinoculated from every four to six days. They are feebly pathogenic for mice and guinea-pigs on introduction into the thoracic or abdominal cavity, and for rabbits on introduction into the blood-stream.

The **clinical diagnosis** is based upon the principle that with the simultaneous existence of some other infectious disease—as, for instance, pneumonia, otitis, tuberculosis—the causative agents of these conditions may be considered the cause of the meningitis. The absence of any such simultaneous organic disease justifies a diagnosis of epidemic cerebrospinal meningitis.

A direct **bacteriologic diagnosis** is possible during life by means of puncture of the spinal canal, which has now been quite extensively practised. Preparations are made from the exudate, and agar-tubes and serum-tubes, or agar-plates, are inoculated, and animals are infected. If tuberculosis is suspected, the specific stain for tubercle-bacilli must be employed. To obtain the causative agents from the meningeal pus after death, the same plan is pursued. As the diplo-

coccus is frequently encountered, it is advisable to inoculate a white mouse or a rabbit immediately with the pus.

BRONCHITIS.

Bronchitis, whatever its nature may be, is likewise dependent upon the activity of the common exciting agents of inflammation : pneumococci, streptococci, staphylococci, pneumobacilli, bacterium coli. Through the action of cold or of some other injurious agency that generally leads to bronchitis, these normal inhabitants of the commencement of the respiratory tract become lodged in the bronchi, and there excite inflammation. Their demonstration in sputum is easy. The patient is instructed to cleanse his mouth thoroughly with a solution of boric acid or of potassium chlorate, and then to expectorate in a sterilized glass dish. The sputum thus obtained is rinsed carefully in several vessels of sterile water, and then a flake from the center of the mass is smeared successively upon each of several agar-tubes or upon an agar-plate (Koch). In this way the usual plate-procedure can be avoided, as only one of the species of bacteria named is, as a rule, found in the bronchitic sputum in each case. The large number of bacteria that are found on microscopic examination of the expectoration are mostly derived from the mouth and the pharynx, and are adherent, therefore, to the outer layers of the sputum. In the majority of cases there develop from the expectoration thus treated pure cultures of staphylococci, Fränkel's diplococci, or streptococci. If mixed infection is present, the colonies develop separately in the tubes last inoculated, and from these they can be readily isolated.

Fetid bronchitis is associated with the presence of the same bacteria, but, in addition, also with putrefactive bacteria (proteus and others).

The green color that is sometimes observed in bronchitic sputum is in some cases due to the bacillus pyocyaneus, in others to the bacillus fluorescens, and to varieties of sarcinæ.

PLEURITIS.

Pleuritis has no uniform bacteriology. It may be primary or secondary ; in the latter event in association with diseases of the lungs, diseases of adjacent organs, trauma-

tism, or general infection. The pleural effusions due to general stasis (transudates attending heart-lesions, nephritis, etc.) are, naturally, sterile, providing secondary infection has not taken place, and for which the serous infiltration of the tissues offers a favorable soil.

The tubercle-bacillus and all of the pyogenic microorganisms are capable of inducing serous as well as purulent pleurisy.

The *primary pleurisies* (so-called pleurisies due to cold) are most frequently dependent upon the tubercle-bacillus. Next in frequency follows the diplococcus pneumoniæ Fränkel, which plays a most important rôle, especially in the pleural inflammations of childhood; and, further, all of the other pyogenic and phlogogenic microorganisms. Whether in all of these cases the pleuritic effusion is really primary can not always be determined with certainty, as the smallest pulmonary lesions—for instance, slight disease of the apex or a bronchopneumonia—that are scarcely susceptible of diagnosis clinically may induce pleuritis.

The *secondary pleurisies* are associated in a portion of the cases with the presence of the same bacteria that are responsible for the primary disease. In the effusions that so frequently occur in the sequence of pneumonia, in the so-called metapneumonic exudates, there is encountered the diplococcus; in the effusions attending pulmonary tuberculosis, the tubercle-bacillus; in the uncommon effusions complicating typhoid fever, the bacillus of Gaffky-Eberth; in the pleurisies that originate in purulent processes within the abdominal cavity, the bacterium coli; and so on. In those diseases whose causative agents are as yet unknown —as, for instance, articular rheumatism and carcinoma— the accompanying pleural effusion has been examined for bacteria, but as yet mostly with negative results. In another portion of the cases these concomitant pleurisies are dependent upon secondary or upon mixed infection. The pleural effusion then contains the common exciting agents of inflammation. Thus, in the empyema following scarlet fever the streptococcus pyogenes is often found; in that following smallpox, staphylococci; in that following influenza, the diplococcus lanceolatus Fränkel; and so on. *Metastatic pleurisy*, as part manifestation of a pyemic general infection, is caused by the staphylococcus or the streptococcus pyogenes. The same statement applies also

to pleurisies that arise in consequence of penetrating
wounds of the chest-wall. The putrid effusions contain,
in addition to the causative agents of suppuration, also
putrefactive bacteria, and generally the proteus.

Method of Bacteriologic Investigation.—A hypoder-
mic syringe with a capacity of from one to six cubic centi-
meters is kept filled for from six to twelve hours in five
per cent. carbolic acid, and is then carefully cleansed with
sterilized water in order to remove all of the disinfectant;
or a Roux's syringe is sterilized by thorough boiling. The
point on the chest-wall where the exploratory puncture is
to be made is washed with soap, alcohol, mercuric-chlorid
solution (1 : 1000), and ether, and the puncture, after
thorough disinfection of the hands, is made in the usual
way. The fluid obtained is received into a sterilized dish and
each of four or five agar-tubes successively, or an agar-plate,
is inoculated with a drop thereof, or a drop of the exudate
is permitted to flow directly from the syringe upon the sur-
face to be inoculated. The tubes are introduced into the
thermostat at a temperature of 37° C. (98.6° F.). At the
same time cover-slip preparations are made in the usual
manner, and examined for tubercle-bacilli and other bac-
teria. With the remainder of the fluid, if tuberculosis be
suspected, two or three guinea-pigs may be inoculated
through the peritoneum (see later). Serous effusions con-
tain few, if any, microorganisms. It is, therefore, a more
reliable procedure to remove considerable amounts of the
fluid, to centrifugate or sediment it, and to study the pre-
cipitate only.

**Diagnostic and Prognostic Significance of the Bac-
teriologic Findings.**—Bacteriologic investigation is not of
great importance in the diagnosis of serous effusions. The
large majority of serofibrinous pleurisies prove to be sterile.
The metapneumonic serous effusions contain at times the
diplococcus lanceolatus Fränkel, and before, as well as
after, the crisis. The presence of pyogenic microbes in
serous pleural effusions, as has been repeatedly observed,
does not, in all instances, justify the conclusion that puru-
lent metamorphosis into an empyema will take place. Such
effusions may, under circumstances, recede completely. If
a decision is to be reached in doubtful cases of serofibrinous
pleurisy whether tuberculosis exists or not, it is advisable
to inject into the peritoneum of guinea-pigs some of the

pleural fluid obtained under sterile conditions, and to wait and see whether the animals die of tuberculosis or not. This procedure, however, always occupies several weeks, and even then it is not always entirely reliable, and it frequently fails to resolve the doubt, as in spite of an evident tuberculous origin of the pleuritic effusion the animals often remain well. The reason for this is to be found in the small number in which the tubercle-bacilli are usually present in the effusion. It is, therefore, advisable to centrifugate a considerable amount of the effusion, and to inoculate guinea-pigs with the precipitate thus obtained.

Bacteriologic examination is, however, much more important in the diagnosis of *empyema*. If the agar-tubes inoculated with the pleural pus remain sterile, this indicates that the process is, in all probability, tuberculous. Direct microscopic demonstration of tubercle-bacilli in cover-glass preparations is, however, successful only in a few cases. Not rarely the empyema of tuberculous subjects is due to secondary infection.

Various observers have reached conclusions with regard to both the prognosis and the treatment of empyema from the species and the virulence of the bacteria found in the pleural pus. The presence of the diplococci is believed to indicate a much more favorable prognosis than that of the other pyogenic organisms. In this so-called pneumococcus-empyema less radical treatment is therefore necessary. Thoracotomy would, under these circumstances, be superfluous, simple evacuation by puncture being sufficient. It can actually be admitted that the prognosis of pneumococcus-empyema is usually good. Nevertheless, radical operation is indicated if the effusion is not quickly absorbed spontaneously, or at least after puncture. Too much reliance is not to be placed upon spontaneous attenuation of the bacteria in the pus, or upon their death, although the organisms in question live usually only for a short time in artificial culture. Bacteria with fully preserved virulence have been cultivated from pleural pus after the lapse of as long as three and one-half months. Further, recovery from empyema due to staphylococci, streptococci, and even typhoid bacilli, has been reported in isolated instances as occurring spontaneously or after simple puncture. The question is yet uudecided whether operation should be undertaken for the relief of purulent effusions in tuberculous subjects when

they contain tubercle-bacilli or are sterile. While operation for all other varieties of empyema, even in cases of tuberculosis, is attended with a relatively favorable prognosis, this is unfavorable in cases of empyema associated with the presence of tubercle-bacilli. Generally, healing does not take place, a fistula remains, and the chronic suppuration leads to death. Nevertheless, some surgeons recommend operation even in these cases, as, without doubt, recovery may take place also under such conditions.

Pneumothorax.—Pneumothorax with perforation, which occurs so commonly in tuberculosis, is, as a rule, followed by purulent effusion. The exudate contains tubercle-bacilli, demonstrable microscopically or by experiment on animals, and, in consequence of mixed infection, one or another of the pyogenic cocci, and, besides, at times, putrefactive bacteria, especially the proteus. Only one case of pneumothorax without perforation has been studied bacteriologically. In this the anaerobic, gas-forming bacillus emphysematosus (p. 128) was found to be the exciting agent. The method of examination is the same as in the case of pleurisy.

PNEUMONIA.

Fränkel's pneumococcus may be looked upon as the causative agent of lobar croupous pneumonia, as this organism is demonstrable in more than three-fourths of all the cases in the pulmonary tissues, which, normally, are entirely free from bacteria. In the larger proportion of cases the pneumococcus alone is present in the diseased tissue; in the smaller proportion, staphylococci and streptococci besides are present. Streptococci alone, or in association with staphylococci, rarely staphylococci alone, may, however, be found in pneumonic foci. In addition, the bacillus of Friedländer (Figs. 42, 47) is found in some cases. All of these bacteria, as has been pointed out, exist in the upper air-passages. They have, further, been demonstrated, though less commonly, in the healthy larynx, but bacteria do not occur in the bronchi and in the pulmonary tissues in healthy persons. For the development of pneumonia, it is, therefore, generally necessary that an accidental, indirect cause (cold, traumatism, etc.) shall cooperate that renders it possible for the bacterium to penetrate more deeply into the air-passages, and there to give rise to inflammation.

It is possible, to a certain degree, to differentiate the pneumonias according to their causative agents :

1. *Pneumococcus-pneumonia* corresponds with the characteristic clinical picture of true croupous pneumonia, with fibrinous exudation into the alveoli, and lobar distribution of the morbid process. Clinically, this variety is characterized by blood-streaked sputum, and especially by the sudden onset of the disease with a chill, the high, stormy course, and the critical decline of the fever. That this special type of disease is related etiologically to the activity of pneumococci is proved almost with certainty by the
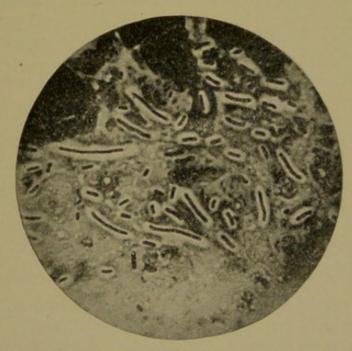
Fig. 47.—Bacillus pneumoniæ of Friedländer, from the expectoration in a case of pneumonia ; × 1000 (Fränkel and Pfeiffer).

following facts : In the first place, in typical cases the diplococcus alone is always found in the diseased area, in which it is demonstrable during life by puncture. In the next place, the general symptoms characteristic of pneumonia are also often present in marked degree, in conjunction with other localizations of the diplococcus, especially pneumococcus-angina. Finally, it may be pointed out in this place that the blood of human beings that have passed through an attack of typical pneumonia with critical defervescence is capable of immunizing animals to pneumococci. There is thus furnished evidence that convalescents from pneumonia are often immune to pneumo-

cocci, and it is thus comprehensible why the symptoms disappear abruptly with the occurrence of the crisis, while the pneumonic infiltration remains unchanged. From these facts it may be inferred that a toxic action on the part of the pneumococci constituted a decisive element in the previous clinical picture; for, when the crisis at once obliterates the disease, no change has been effected in its anatomic basis, in the infiltrate itself; only the toxic activity of the pneumococci has been withdrawn, and the manifestations that have just disappeared may, therefore, reasonably be referred to that.

Finally, it may be mentioned that true pneumonia has been induced experimentally in favorable cases by inhalation of pneumococci. Such experiments are usually attended with difficulty, because the animals commonly employed (rabbits, mice), which are so much more susceptible to the pneumococcus than human beings, die, as a rule, in consequence of the septicemic general infection, and localized disease is induced in them with difficulty.

2. *Streptococcus-pneumonia* corresponds with the clinical picture of bronchopneumonia (cellular, catarrhal pneumonia). The infiltrate is only lobular, usually less dense, and associated with less fibrin; the sputum is mucopurulent and not rusty; the onset is not so pronounced; the course is more insidious and attended with remissions and intermissions in the fever (so-called streptococcus-curve); and, above all, the crisis is wanting. Some cases of streptococcus-pneumonia are characterized by especial severity (*pneumonie infectieuse* of the French). The differentiation of this variety is, however, not readily sustained, in the first place because—

3. *Staphylococcus-pneumonia*, which is exceedingly uncommon as a single infection, resembles it absolutely; and, secondly, because of the occurrence so frequently of—

4. *Mixed forms of pneumonia*, in which two or even three of the bacteria named are found together. These forms of the disease furnish clinically also a mixed picture that occupies a position between croupous pneumonia and bronchopneumonia. As a rule, the occurrence of blood-streaked sputum or the temperature-curve, even when the infiltrate is small, indicates the participation of pneumococci in the morbid process; or, in a case of apparently true croupous pneumonia, which, however, is not a pure

type etiologically, but is a mixed form, the crisis does not appear. On the whole, however, there are no fixed rules for these mixed types. They render impossible the clinical diagnosis with regard to the causative agent in the individual case.

5. *Influenza-pneumonia* is associated with the presence of the influenza-bacillus, partly in pure culture, and partly together with streptococci or pneumococci.

6. *The pneumonia complicating tuberculosis* in the form of caseous pneumonia may be due to the tubercle-bacillus exclusively. On the other hand, pneumonia dependent upon streptococci, pneumococci, and influenza-bacilli is, however, not at all uncommon in tuberculous lungs. When pneumococci take part in the morbid process, the clinical picture assumes the peculiar characters that have been described, and are indicative of an acute severe infection.

7. *The pneumonia complicating other infectious diseases* is less commonly due to the cause of those diseases—as, for instance, typhoid pneumonia due to typhoid-bacilli—and more commonly to the organisms usually causative of inflammation. The pneumonia that occurs in the sequence of diseases of the abdominal organs is often associated with the presence of the bacterium coli—as, for instance, the bronchopneumonia not rarely observed in connection with incarcerated hernia.

The **bacteriologic diagnosis** can be made from examination of the sputum, but the procedure of Koch, as described by Kitasato, for the culture of tubercle-bacilli directly from the sputum must be observed—that is, the mass of sputum must be thoroughly rinsed in dishes with sterilized water, in order to remove the large number of bacteria that have been added from the pharynx and the mouth. Then a flake is isolated from the interior of the mass, and this is smeared several times upon an agar-plate or upon several glycerin-agar tubes in slants ; that which is left is examined microscopically.

Frequently direct puncture of the diseased lung has been made with a thoroughly sterilized hypodermic syringe, and a few drops of the exudate are obtained ; and these are treated bacteriologically in the manner described. This procedure—perfect asepsis being observed—need not occasion the slightest concern, as it is often practised involun-

tarily in the search for pleuritic effusions (exploratory puncture) without ever doing any harm.

If the information be sought whether the pneumococcus participates in the process, it is best to inject subcutaneously some of the sputum or pulmonary fluid into a mouse, or, better, into the still more susceptible rabbit. If the pneumococcus is present, the animal will die in from twenty-four to forty-eight hours in consequence of diplococcus-septicemia, which is readily demonstrable microscopically in stained cover-glass preparations from the heart's blood of the animal.

Prognostic significance is to be attached in only limited degree to the results of bacteriologic examination, because in the case of pneumonia also much depends upon the virulence of the exciting agent. However, a crisis may be looked for with greater assurance when pneumococci alone are found than under any other conditions. It must, however, not be forgotten that even in cases of pure pneumococcus-pneumonia the crisis may not occur ; while, on the other hand, it may be present in cases of streptococcus-pneumonia. The general condition of the patient, evidently, is of considerable importance in this connection, and it must be given due consideration in every aspect in predicting the crisis. Thus, the prognosis is unfavorable in the pneumonia of alcoholics, in that of the aged, and of those with kyphosis, even when pneumococci alone are found. The patients often die before the crisis, or the disease pursues a protracted course, and terminates late without the occurrence of a crisis. The pneumococcus-pneumonia of tuberculous subjects justifies a more favorable prognosis than a purely tuberculous pneumonia under the same conditions.

Reference has already been made to the conception of the crisis as indicating the advent of immunity, as well as to the evidence of the immunizing property of the blood-serum after the crisis in a number of cases of pneumonia. Why this immunity is so transient, disappearing in some cases in the course of a few days, is not yet known. In any event it has been established clinically that pneumonia has a tendency to recur, and it may almost be included among those diseases of which one attack rather predisposes to subsequent attack. In a pneumococcus-culture the bacteria die in the course of a few days (from four to seven),

but this takes place only in cultures, and not in the body, and it is incapable, therefore, of explaining the crisis. The focus of disease in the lung, as well as the sputum, contains living bacteria both during and long after the occurrence of the crisis. Often these retain their virulence throughout the entire period. Should the virulence be diminished during the crisis, it is, however, soon again augmented, as experience has shown. It is, therefore, not the cocci, but the human organism that undergoes some change in the crisis : it becomes insusceptible to the diplococcus-virus—that is, immune.

Transmission of Pneumonia.—Infection with pneumonia takes place principally through the respiratory passages. Only in some cases of secondary, complicating pneumonia may the microorganisms gain entrance into the lungs through the blood-stream. *Direct transmission* of pneumonia from one individual to another appears possible, and a considerable number of *house-epidemics* of pneumonia have been reported. In the majority of cases an attack of pneumonia probably results from the inhalation of pneumococci with the air ; or, more frequently, pneumococci that have long been present in the mouth, the pharynx, or the nose, are permitted by some accidental occurrence to gain entrance into the lungs and there to set up inflammation. Naturally, in connection with the *epidemic distribution* of pneumonia, the possibility can not be excluded that the accidental influence has been operative in all cases in common, that the pneumococcus need not be transmitted from one person to another, but that it was present previously in all affected.

The *hereditary transmission* of pneumonia from the mother to the fetus is a matter of great interest. A few cases have been reported in which the children of pneumonic mothers have been born with pneumonia. In general the pneumococcus is not disseminated outside the lungs of the infected individual. The slighter degree of susceptibility of human beings protects them, as a rule, against septicemic infection, which occurs regularly, for instance, in the more susceptible rabbit. In some cases, however, the pneumococcus has been found, also, in the blood of pneumonic patients during life. Such cases have always been marked by especial severity, with a fatal termination, so that the discovery of pneumococci in the cir-

culating blood must always be considered as of unfavorable prognostic omen. In the small number of cases of *congenital pneumonia* reported the bacteria must have gained entrance into the placenta, through a lesion of which they have passed over into the fetal circulation. Without such lesion the placenta, as is quite generally admitted, is impassable to microorganisms. Like all febrile diseases, pneumonia readily induces abortion. The child that has been infected with diplococci can, however, suffer from true pneumonia only if it has already breathed. As a matter of fact, in only two of the children in the reported cases was pneumonic infiltration present; in the others diplococci were found in the blood—there existed a septicemia.

ENDOCARDITIS.

Endocarditis occurs usually as a *secondary infection* in the course of various other diseases. The most important of these is *acute articular rheumatism*, whose predominant etiologic significance in the generation of endocarditis is well known. The causative agents of rheumatism have not yet been isolated, and rheumatic endocarditis also is one of those infectious diseases whose intimate etiology remains to be cleared up. The same statement may be made with regard to *chorea*, whose endocarditic manifestations also are probably to be attributed to the same rheumatic etiology; and also with regard to *erythema nodosum*.

In the deposits upon the valves of the heart in the sequence of *erysipelas* the streptococcus pyogenes is demonstrable both on culture and on microscopic examination.

In the endocarditis following *suppurative processes* (pyemia, septicemia, puerperal fever) the streptococcus or the staphylococcus pyogenes has been found in the vegetations : in that following *osteomyelitis*, generally the staphylococcus ; and after *suppuration* in the abdominal cavity, the bacterium coli commune.

Endocarditis is comparatively frequent in the sequence of *croupous pneumonia*. The lesions, which preferably involve the aortic valve, contain, as a rule, the diplococcus lanceolatus Fränkel. Bacteriologic examinations with reference to the endocarditis following *influenza* are yet wanting.

Diphtheric endocarditis is extremely uncommon. Only one case of true diphtheric endocarditis (mitral valve), with

demonstration of diphtheria-bacilli, has been recorded in the literature.

Almost equally as uncommon as diphtheric endocarditis is also true *typhoid endocarditis*—that is, that caused by the Gaffky-Eberth bacillus.

Tuberculous endocarditis has long been known. It attacks with a certain degree of exclusiveness the thin margins of the mitral leaflets, and particularly on their auricular surface. Tubercle-bacilli have been repeatedly demonstrated in the lesions.

True *gonorrheal endocarditis* is dependent upon the activity of the gonococcus, whose presence in the endocarditic vegetations has been demonstrated by Leyden.

The causative agent of the endocarditis following measles, scarlet fever, smallpox, as well as the causes of the primary disease, are still unknown.

The endocarditis complicating *acute nephritis* is generally caused by the same microorganisms (the exciting agents of suppuration and inflammation) that give rise to the primary morbid process in the kidneys.

It is, however, to be emphasized that in all of the diseases named, as well as in malaria and in carcinoma, the concomitant endocarditis need not be due to the original infecting pathogenic germ, but it may be the expression of a secondary or of a mixed infection that has been engrafted upon the endocardium, whose resistance has been lowered by reason of the primary infection. Under such circumstances staphylococci, streptococci, diplococci, or the bacterium coli commune will be found in the vegetations upon the valves : in short, those microorganisms that have repeatedly been shown to be the cause of secondary infection.

So-called *malignant, ulcerative endocarditis* is etiologically and clinically only a variety of ordinary endocarditis pursuing a malignant course, and giving rise to necrosis of the vegetations. If it is due to the common exciting agents of inflammation—staphylococci, streptococci, pneumococci, or the bacterium coli commune—it may be viewed as a special form of pyemia, characterized by the localization of the metastases on the valves of the heart. In isolated cases of this kind the microorganisms were demonstrated in the circulating blood during life.

Experimental Development of Endocarditis by Means of Bacteria.—After a previous lesion of the valves (through

catheterization by way of the right carotid), it has been possible to induce endocarditic changes of malignant character in experiments on animals by intravenous injection of the exciting agents of inflammation and suppuration. The same result may be attained without this serious procedure if the bacterial material is introduced into the vein in the form of a suspension that contains coarse particles— for instance, from a potato-culture. The particles, with the contained and adherent microorganisms, lodge upon the valves, and thus form the starting-point of endocarditic changes.

Bacteriologic Diagnosis.—In doubtful cases it is advisable to examine the blood bacteriologically (p. 162). As has been mentioned, it is sometimes possible to demonstrate the microorganisms in the circulating blood, and the diagnosis of ulcerative endocarditis is then justified. In the majority of cases, however, examination of the blood yields negative results, and even in such cases as exhibit a distinctly malignant character. The clinical diagnosis may, therefore, receive but little support from bacteriologic examination in connection with this disease.

PERICARDITIS.

Also with regard to pericarditis, the distinction between *primary* and *secondary* inflammation can not readily be made from the etiologic standpoint. The principal factor in the causation of pericarditis likewise is *acute articular rheumatism*, whose exciting agent, as has been stated, is unknown. *Traumatic pericarditis* is either to be referred to the action of bacteria that gain entrance into the pericardial cavity simultaneously with the inception of the injury, or, in the case of nonpenetrating wounds of the thorax, a point of lessened resistance is established in the pericardium in consequence of rupture of blood-vessels, ecchymosis, etc., resulting from the contusion of the chest, in which microorganisms circulating in the blood lodge and give rise to inflammation. Pericarditis in the sequence of *erysipelas* is dependent upon the streptococcus pyogenes ; the pericarditis attending *pneumonia*, upon the diplococcus of Fränkel ; *puerperal and pyemic pericarditis*, upon the exciting agents of suppuration.

A distinct position is occupied by *tuberculous pericarditis*,

which, next to the rheumatic, is probably the most common variety. Its exciting agent, the tubercle-bacillus, gains entrance into the pericardium through direct extension of the tuberculous process from the adjacent lung, or by way of the blood or the lymph.

The **bacteriologic diagnosis** is possible during life only on puncture or operation for the relief of the pericardial effusion.

MYOCARDITIS.

Suppurative Myocarditis.—A greater or smaller number of purulent foci of varying size may be present in the heart-muscle in association with pyemic processes of diverse origin. They contain pyogenic microbes.

Acute Diffuse Myocarditis.—Acute inflammation of the myocardium may complicate all infectious diseases of rapid course. Its bacteriologic relations are as yet but little known. The bacillus of Gaffky-Eberth has been repeatedly found in the myocardium in cases of typhoid fever. Frequently, however, the infection appears to be secondary and dependent upon the common exciting agents of inflammation. The metabolic products of microorganisms may likewise give rise to similar myocarditic alterations—as, for instance, the toxin of the diphtheria-bacillus, which is the cause of diphtheric myocarditis.

PERITONITIS.

The inflammatory processes involving the peritoneum may be induced through chemic influences exerted by the metabolic products of bacteria—*aseptic peritonitis*, for instance, in consequence of absorption of decomposition-products from the intestine in cases of ileus. In the majority of cases, however, such processes result directly from the activity of bacteria (*bacterial* or *septic peritonitis*). In accordance with the source of the exciting agents, peritonitis is conveniently divided into several varieties : (1) That which arises from the digestive tract — from the stomach, the duodenum, and the small intestine, as well as from the cecum, the vermiform appendix, the colon, and the rectum ; (2) that which arises from the gall-bladder and the liver ; (3) that which arises from the kidneys and the urinary bladder ; (4) that which arises from the female

genito-urinary tract; to these may be added (5) the rare cases in which the infection is of *hematogenous* origin; and (6) the still rarer, in which the peritonitis is the result of operative intervention. In the first four groups named the bacteria may migrate to the peritoneum (as in the case of puerperal peritonitis, in which the exciting agents reach the peritoneum from the uterus by way of the lymphatics) in the absence of a breach in the continuity of the organ primarily infected; or there may be such a breach in continuity, and *perforative peritonitis* results. The latter is the more dangerous variety, because with the microorganisms other materials—as, for instance, intestinal contents—gain entrance into the peritoneal cavity through the perforation, and these act as chemic and mechanical irritants, and thus make possible and easy the proliferation of the bacteria. By reason of the extraordinarily great absorptive power and the extent of the absorbing surface of the peritoneum, the entrance of bacteria into the peritoneal cavity in experiments on animals does not invariably lead to peritonitis, as has been demonstrated by the injection of moderate amounts of pyogenic cocci into the abdominal cavity of dogs, rabbits, and guinea-pigs. For the development of peritonitis a predisposing factor is additionally necessary, such as especial virulence of the bacteria—as, for instance, in general septicemia—the simultaneous entrance of intestinal contents, and, experimentally, the conjoint introduction of considerable amounts of preformed bacterial poisons, etc.

The distinction between *diffuse* and *circumscribed* peritonitis, which is so important clinically, is not supported by bacteriologic examination, as the causative agents are the same in both instances.

The **bacteriologic diagnosis** can only be made in connection with operation or on exploratory puncture. If it is desired for special reasons in a given case to make an examination for bacteria after death, this must be done as soon as possible after dissolution has taken place, as the result may be invalidated by the migration of bacteria from the intestine after the lapse of a few hours. From the effusion or from the deposits upon the peritoneum—in addition to microscopic examination—plates are simply cast or tube-slants are smeared.

The many bacteriologic investigations that have been

carried out in the study of peritonitis have thus far not yielded practically available results. In some of the cases there existed multiple infection—that is, infection with a number of varieties of bacteria. Coli-bacteria, as well as staphylococci and streptococci, have been mostly found in the effusion. In addition, pneumococci have been observed, as well as a large number of other bacteria, of which the gonococcus, the proteus vulgaris, the bacillus pyocyaneus, as well as a number of nonpathogenic varieties described by Tavel and Lanz, a bacillus resembling that of diphtheria, one resembling the tetanus-bacillus, and one the actinomyces-fungus, may be mentioned. The bacterium coli commune, which is the most common attendant upon peritonitis, is not a bacteriologic entity, but so variable in form and properties that the designation, bacterium coli, must be employed only as the collective name for a large group of related bacteria. When infection takes place through the blood-stream, only one exciting agent is found, and principally the streptococcus or the pneumococcus. In cases of operative peritonitis, usually streptococci have been found ; and in cases of puerperal peritonitis, in addition also the other exciting agents of inflammation. Tuberculous peritonitis is caused by the tubercle-bacillus.

From all that has been said it may be seen that peritonitis is not due to a single specific exciting agent, as the organ from which it arises (bladder, intestine) generally contains various bacteria. The infections that originate from the female genital organs are associated especially with cocci ; those originating from the intestine, especially with coli-bacteria. Further, the peritonitis arising from the small intestine is believed to be attended with the presence of fewer bacteria, while that arising from the large intestine is attended with the presence of more, as the contents of the large intestine exhibit a larger number of microorganisms than those of the small intestine. Both points of distinction are, however, not conclusive, and the results of bacteriologic examination of the peritonitic exudate can not be employed without reservation as a certain guide for diagnostic purposes, nor have these results yet acquired noteworthy significance from a prognostic or a therapeutic point of view.

PERITYPHLITIS.

Perityphlitis, like other inflammations arising from the intestine, is due to the activity of the bacterium coli commune. Thus far only perityphlitic abscesses and their complications have been submitted to examination, coli-bacilli being predominantly found. Actinomycotic and tuberculous perityphlitis are associated with the presence of their special exciting agents.

CHOLECYSTITIS AND CHOLANGITIS.

Normally the bile is sterile, the bacterium coli and cocci being found only in the lowest portion of the choledoch duct. Every obstruction, however, that interferes with the free discharge of bile (gall-stones, etc.) renders possible infection of the biliary passages. The bacteria present in the bowel, particularly the bacterium coli commune, less commonly staphylococci, streptococci, and pneumococci, gain entrance into the gall-bladder and the biliary passages, where they induce inflammation and suppuration.

Normal bile possesses no bactericidal activity ; when obtained in a sterile condition, it constitutes a fairly good nutrient medium for coli-bacilli and staphylococci.

Infection of the unobstructed biliary passages takes place in human beings in individual cases of typhoid fever, cholera, and croupous pneumonia. It may be assumed that in these cases also the infection takes place through the choledoch duct. Typhoid-bacilli and cholera-bacilli are systematically present in a virulent state in the intestinal canal throughout the course of the respective diseases to which they give rise. The pneumococcus occurs but seldom in the intestinal tract, although it has been found there with certainty in individual cases of pneumonia. As a result of the constitutional disease, the function of the liver is more or less impaired, the excretion of bile is not normal, and, in consequence, infection from the intestine is possible.

Infection through the blood is probably of rare occurrence in human pathology. In experiments on animals this may be attained by introducing the bacteria in large number, or by previous injury of the biliary passages—as, for instance, by the establishment of a biliary fistula.

Experiments on Animals.—If, after ligation of the choledoch duct, coli-bacilli are introduced in the central portion of the duct, the animals (dogs, rabbits) die as a result of purulent cholecystitis and cholangitis.   If the bacilli are injected directly into the mouth of the choledoch duct after the duodenum has been opened, the same result is obtained if the coli-cultures are sufficiently virulent.

The **bacteriologic diagnosis** can be made only in cases of empyema of the gall-bladder, aseptic puncture being practised and plates being cast with the bile obtained. Such puncture, however, should not be undertaken unless urgently indicated, as even when made with most careful aseptic precautions, signs of peritoneal irritation, usually mild, follow.

ABSCESS OF THE LIVER.

So-called *biliary abscesses* belong in the domain of cholangitis. *Pyemic abscesses* of the liver constitute part of a purulent general infection. (See Pyemia and Its Causes, p. 161.) Hepatic abscesses in the sequence of *gastro-intestinal lesions* arise through the intermediation of the portal vein ; coli-bacilli act as exciting agents. With regard to *tropical abscesses* of the liver, the section on Dysentery may be consulted.

In the **bacteriologic diagnosis** the pus obtained on exploratory puncture with a sterile hypodermic syringe in the search for the abscess is subjected to examination.

CYSTITIS.

Numerous investigations in recent years have shown that the bacterium coli commune, or the bacterium aerogenes, plays the most important part in the etiology of cystitis. The other microbes of suppuration and inflammation also deserve consideration in this connection, but they occur less frequently than the aerogenes and the coli. An exception is formed by *puerperal cystitis*, following parturition, with which the streptococcus pyogenes and the staphylococcus pyogenes are equally associated ; also by *gonorrheal cystitis*, which, in part at least, is dependent upon the gonococcus, and by *tuberculous cystitis*, which is caused by the tubercle-bacillus.

The bacteria gain entrance into the urinary bladder—

1. Through unclean instruments employed in catheterization.

2. By ascending through the urethra—this is especially the case in women.

3. Through the intermediation of the kidneys; some bacteria are capable of passing through the renal filter, and thus gain entrance with the urine into the bladder.

Among influences favoring the development of cystitis are cold, traumatism, and retention of urine. In addition to the common exciting agents of suppuration other less common microorganisms have been found in individual cases of cystitis: the so-called micrococcus albicans amplus, the diplococcus subflavus, the proteus, and others. These bacteria are, however, not pathogenic in themselves, and probably acquire importance only in mixed infection. The proteus causes putrid decomposition of the urine.

Bacteriologic Diagnosis.—Catheterization is practised by means of a catheter sterilized by boiling, the urine being received into a sterile vessel. Men are permitted simply to pass urine spontaneously, the last portion evacuated being kept for examination, and the orifice of the urethra having previously been thoroughly cleansed. From the urine plate-cultures are made.

Experiments on Animals.—By injecting pyogenic cocci into the bladder it is possible to induce cystitis in male animals with certainty, if the escape of urine is prevented for from twelve to twenty-four hours by ligation of the penis.

Ammoniacal Fermentation of Urine.—The bacterium coli possesses, in only slight degree, the faculty of causing decomposition of urine. Ammoniacal fermentation results from the action of individual varieties of the bacterium coli in the presence of a feebly acid, or an alkaline, reaction of the urine. Other varieties of the bacterium coli are incapable of causing decomposition of urine. Ammoniacal cystitis, however, develops in the presence of the staphylococcus pyogenes or the proteus, whether in pure culture or in mixed infection with the coli commune.

Pneumaturia.—In some cases of cystitis, especially with coincident presence of sugar in the urine, but also in the absence of glycosuria, the formation of gas may take place in the bladder. The urine under such circumstances is evacuated with an audible sound (pneumaturia). This

phenomenon is the result, in some cases, of fermentation of the sugar in the urine through the activity of microorganisms ; in other cases special varieties of coli-bacilli (aerogenes, etc.), characterized by abundant production of gas, have been cultivated from such urine. In one such case the gases formed consisted, in addition to carbon dioxid, of free nitrogen and hydrogen.

NEPHRITIS.

A bacterial origin has been ascribed to—

1. Primary infectious nephritis.

2. The nephritis occurring as a complication of infectious diseases, including the septic.

Streptococci have repeatedly been found in association with *acute nephritis*, and both in the urine, from which they disappeared with the termination of the disease, and after death in the kidneys, in which they were visible in the vessels, in the epithelium, and in tube-casts. Acute nephritis has been induced also experimentally in animals by injection of streptococci into the blood. Under these conditions a large number of streptococci at first appeared in the urine, but these disappeared later. The disease, however, progressed and terminated fatally, but no cocci were demonstrable in the kidneys after death. This observation indicates that nephritis may be of bacterial origin without bacteria being found in the kidneys after death (Mannaberg). The passage of the bacteria through the kidneys may suffice under certain circumstances to excite the anatomic inflammatory process, which may then pursue its further course independently of them. In the majority of cases of acute primary nephritis bacteria are, as a rule, found in the kidneys.

A variety of inflammation of the kidney excited by bacteria appears further at times to present itself from the beginning as a chronic nephritis. In the morbid condition of slow course induced by Charrin in rabbits by inoculation with pyocyaneus, chronic nephritis was found repeatedly.

In some cases of *complicating nephritis* the exciting agents of the primary disease have been present. Thus, typhoid-bacilli have been found in the kidneys in association with typhoid nephritis ; diplococci, in association with pneumonic nephritis ; and spirilla of relapsing fever, in the

urine of a patient suffering from nephritis complicating that disease. In other cases streptococci were found in the kidneys (smallpox, rheumatism, some cases of scarlet fever, etc.); under these conditions it is possible that a secondary or a mixed infection existed. Finally, complicating nephritis need not be excited directly by the bacteria, but it may be of toxic origin, resulting from the elimination through the kidneys of the toxins generated by the bacteria at the site of the primary disease. This is true peculiarly of diphtheric nephritis; in conjunction with which, likewise, as is usual with scarlatinal nephritis, bacteria can not be found in the majority of the kidneys examined.

The **bacteriologic diagnosis** can be made during life from examination of the urine (possibly after centrifugation), when there is no disease of the conducting passages, and especially of the bladder. Under normal conditions the urine that enters the bladder is and remains sterile, only becoming contaminated with bacteria in the urethra. For this reason the urine removed from the healthy bladder with a sterile catheter may be transferred directly to a nutrient medium, and such bacteria as develop may be considered as being derived from the kidney. In men, generally, irrigation of the urethra with the first half of the urine contained in the bladder is sufficient; the last half is, as a rule, sterile in healthy individuals.

Diagnostic or prognostic significance can not be attached to the demonstration of bacteria in the urine in cases of nephritis. According to Mannaberg, streptococci are found in the urine only in cases of true acute nephritis that pursue a rapid and favorable course, while they are wanting from the beginning in the apparently acute cases that subsequently prove to be chronic. This statement has, however, not been confirmed by other observers.

PERINEPHRITIS.

Perinephritis consequent upon disease of adjacent organs (kidney, intestine) is generally due to the bacterium coli. Perinephritis following traumatism, or attending general infection, may be due to any pyogenic microorganism.

PYELONEPHRITIS.

A distinction is to be made between an *ascending* and a *descending* pyelonephritis. The first variety, by far the more common, is associated with the presence of identically the same microorganisms as is cystitis, upon which it is dependent, and of which it represents the final, incurable stage. The microorganism most frequently associated is the bacterium coli or aerogenes. The descending variety, with infection from the kidney, is usually a pyemic process, and is dependent upon the related microorganism.

In ascending pyelonephritis the bacteria gain entrance into the pelvis of the kidney as the result of retention of urine. No longer disturbed by the discharge of urine, the microorganisms that have caused the inflammation of the bladder wander into the ureter and, multiplying therein, they finally, by extension upward, invade the pelvis of the kidney.

Chronic occlusion of the ureter may be followed by a pure pyelonephritis, without preceding cystitis, when the exciting agents of inflammation are present in the circulating blood, are eliminated through the kidneys, and collect in the stagnating urine in the pelvis of the kidney.

The **bacteriologic diagnosis** is only possible during life if operation be performed.

Experiments on Animals.—After ligation of the ureter, pyelonephritis can be induced in rabbits by means of coli-bacilli or of pyogenic cocci, both by injection of these bacteria directly into the pelvis of the kidney or into the ureter above the ligature, and by intravenous injection.

INFLAMMATIONS OF THE FEMALE GENITAL ORGANS.

Vulvitis.—Suppurative inflammation of the vagina is caused by the exciting agents of suppuration; diphtheric inflammation, by the diphtheria-bacillus; gonorrheal inflammation, by the gonococcus.

Endometritis.—Puerperal endometritis is always of bacterial origin (streptococci, staphylococci, coli-bacilli); of the remaining varieties of endometritis the majority are to be attributed to gonorrhea.

Salpingitis and Oophoritis.—Inflammation of the tubes and ovaries usually represents an extension of the endo-

metritic process. The bacteria pass by continuity from the mucous membrane of the uterus to that of the tube, and from this to the ovary. The most frequent cause is, therefore, gonorrhea. In addition, the presence of streptococci, staphylococci, coli-bacilli, and Fränkel's pneumobacilli has been demonstrated. A special position is occupied by tuberculosis of the tubes. Exceptionally, the oviducts are attacked by actinomycosis. The oophoritis that occurs in the course of some infectious diseases is due to the exciting agents of the primary disease, or they are caused secondarily by pyogenic microorganisms.

Perimetritis and Parametritis.—Primary inflammation of the perimetrium and the parametral connective tissue is, almost without exception, dependent upon puerperal infection (streptococci, staphylococci, bacterium coli, in mixed infection also proteus), in the course of which the exciting agents of suppuration gain access to the perimetrium and the pelvic connective tissue by way of the lymph-channels. Secondary inflammation exhibits the same etiologic relations as salpingitis and oophoritis, of which it constitutes a complication. Gonorrhea frequently and tuberculosis less commonly are of etiologic significance in this connection.

INFLAMMATORY DISEASES OF THE EYE.

Conjunctivitis.—The following microorganisms have been cultivated in cases of simple conjunctivitis : Staphylococci, streptococci, pneumococci. Diphtheric conjunctivitis is excited by the diphtheria-bacillus, gonorrheal conjunctivitis by the gonococcus, and tuberculous conjunctivitis by the tubercle-bacillus.

Keratitis.—Some cases of keratitis owe their origin to the common exciting agents of inflammation and suppuration, which at times gain access to the tissues through a lesion of the cornea. Hypopyon originates in a similar manner.

Iritis and Choroiditis.—Inflammation of the deeper structures of the eye arises secondarily by extension of the pyogenic organisms from the cornea (contact-infection), or metastatically through dissemination of the bacteria by means of emboli (puerperal fever, pyemia, etc.). The same statement applies to panophthalmitis. Primary inflammation of the choroid and retina is usually dependent upon

the activity of the metabolic products of bacteria, which do not themselves advance, as a rule, beyond the cornea.

Sympathetic Ophthalmia.—Deutschmann observed in cases of sympathetic ophthalmia an infiltration of the pia and the presence of phlogogenic bacteria in the optic-nerve sheath of the sympathetically affected eye. He attributed the sympathetic inflammation to direct invasion of the microbes by way of the optic-nerve path from the primarily affected eye. These observations have, however, not been confirmed, and the parasitic origin of sympathetic ophthalmia must still be considered as undemonstrated.

Chalazion is looked upon as the expression of a chronic inflammation of the tarsal connective tissue that is caused by the entrance of the exciting agents of inflammation into the excretory ducts of the Meibomian glands, and in the hair-follicles of the eyelashes. Giant-cells are invariably present in the granulation-tissue of the chalazion, and Tangl observed therein the presence of tubercle-bacilli. This observation, however, has not been repeated, and numerous inoculations of chalazion-tissue upon animals have never led to the development of tuberculosis in these.

Trachoma.—Diplococci closely resembling gonococci have been repeatedly demonstrated in the contents of the trachoma-follicle. The transmission of the disease by means of these microorganisms appears to have been successful in several instances, but their specific significance is, on the whole, still doubtful.

PYEMIA AND SEPTICEMIA.

Pyemia and septicemia are not sharply separable from each other either clinically or etiologically. Both are caused by the exciting agents of suppuration, with the difference, however, that in relation with septicemia the element of intoxication is the more conspicuous ; whereas, in relation with pyemia dissemination of the bacteria by way of the blood-stream and development of multiple foci of suppuration (metastases) take place. So-called putrid intoxication and acute malignant edema (*gangrène fou-droyante* of the French) must not be considered as varieties of ordinary septicemia. Both of these disorders occupy a peculiar position from the etiologic point of view, as the first is due to mixed infection with putrefactive bacteria,

especially the proteus of Hauser (see Proteus-infections), and the second is dependent upon the activity of a special specific cause—the bacillus of malignant edema (vibrion septique, see Malignant Edema). In connection with the pyemia and septicemia of human beings, therefore, there are to be considered the streptococcus pyogenes, the staphylococcus pyogenes, the diplococcus lanceolatus Fränkel, the bacterium coli commune, and the diplobacillus pneumoniæ Friedländer. The starting-point of pyemia and septicemia is usually a primary focus of suppuration or of inflammation. If such a lesion can not be demonstrated, the condition is designated *cryptogenetic septicemia*. Under these circumstances the primary focus has, in the majority of cases, merely escaped detection because of its obscure situation. Among such concealed sources of septic disorders may be mentioned mediastinitis, prostatic abscesses, collections of pus in the accessory cavities of the nose (antrum of Highmore, ethmoid sinuses, etc.). Only in rare cases does pyemia or septicemia result from direct absorption of the exciting agents of inflammation into the blood from without or from an internal surface without the formation of a primary focus.

Bacteriologic Diagnosis.—The bacteria can by no means be demonstrated in the blood in all of the cases. This should not occasion surprise in cases of septic intoxication, as the microorganisms are present in the blood in small number, if at all. In cases of pyemia the bacteria can be more frequently demonstrated in the blood, especially at the time of the chills, when the infected thrombi are set free and give rise to fresh metastases; but even here the results of blood-inoculation are often negative.

The technic for examination of the blood is simple. The finger of the patient is cleansed by means of soap, alcohol, mercuric-chlorid solution, and ether, its tip is slightly punctured with a lancet sterilized in the flame, and the escaping blood is smeared upon culture-media by means of the platinum loop heated in the flame; eventually, plates are made. It is better, because of the small number of bacteria circulating in the blood at any time, to employ a considerable amount of blood. This is aspirated by means of a sterilized Roux syringe from a vein distended by compression.

Metastatic abscesses are opened with aseptic precautions,

and from the pus cultures are made directly, or plates are poured and cover-slip preparations are prepared.

The excretory products of septic patients—urine, sweat, and saliva—at times contain the exciting agents of the existing disease, and in suitable cases it is, therefore, advisable to examine these secretions also. The elimination of the bacteria through these channels constitutes a mode by which the organism spontaneously gets rid of the exciting agents of the disease.

Experiments on Animals.—Experimental septicemia, such as may be induced in animals by means of the most varied microorganisms, can not without qualification be considered analogous to septicemia in human beings. In experiments on animals unlimited multiplication of the bacteria in the blood takes place, although it is to be borne in mind that enormous multiplication sets in only a short time before the death of the animal. The conditions are much simpler in the case of pyemia, as this disorder may be induced in animals through all portals of infection and with all pyogenic microorganisms. It is a necessary condition, however, that the microorganisms employed in the experiment shall possess a sufficient degree of virulence.

PUERPERAL FEVER.

Puerperal fever is only clinically a special form of pyemia or septicemia ; but in relation to its causative agents, analogous to all other varieties. The streptococcus pyogenes, the staphylococcus pyogenes, and, less commonly, the bacterium coli have been found to be its exciting agents. The severity of the disorder is dependent upon the fact that the bacteria gain entrance directly into the open lumen of the vessels of the uterine mucous membrane injured in the process of parturition, and by this means into the general circulation. The process pursues a relatively favorable course when the vessels are already occluded by thrombi, and the larger lymph-trunks are again closed. The bacteria then wander through the lymph-spaces between the muscle-fibrils, reach the pelvic connective tissue, and there give rise to a localized, circumscribed, suppurative process, so-called puerperal parametritis. The occurrence simultaneously, or in rapid succession, of widely separated foci of suppuration—as, for instance, the periton-

itis, the empyema, the arthritis, etc., of puerperal sepsis—
can only take place through the dissemination of the
germs by way of the blood-stream.

It may be mentioned that, in the milk of nursing women
suffering from puerperal fever, pyogenic cocci can not rarely
be found.

OSTEOMYELITIS.

Osteomyelitis is not a specific disease. Staphylococci
have been most frequently found to be its exciting cause
—both the aureus and the albus ; less commonly, the
streptococcus pyogenes, the diplococcus Fränkel, the
typhoid-bacillus, and the bacterium coli commune have
been cultivated from osteomyelitic foci. In accordance
with these observations osteomyelitis may be considered
as a form of pyemia characterized clinically by its localiza-
tion in the bone-marrow. The skin and the open cavities
of the body constitute portals of entry for the bacteria. It
is, however, by no means necessary that primary foci
(furuncle, panaris, etc.) should exist in every instance.
The forms of life normally present upon the skin and
mucous membranes render superfluous such an assumption
in the individual case. Osteomyelitis occurs exclusively
in young individuals, and it has, therefore, been designated
the pyemia of the developmental period. In young in-
dividuals the growing zone of bone represents a point of
lessened resistance at which bacteria, when they gain
entrance into the circulation from any source, may lodge
and multiply.

Experimental Evidence.—If young animals (rabbits or
dogs) are inoculated by intravenous injection with pyogenic
microorganisms, subperiosteal abscesses and purulent in-
flammation of the medulla of bone occur. In older ani-
mals similar results are obtained only if previously a frac-
ture has been induced ; thus, to a certain degree, a point
of lessened resistance has been established artificially.
Under such conditions osteomyelitic changes take place in
the situation of the fracture. These experimental obser-
vations are in accord with the facts of human pathology—
as osteomyelitis, as has been mentioned, is a disease of
early life.

PYOCYANEOUS GENERAL INFECTION.

The bacillus pyocyaneus is, in general, quite a harmless bacterium. Its presence in the pus of wounds, in cases of otitis media, etc., retards the process of healing in the respective diseases only inconsiderably, and gives rise to the well-known greenish or bluish discoloration of the pus and the dressings. In the bodies of children the bacillus pyocyaneus at times exhibits pernicious activity, and it appears under some circumstances capable of causing severe general infection. Neumann obtained this microorganism from the blood and the internal organs of a new-born infant dead of hemorrhagic septicemia. H. Kossel found it in children in the meningeal exudation in the sequence of otitis, in diarrheal stools, in cases of nephritis, and in the presence of inflammatory affections of the nasopharyngeal space. He believes that the bacillus is capable of causing serious injury to the organism of the child, either directly through the intermediation of the blood-stream or indirectly through its metabolic products.

PART III.

SPECIFIC DISEASES OF BACTERIAL ORIGIN.

TYPHOID FEVER.

Morphology of the Typhoid-bacillus.—The bacillus of typhoid fever was first observed by Koch and Eberth, and grown in pure culture by Gaffky in 1884.
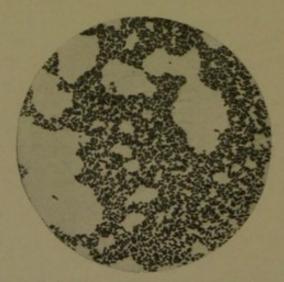
Fig. 48.—Bacillus typhi, from an agar-agar culture twenty-four hours old; × 650 (Heim).

The typhoid-bacilli are small, plump rods, with rounded extremities (0.5–0.9 × 1–3 μ). In the tissues the organisms lie, as a rule, isolated, while in cultures they are arranged in pairs and not rarely in long filaments. They possess from eight to eighteen terminal and lateral flagella, and, in consequence, they are most actively motile, their movement being serpentine in character. They do not stain so well as other bacteria and they take the stain with some difficulty. It is, therefore, advisable to warm the aqueous staining solutions and also the diluted carbol-fuchsin solution. The bacilli do not stain by Gram's method.

166

Spore-formation.—Gaffky considered as spores certain terminal bright, oval bodies that are said to remain unstained. These bodies (polar granules) have, in accordance with subsequent investigations, come to be looked upon as involution-forms. At any rate the bacilli that contain such bodies are not noted especially for their powers of resistance, as they are destroyed with certainty by exposure for ten minutes to a temperature of 60° C. (140° F.).

The typhoid-bacillus grows in the absence of oxygen, though by no means so well as in its presence (facultative anaerobiosis). The temperature-optimum for the typhoid-bacillus is that of the body. The organism thrives well, however, at room-temperature. The temperature-maximum is 46° C. (114.8° F.).

The Appearance of Typhoid-bacilli in Cultures.—The bacillus of typhoid fever—in contrast with most other pathogenic bacteria—exhibits vigorous growth on slightly acid nutrient media.

On *gelatin-plates deep colonies* appear as small, punctate, sharply circumscribed dots ; with low powers of the microscope they may exhibit a brownish-yellow color and a whetstone shape. *Superficial colonies* are much larger, and form a bluish, iridescent, delicate coating, with an irregularly serrated border. Only the central portion of the colony appears, with low powers of the microscope, of a yellowish color, while toward the margin a delicate linear network can be observed, giving rise to a leaf-like appearance. The gelatin is not liquefied.

In *gelatin stab-cultures* development takes place along the entire line of inoculation. Superficial growth is pronounced, and presents characteristics similar to those observed in superficial colonies on plates.

Gelatin Streak-cultures.—From the center the entire surface of the gelatin is covered by a delicate, iridescent, bluish coating.

In all gelatin-cultures a peculiar milky turbidity of the nutrient medium frequently occurs in the neighborhood of the culture.

In *agar streak-cultures* and in *blood-serum* a whitish coating of inconsiderable density forms, without characteristic peculiarities.

The appearance of *potato-cultures* is of importance. In these the typhoid-bacillus grows in an invisible layer, with an appearance as if nothing at all had developed upon the surface of the potato. If, however, an attempt is made to remove material with a platinum loop, it is at once found that the potato is entirely covered by a layer of some kind. Microscopic examination confirms this observation, and discloses the presence of large numbers of actively motile rods. This mode of development is quite peculiar, and occurs, so far as is as yet known,

only with typhoid-bacilli. It is, however, not constant. There are varieties of potato upon which the bacilli of typhoid fever develop in yellowish or brownish, raised, and sharply circumscribed deposits, and generally upon potatoes whose surface yields a neutral or even an alkaline reaction. Such visible growth can be obtained also artificially by rendering the surface to be inoculated of alkaline reaction. Typical characteristic growth takes place only when the potatoes exhibit an acid reaction, which they do, as a rule.

In *milk* the typhoid-bacillus induces slight formation of acid, but never coagulation. In Petruschky's *whey* (p. 86) the typhoid-bacillus generates not more than three per cent. of acid, whereas the bacterium coli commune generates more than seven per cent.

Bouillon is rendered turbid by cultures of the typhoid-bacillus.

The typhoid-bacillus does not induce fermentation in nutrient media containing grape-sugar, milk-sugar, or cane-sugar ; nor does it form indol in a solution of peptone and sodium chlorid.

If potassium nitrite and sulphuric acid are added to peptone-cultures, a red coloration does not occur (p. 122, bacterium coli commune).

The typhoid-bacillus, as well as the bacterium coli commune and the entire group of bacteria resembling both, is characterized by a certain degree of resistance to carbolic acid, addition of which to the nutrient medium in the proportion of $\frac{1}{4}$ per cent. not inhibiting the bacilli in their growth.

Vital Activity of the Typhoid-bacillus.—Typhoid-bacilli retain their vitality in *sterilized water* for a considerable time (up to three months) ; they may even, at least at first, multiply therein. In *unsterilized water* they die in the course of two weeks, in consequence of the activity of the water-bacteria, by which they are suppressed, and more quickly in running water than in stagnant water. Under favorable conditions, protected from light, evaporation, and competition, they may persist for a long time. *Milk* may at times contain living typhoid-bacilli for as long a period as five weeks. In the slime of streams and of wells typhoid-bacilli retain their capability of development for not less than three weeks. Buried in the superficial layers of the *earth*, they have been demonstrated in a living state after five and a half months. They appear also capable of persisting for an equal length of time in the *feces*—for three months and more ; naturally only when too many putrefactive bacteria are not present at the same time. Typhoid-

bacilli bear *cold* quite well; they are not injured by freezing for two or three times, and subsequent thawing. They are less resistant to *heat*, as has been mentioned. (See Spore-formation.)

Dried in a thin layer, typhoid-bacilli have been found to preserve their vital activity (Uffelmann)—

In garden-soil for twenty-one days.

In sweepings for more than thirty days.

In white filter-sand for eighty-two days.

Upon linen for from sixty to seventy-two days.

Upon buckskin for from eighty to eighty-five days.

Upon wood for thirty-two days.

According to Kruse, they die in thin layers, dried, within from five to fifteen days.

Portals of Infection and Dissemination of Typhoid-bacilli.—Dried typhoid-germs may be carried through the air with floor-dust, street-sweepings, particles of clothing, etc. They may, thus, possibly be inhaled. *Infection by way of the lungs*, however, is rather improbable with regard to typhoid fever, although it played an important part in the earlier theories that denied the transmission from case to case, and considered necessary the presence of the typhoid-bacilli in the earth for their complete maturation; but this has not been demonstrated. Besides, in human beings the *digestive tract* appears to be the only portal of entry for the typhoid-bacillus. The typhoid-germs must be swallowed, and gain entrance into the intestine. To this end it is necessary that they lodge upon, and be taken up with, food. Apparent infection through the lungs may also be so interpreted that the typhoid-germs contained in dust and with this inhaled are restrained in the upper part of the respiratory tract, to be subsequently carried with food into the digestive tract. It is an important fact that the typhoid-bacillus is not destroyed with certainty by the hydrochloric acid of the gastric juice. The barrier interposed by the stomach thus fails to afford reliable protection against typhoid infection, even when the function of that organ is completely normal.

Articles of food may be contaminated with typhoid-bacilli through the intermediation of the air; dust containing typhoid-bacilli may be deposited upon articles of food. More frequent, however, is direct contamination by means of the feces, which become attached to the hands of the at-

tendants in the emptying of bed-pans, and in the cleansing of soiled linen, and transference to food from want of cleanliness. In this way infection from case to case takes place, as may often enough be demonstrated. The disease occurs in *epidemic distribution* when a common article of food is contaminated. Thus, epidemics of typhoid fever have been caused by milk, by oysters obtained from infected water, and more frequently by *drinking-water*. Repeatedly, a communication between wells or sources of water-supply and neighboring cesspools into which undisinfected stools have been emptied has been found to be the cause of typhoid epidemics. In other epidemics contamination of the public water-supply by means of typhoid dejections, or through the washing of infected clothes, has most probably taken place.

Recently, typhoid-bacilli have been observed a number of times without a direct relation to cases of typhoid fever being demonstrable or even suspected. Lösener found bacilli that corresponded in all respects with Eberth-Gaffky bacilli in a specimen of earth obtained from an untilled field, and, further, in the water-supply of Berlin obtained from a conduit in his laboratory. Remlinger and Schneider, using perfect methods, have recently cultivated the same microorganisms from earth, dust, and water. They even found them in the intestines of five individuals who had never suffered from typhoid fever. Observations like these are, however, exceptional, and are as yet beyond explanation.

In order that typhoid fever may develop, in addition to the taking up of the bacilli a *special predisposition* on the part of the individual certainly appears necessary ; or the bacilli must possess especial virulence or be taken up in excessive number. In general, human beings may be considered as not particularly susceptible to typhoid fever. The requirement of an especial *temporal and local predisposition* (elevation of the ground-water), as demanded by the theory that the condition of the soil bears some relation to the occurrence of typhoid fever, can no longer be sustained.

The Occurrence of the Bacilli in Typhoid Patients.— The bacilli are found systematically in *Peyer's patches*, the *mesenteric glands*, the *spleen*, the *liver*, and the *bone-marrow* of typhoid patients. They are always collected in groups that frequently exhibit a relation with blood-vessels. In

the *feces* they are not found before the second week of the disease, usually after the tenth day, but, as a rule, not in large number. In most cases they disappear from the stools as early as the fourth week of the disease, and in rare cases they are demonstrable until after defervescence. The *blood* of typhoid patients is generally sterile, although typhoid-bacilli have been found in the blood of the rose-spots and from the veins. It is necessary to employ con-siderable amounts of blood (1 cu. cm.) for the purpose of examination. The *urine* during life and the *kidneys* after death frequently contain the bacilli, especially in cases complicated by albuminuria. Rarely the bacilli have been found, besides, in the *lungs* in some cases of typhoid pneu-monia or bronchopneumonia, in the *meninges* in cases of typhoid meningitis, in the *myocardium* in cases of typhoid myocarditis, and in the *testicles* in cases of typhoid orchitis.

Typhoid-bacilli have been found repeatedly in the pus of *suppurative processes* that develop in the course, or as a sequel, of an attack of typhoid fever: as, for instance, osteoperiostitic processes, encapsulated peritonitis, abscesses of the spleen and the liver, inflammation of joints, thyroiditis, empyema, etc. These observations show that the typhoid-bacillus is also capable of manifesting pyogenic activity. Typhoid-bacilli have been found in such posttyphoid sup-purative processes fifteen months after the termination of the disease, and in one case of old bone-disease as long as seven years afterward.*

Mixed Infection and Secondary Infection.—The course of typhoid fever is frequently modified by complicating in-fections due to other microorganisms, usually those exciting inflammation. At times these complications appear at the commencement of the disease as the expression of a mixed infection ; at other times they occur later as secondary in-fections. The bacterium coli commune plays the most im-portant part in the development of these conditions. It is found to be the cause of peritonitis, cholangitis, etc. Next in importance is the streptococcus pyogenes, which has been frequently demonstrated in cases presenting secondary empyema, otitis, and bronchopneumonia. Mixed infection

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* Hunner, " Bulletin of the Johns Hopkins Hospital," Aug.-Sept., 1899, p. 163, has reported a case of acute suppurative cholecystitis in which typhoid-bacilli were isolated from the contents of the gall-bladder eighteen years after an attack of typhoid fever.—A. A. E.

with streptococci is responsible for the development of the dangerous condition known as streptococcous typhoid septicemia. The investigations of Vincent have shown that the presence of the streptococcus and artificial mixed cultures materially increases the virulence of typhoid-bacilli. The staphylococcus pyogenes, the diplococcus lanceolatus Fränkel, and the proteus, likewise frequently find opportunity to gain lodgment in the organism enfeebled by typhoid fever, and to give rise to the development of furuncles, cutaneous abscesses, bronchopneumonia, catarrh of the middle ear, gangrene of the skin, and the like.

Experiments on Animals.—Mice, guinea-pigs, rabbits, goats, etc., die after the introduction of virulent Eberth-Gaffky bacilli, with decline of temperature, convulsions, and diarrhea. Considerable numbers of bacteria are necessary in *subcutaneous introduction*, while smaller numbers suffice in *intraperitoneal* and *intravenous inoculation*. The fatal result is brought about under these conditions through *intoxication*. If the virulence of the infectious agents is marked, the animals, and especially white mice, may, however, be destroyed by intraperitoneal injection of quite small numbers of bacteria, although large numbers of the bacilli are found in the blood after death. It is certain that under these conditions an increase has taken place in the number of bacteria, and it can, therefore, not be denied that the disease induced in animals by typhoid-bacilli displays the characters of a true infection.

The development of actual typhoid fever in animals (and also mere typhoid-bacilli intoxication) in consequence of introduction of the bacteria through the mouth is attended with serious difficulties. It is, however, to be observed that a true typhoid disease appears not to exist naturally in animals. If preliminary treatment is employed similar to that adopted in the experimental development of cholera— alkalinization of the gastric contents, and injection of tincture of opium—changes are at times induced in guinea-pigs by the introduction of typhoid-bacilli through the mouth that in some degree are suggestive of those of typhoid fever in human beings. Experiments on animals of a completely positive character, with the pathologic-anatomic lesions of true typhoid fever, have not been recorded in the literature.

The *pyogenic activity* of the typhoid-bacillus can be readily demonstrated in experiments on animals.

Etiologic Relations of the Bacilli to Typhoid Fever in Human Beings.—In view of their constant presence in all cases of typhoid fever, and of their occurrence exclusively in this disease, the Eberth-Gaffky bacilli may be considered as the exciting agents of typhoid fever, although experimental development of typhoid fever by means of the bacilli has not been induced. The bacteria that gain entrance into the digestive tract with the food obtain lodgment, in the presence of the necessary predisposition on the part of the individual, in the follicles and plaques of the intestinal wall. • The period of incubation for typhoid fever is from one to three weeks. During this time the bacteria slowly give rise to the anatomic process that constitutes the basis of the disease, the swelling of the plaques, and, later, their ulceration. At the same time the bacteria multiply and gain entrance—probably by way of the lymph-paths especially—into the mesenteric glands, also into the liver and the spleen. To this point typhoid fever is a truly infectious disease. The toxic activity of the bacteria is, however, of equally marked importance. Brieger and Fränkel have demonstrated in bouillon-cultures of typhoid-bacilli a chemic poison that they place in the class of toxalbumins. R. Pfeiffer also held the view with regard to typhoid fever that the peculiar poison resides in the bodies of the bacteria. (See General Section, p. 31.) This can be readily obtained by destroying young agar-cultures carefully by means of chloroform-vapor or by exposure for an hour to a temperature of 54° C. (129.2° F.). From eight to ten mg. of the bacterial mass suffice to destroy a guinea-pig. There is no doubt that also in the body of the typhoid patient a specific poison is generated by the bacteria, and is absorbed. The characteristic mental dulness, and the peculiar febrile course may be considered as expressions of this intoxication. Not rarely cases of typhoid fever are observed in which the intestinal lesions are subordinate to the toxic state. These are instances of typhoid fever with most profound mental confusion and high fever, but with mild intestinal manifestations, in which after death only a few ulcers of small extent are found in the bowel. Cases have even been reported in which during life the characteristic stools, and after death the typhoid ulcers, were entirely wanting : the diagnosis of typhoid fever being based solely upon the demonstration at the autopsy of typhoid-bacilli in the greatly enlarged spleen.

Bacteriologic Diagnosis of Typhoid Fever (Differentiation of Typhoid-bacilli and Bacterium Coli Commune).
—The bacteriologic diagnosis of typhoid fever is usually complicated by so many difficulties that it can not be considered as an aid in the clinical diagnosis. Isolation of the typhoid-bacillus in the feces is a matter of great difficulty by reason of the extraordinary resemblance of the bacillus to the bacterium coli commune—a resemblance that is so marked that some observers (particularly the Lyons school) have considered the two microorganisms identical. The microscopic appearances, the agar-culture and the gelatin-culture of both bacteria are absolutely alike. Potato-cultures are, generally, though by no means always, different: the bacterium coli gives rise to a thick, raised, circumscribed, greasy, brownish deposit; the typhoid-bacillus, on the other hand, to an invisible, extensive coating. In using potatoes for differential diagnostic purposes one-half of the surface must be inoculated with the suspected bacteria, and the other half with actual typhoid-bacilli. If the same growth takes place on each half of the potato, it is highly probable, providing there is agreement in other respects, that the suspected organism is really the specific Eberth-Gaffky bacillus. Among further points of differential diagnosis as between typhoid-bacilli and coli-bacilli are the following: (1) The bacterium coli coagulates milk; the typhoid-bacillus does not. (2) The bacterium coli generates gas in nutrient media containing peptone, and especially in those containing grape-sugar, after exposure in the thermostat for even a few hours; the typhoid-bacillus does not. It must, however, not be overlooked that, though quite seldom, there occur varieties of coli-bacilli that do not coagulate milk and do not cause fermentation of grape-sugar, and thus can not, by any of the methods thus far known, be absolutely differentiated from the Eberth-Gaffky bacillus. A further point of differentiation consists in the fact that the typhoid-bacillus does not yield the indol-reaction, whereas the bacterium coli does. There is, however, a variety of bacterium coli that does not generate indol. The differentiation between the two bacteria thus remains extremely difficult, and only such bacteria can be decided with perfect certainty to be typhoid-bacilli as possess all of the peculiarities described, and as have been cultivated from the spleen of human beings who

exhibit, or have exhibited, the clinical symptoms of typhoid fever.

Recent and reliable aids in the differential diagnosis between the bacillus of Eberth-Gaffky and the great horde of bacteria resembling the typhoid-bacillus and the bacterium coli are furnished by the reaction of Pfeiffer and that of Gruber. (See General Section, pp. 62 and 63.) Ten times the minimal lethal dose amount of the suspected bacteria are mixed with a small amount—less than 0.1 cu. cm.—of serum obtained from an animal highly immunized against typhoid fever, and the mixture is injected into the peritoneum of a guinea-pig. If after from ten to twenty minutes the characteristic disintegration of the bacteria into granules becomes apparent in the fluid obtained from the abdominal cavity by means of a capillary tube, the conclusion may be reached that the organisms are actually typhoid-bacilli. If the virulence of the microorganisms is slight or wanting, Pfeiffer's reaction is obviously not available. Under these conditions Gruber's reaction is employed. The technic has been described in the general section (p. 64). If agglutination takes place, the diagnosis may be considered as positive.

Typhoid-bacilli are isolable from the feces only with great difficulty on account of their close resemblance to the bacterium coli commune. The procedure most likely to prove successful is that recommended by Elsner. Plates are made in the usual manner with potato-gelatin (see Methods of Culture and of Examination, p. 81), to which shortly before use potassium iodid has been added in the proportion of one per cent. This culture-medium appears especially adapted for the growth of typhoid bacilli and coli-bacteria, with the qualification that the latter grow more vigorously than the former. After the lapse of forty-eight hours the colonies of coli-bacilli appear as dark-brown spherical masses, while those of the typhoid-bacilli appear as small, transparent, water-like drops. The method of Elsner is, however, not absolutely trustworthy. Its application requires much practice, and it does not render superfluous further precise identification of the apparent typhoid-colonies according to all of the rules mentioned.

The bacteriologic diagnosis of typhoid fever may be made quickly and easily by *puncture of the spleen*, and the development of cultures from the fluid obtained. If the

disease present is typhoid fever, pure cultures may thus be obtained at once, which do not coagulate milk and do not generate gas in peptone-bouillon. In rare cases of mixed infection, in addition to typhoid-bacilli the streptococcus pyogenes or the staphylococcus is also found in the fluid from the spleen. The method of puncturing the spleen is the same as that employed in every other form of exploratory puncture. On aspiration the splenic fluid, mixed with blood, is readily obtained, and this is injected into a sterilized dish, five agar-tubes being each then smeared successively with a drop of the fluid ; with the remainder plates are made, and milk-flasks and peptone-bouillon tubes are inoculated. Notwithstanding the trustworthy character of its results, puncture of the spleen is, however, not to be recommended. The procedure is by no means free from danger. It should not be forgotten that the typhoid-bacillus is also capable of pyogenic activity, and that the puncture-track in the spleen may give rise to purulent complications, possibly in the peritoneum.

The most valuable service in the clinical diagnosis of typhoid fever appears to be rendered, however, by the procedure of Widal, who, as has already been mentioned (p. 66), showed that also the blood-serum of typhoid patients yields the *agglutination-phenomenon* of Gruber. Blood-serum from the patient suspected to be suffering from typhoid fever is obtained by aseptic puncture of the tip of a finger, or of a vein by means of a sterilized syringe. The blood thus obtained is permitted to flow into a test-tube, and to coagulate in a slanting position ; in this way the largest proportion of serum will be obtained. The test is then made according to one of the methods described on page 64 ; the one preferred is a matter of indifference. The desired information is gained most quickly through the microscope, and only by this means is it possible to determine the extreme limit of agglutinating activity—that is, the highest degree of dilution of the serum with which the reaction can still be induced. It has been emphasized that the reaction of Gruber is a quantitative one, and that, therefore, everything depends upon determining quantitatively with precision the agglutinating activity of the serum. A proportion of 1 to 50 is sufficient for the positive diagnosis of typhoid fever. Many thousand specimens of serum have now been examined according to the method

of Widal, and in no instance has this dilution yielded the reaction with serum not from a case of typhoid fever. It is possible, however, that the agglutinating activity in cases of typhoid fever may be below these figures; with dilutions between 1 : 50 and 1 : 10 Widal and Sicard recommend that the case be considered as suspicious with regard to typhoid fever, and that the observation be repeated in the course of a few days. The serum of typhoid patients, even when not sterile, retains its agglutinating power unchanged for several months. It may, therefore, be preserved and sent from one place to another. The time when the agglutinating property appears in the serum is of importance in diagnosis. As a rule, it is demonstrable after the seventh day, although it may appear later or earlier. The earliest that it has been observed was on the second day by C. Fränkel, and the latest in the first days of convalescence by Achard.

The Widal-Gruber reaction grows feebler in the first weeks or months of convalescence, finally to disappear completely in some cases. Frequently, however, it persists, and it may be demonstrable after the lapse of years, or even of decades, and it can thus be utilized as an evidence that the individual in question has previously suffered from an attack of typhoid fever. On this account it is absolutely necessary, in the application of serum-diagnosis, to learn from the patient's history whether he has not already at some time passed through an attack of typhoid fever, however mild; otherwise, there is danger that a reaction of older date may be employed diagnostically in relation to the disease under observation. Widal and Sicard divide their cases of typhoid fever into five groups, accordingly as the serum exhibits a greater or lesser agglutinating activity. In the first group the agglutinating power is very slight, below 1 to 100; in the second group it is feeble, between 1 to 100 and 1 to 200; in the third group it is moderately great, up to 1 to 500; in the fourth group it is great, up to 1 to 2000; and, finally, in the fifth group it is above 1 to 2000. Each of these five groups includes both mild and severe cases. The agglutinating curve in individual cases of typhoid fever, observed throughout the whole course of the disease, likewise exhibits the widest variations. Each case bears in this respect, as Widal and Sicard believe, its individual impress. Its agglutinating

12

power may appear earlier or later, and in greater or in less degree, and it may even be entirely absent, as these observers have noted, although but once among 163 cases.

The examination of water for typhoid-bacilli is of great practical importance, as in most epidemics the drinking-water is to be considered as the vehicle for the typhoid virus. For this purpose carbolic acid is employed, being added to the suspected water in such amount that this shall contain from 0.05 to 0.25 per cent. of the acid. This addition is made for the purpose of inhibiting the activity of the bacteria present in water that liquefy gelatin ; the typhoid-bacilli themselves readily withstand such slight additions of carbolic acid. With the carbolized water three plates are made in the customary manner, according to the method of Elsner. As by this means only small amounts of water are subjected to examination, it is easily possible that typhoid-bacilli may escape detection, even when present. It is, therefore, well to subject considerable amounts of the suspected water to examination. To this end a sterilized, alkaline, concentrated solution of peptone and sodium chlorid is employed that contains in a specified number of cubic centimeters one gram of peptone and one gram of sodium chlorid. This amount is added to 100 cu. cm. of the carbolized water in an Erlenmeyer flask, and the mixture is placed in the thermostat for from eighteen to twenty-four hours. If typhoid-bacilli are present in the water, in some degree protected against the competition of the other bacteria by the addition of carbolic acid, they undergo multiplication, and they can be more readily demonstrated on plates prepared from the mixed cultures. The identification with certainty of the developing suspicious colonies as typhoid-colonies is, however, again attended with considerable difficulty. The water contaminated by typhoid dejections naturally always contains also the bacterium coli commune, and besides there are frequently present in the water other nonpathogenic bacilli that bear an extraordinary resemblance morphologically and in culture to the typhoid-bacillus—so-called pseudotyphoid-bacilli. These, however, sometimes yield the indol-reaction and sometimes not. A considerable number of these bacteria also develop in the mixed cultures, and a number even much better than the specific typhoid-bacilli. A decision as to the presence of typhoid-bacilli in water may

therefore be finally reached only when, after comparison with an unequivocal pure culture (from the spleen of a typhoid patient), all doubt has been removed that both the culture obtained from the water and the earlier pure culture agree in every detail, and with regard to both Pfeiffer's and Gruber's reactions. Notwithstanding these difficulties, typhoid-bacilli have been demonstrated in drinking-water in several instances by competent observers.

The prophylaxis of typhoid fever, in accordance with what has already been said, consists especially in the *antisepsis of the sick-room*. The feces and the urine from typhoid patients, all materials contaminated by these discharges (body-clothing and bed-clothing, etc.), in fact, everything that has come in contact with the patient, must be most thoroughly disinfected, as the adherent bacilli may constitute the source of new infections. Methods of disinfection are described in the Appendix.

In the second place, the prophylaxis concerns itself principally with the hygienic relations of the *drinking-water*, which must be boiled before being used whenever suspicion of contamination exists in times of epidemics.

Immunity and Cure.—Typhoid fever is one of those diseases, as shown by clinical experience, that attack the same individual but once. Two attacks have occurred in the same individual in about two per cent. of all the cases; the occurrence of three attacks in the same individual has, according to a recent report, been observed only five times, and four attacks in the same individual but once. It may, therefore, be concluded that recovery from an attack of typhoid fever confers a certain degree of immunity. Support for this view is found in the fact that the blood-serum of some individuals who have recovered from typhoid fever exhibits immunizing properties with relation to the disease induced experimentally in animals with typhoid-bacilli.

The immunization of animals to typhoid-bacilli is readily effected. Bouillon-cultures heated to a temperature of 60° C. (140° F.), or agar-cultures exposed to a temperature of from 54° C. (129.2° F.) to 56° C. (132.8° F.) have been employed for this purpose, and also the filtrate of unheated virulent cultures, or of cultures in thymus-bouillon.* The simplest

* According to a recent communication, Buchner and Hahn obtained immunity with the cell-juice of typhoid-bacteria rubbed up and expressed by the method of E. Buchner (p. 32). (The plasmatic cell-juices of the bacteria

method of immunization consists in the employment of the ordinary unheated and unfiltered bouillon-culture. Many experimental animals possess a considerable degree of immunity to the typhoid-bacillus, and there is no great difficulty in increasing this. The animal is treated once or twice with an intraperitoneal injection of half that amount of bouillon-culture that is just necessary to cause death. After from three to five days the animal will be able to withstand this previously lethal amount, and after several days, one and a half times, then twice, thrice, etc., this dose can be injected. In this way a high degree of immunity can rapidly be induced. The blood-serum of the immunized animals is in turn capable of conferring immunity upon untreated animals.

As R. Pfeiffer and his pupils assume, the blood-serum of typhoid convalescents and of animals immune to typhoid fever does not contain antitoxic, but only lysogenic protective substances (p. 62)—that is, the serum, injected into the peritoneal cavity of guinea-pigs simultaneously with living typhoid-bacilli, causes dissolution of the specific microorganisms. If two milligrams of a fresh, virulent agar-culture that has been sterilized by exposure for several hours in the thermostat at a temperature of 56° C. (132.8° F.) are injected into human beings, after a brief period of indisposition the blood-serum of such persons likewise possesses lysogenic properties. The strength of the lysogenicity attains the same degree as is present in the typhoid convalescent, and the standard of the serum equals about 0.01 (p. 63). If the appearance of the specific bactericidal (lysogenic) substances in the blood of individuals that have suffered from typhoid fever is really, as Pfeiffer assumes, the essential cause of immunity, then, according to the experiments just described, it must also be possible, by means of prophylactic injections of minimal amounts of the dead bodies of typhoid-bacilli, to induce immunity of like degree and duration. The decision of this most important question must await the results of further investigation.

Attempts at cure with protective serum or with milk obtained from an immunized animal have been made only on a small scale, and distinct success has not as yet been observed. This is in harmony with the view already expressed that

are designated plasmins, and the expressed juice of typhoid-bacilli correspondingly as typhoplasmin.)

the serum of those immune to typhoid fever does not possess antitoxic properties. The therapeutic experiments with typhoid-cultures exposed to a temperature of 60° C. (140° F.) that have thus far been made upon human beings have also failed. This want of success can not be opposed to the possibility already suggested of prophylactic immunization by means of dead bacilli, inasmuch as the already diseased and poisoned organism may react differently than the healthy body to injections of typhoid poison.

ASIATIC CHOLERA.

The exciting agent of Asiatic cholera is the comma-bacillus discovered by Koch in 1883.

The *cholera-bacilli* are more or less markedly curved rods (vibrios), from one-half to at most two-thirds as large as tubercle-bacilli (from 0.8 to 2 μ), although thicker than these. The comma-form is not well defined in all bacilli. Every preparation contains forms, especially the quite young bacilli, that appear as simple, straight, extended bacilli. The most characteristic and typical commas are present in freshly made artificial cultures. Besides, the form of the growing vibrios varies in accordance with their source in one or another epidemic. In certain epidemics the cholera-bacteria assume throughout an almost straight form. Frequently the comma-bacilli are arranged in pairs; when two commas are so applied to each other that the corresponding curves are opposed, the so-called S-form results.

If in the growth of the vibrios the individual newly formed bacteria adhere to one another after division, the so-called cholera-spirilla result. Though observed very seldom in the dejections of cholera-patients, the spirilla occur frequently in artificial cultures, especially when these have become old, the nutrient material exhausted, or if an antiseptic in dilute concentration (as, for instance, alcohol) has been added. The spirilla occur with especial frequency in the peritonitic exudate of guinea-pigs inoculated with cholera-bacilli. The spirilla are generally looked upon as involution-forms, principally because they are thicker in cultures than the young individual commas. The cholera-vibrios exhibit extraordinarily active motility. This is especially noticeable in the hanging drop, in which their appearance suggests a swarm of dancing gnats. This motility is dependent upon the presence of terminal flagella that can be readily demonstrated at one extremity by means of Löffler's method of staining.

The comma-bacillus does not possess *spores*. The arthro-spore-formation that Hüppe assumed to exist as a result of his earlier investigations has not been confirmed by other observers.

The comma-bacilli are most readily *stained* with a saturated aqueous solution of fuchsin or with carbolfuchsin. The exposure to the stain should be longer than usual. The bacilli do not stain by Gram's method.

Cholera-bacteria grow upon all of the usual nutrient media, and also in the absence of oxygen (facultative anaerobiosis), although, according to recent investigations, it appears that oxygen can never be entirely wanting. The cholera-bacilli always require a distinctly alkaline nutrient medium for their development, as they are exceedingly sensitive to the presence of even slight

Fig. 49.—Comma-bacilli (from the mouth); × 1000 (Günther).

amounts of acid. The degree of alkalinity most suitable for the cultivation of cholera-bacilli is secured by adding one gram of crystallized sodium carbonate to 100 cu. cm. of carefully neutralized gelatin (Dahmen), or by preparing a 10.6 per cent. solution of soda (from calcined sodium carbonate) and adding 55 cu. cm. of this to one liter of gelatin (Flügge). For ordinary purposes simple determination of the alkalinity by means of litmus-paper is sufficient, but the paper must be made distinctly blue.

The temperature-minimum for cholera-cultures is 8° C. (46.4° F.); the temperature-optimum from 30° C. (86° F.) to 40° C. (104° F.).

Appearances of Comma-bacilli in Cultures.—*Gelatin-plates.*—The plates are best permitted to develop at 22° C.

(71.6° F.)—a temperature at which the gelatin still retains its solid consistence. After from twenty-four to thirty hours the colonies appear, on microscopic examination, as small, whitish-yellow dots, with an irregular, rough margin. Their contents are coarsely granular. After a time the granules become glistening, so that the colonies appear as if they had been strewn with small bits of glass. With the further development of the culture the gelatin undergoes liquefaction, and this occurs the more rapidly the more favorable the temperature and the more nearly the degree of alkalinity approximates its optimum. At the beginning liquefaction progresses quite slowly; small, funnel-shaped depressions constitute the first peculiarity visible to the naked eye. Viewed with oblique light, the plate appears as if it has been punctured superficially with a fine needle. This beginning liquefaction is characterized by a bright boundary surrounding the individual colonies, as viewed with low powers of the microscope. The colonies have now become somewhat darker and opaque, and their irregular margin is not rarely marked by fine, pointed processes. Later the liquefaction becomes more active, the funnel-shaped depressions become larger, and the colonies sink to the bottom of the depressions. The border surrounding the colony (area of liquefaction) is no longer bright, but filled with small, grayish masses. These consist of groups of bacteria that have become detached from the colony and admixed with the liquefied gelatin. The colony itself, a brown, irregular mass, lies at the bottom of the funnel. In order to bring it clearly into view, the tube of the microscope must be pushed downward. In employing gelatin prepared according to the method of Forster (p. 81) the plates may be exposed to a temperature of 25° C. (77° F.) or 26° C. (78.8° F.). Under these circumstances growth takes place much more actively, and the peculiarities of the colonies described appear much more quickly.

Gelatin Stab-culture.—Growth takes place along the entire line of inoculation in the form of a white thread that grows thinner downward. After from twenty-four to forty-eight hours liquefaction slowly sets in in the upper portions, and here also leads to the formation of a funnel. This is naturally more extensive than the funnel of liquefaction of the individual colonies in plates. Liquefaction takes place so slowly that at first the fluid formed has time to undergo evaporation. The upper portion of the liquefaction-funnel is, therefore, empty, and an appearance is created as if the puncture of inoculation contained an air-bubble. The line of inoculation itself appears in slight degree liquefied, and, in consequence, somewhat enlarged. Its lower portion contains the bacterial masses that have gravitated thither, and which here assume the form of a spirally

wound thread. In the further course of the growth—not before several weeks—the gelatin is completely liquefied.

On *agar-plates* growth is not so characteristic as in gelatin-plates. The superficial colonies present a peculiar, light gray-ish-brown, transparent appearance.

Agar streak-cultures exhibit a grayish-white, moist, glistening coating.

Blood-serum is gradually liquefied.

Potatoes.—In spite of the usually acid reaction of potatoes the cholera-vibrios generally thrive upon this culture-medium, but only at temperatures above 21° C. (69.8° F.). They then form a grayish or grayish-brown, thin, translucent coating. Upon some varieties of potatoes, however, the comma-bacilli do not thrive, but they can readily be cultivated if the potatoes are rendered slightly alkaline by means of a solution of soda, or if they are boiled in a three per cent. solution of sodium chlorid.

Milk is coagulated by cholera-bacteria obtained in certain epidemics, but not by others. The latter appears to be the rule.

Bouillon is rendered turbid, and in the majority of cases a superficial membrane forms at body-heat. Like the majority of vibrios and spirilla, the cholera-vibrios also possess the property of multiplying with especial activity in quite dilute bouillon (from six to eight times). A one per cent. aqueous solution of peptone, with addition of one-half per cent. sodium chlorid, also favors the development of the comma-bacilli. If the peptone is not alkaline originally, the last-mentioned nutrient medium must be rendered so by addition of soda.

Cholera-red Reaction.—If a few drops of pure dilute hydrochloric acid or sulphuric acid are added to cholera-cultures that have grown in nutritive media containing peptone, a rose-red or purple-red coloration appears within a short time. Bouillon-cultures yield this reaction after exposure for twelve hours in the thermostat. This so-called cholera-red reaction is nothing more than an ordinary nitroso-indol reaction. The comma-bacilli possess the property of forming indol and of converting into nitrites the nitrates always present, at least in small amount, in nutrient solutions. In addition to the comma-bacilli, there are other vibrios that likewise yield the nitroso-indol reaction : as, for instance, the vibrio Metschnikoff, and the vibrio berolinensis found in the water-supply of Berlin. (See water-bacteria, Appendix.) The Finkler-Prior bacillus and the Denecke cheese-bacillus also form indol, but no nitrite, so that addition of pure acids free from nitrous acid does not cause a red coloration with them. The one per cent. aqueous solution of peptone and sodium chlorid previously mentioned is especially suited for the formation of cholera-red. The reaction is at times wanting in bouillon when too much or too little nitrate is present.

Tenacity of the Cholera-vibrios.—The comma-bacilli are endowed with extremely little resistance. They can be destroyed in four minutes in *water* at a temperature of 52° C. (125.6° F.). Lower temperatures are, however, better borne, although the bacilli die in ice after the lapse of a few days. The susceptibility of comma-bacilli to small amounts of *acids* has already been mentioned. The addition of not more than 0.07 or 0.08 per cent. of hydrochloric or nitric acid to neutral culture-media is capable of preventing all development. These facts explain why the normal gastric juice, with its hydrochloric-acid content of about 0.2 per cent., constitutes an insurmountable obstacle to the cholera-vibrios. If cholera-bacilli are spread in a *thin layer* upon a basis of any sort so that they are rendered completely adherent and dry, they lose their capability of development in the course of three hours. *Dried upon the hand*, they survive only for one or two hours. The same rapid destruction (in any event within twenty-four hours) takes place in connection with the contamination of *smooth surfaces*—such as floors, paper, etc.—with comma-bacilli. From these considerations it may be concluded that the transmission of cholera by way of the air, through the intermediation of dried particles of dust, is scarcely possible. Surrounded by *moisture* the comma-bacilli may, under favorable conditions, retain their vitality for a long time, up to nine months—as, for instance, in moist linen rolled together compactly and kept in a cool place, and containing the vibrios in pure culture, so that they are not overgrown by other bacteria. They may be found alive in agar-cultures and gelatin-cultures after the lapse of six months.. The usual *antiseptics*, even in feeble concentration, destroy the cholera-vibrios within a short time : one-half per cent. carbolic acid, for instance, in the course of a few minutes. In *fresh milk* the comma-bacilli retain their vitality for twenty-four hours ; in boiled milk, for two or three days ; upon *articles of food* protected from evaporation under a glass jar, for from four to eight days. The bacilli sometimes survive for weeks in the *dejections* of cholera-patients, although this occurs only under peculiarly favorable conditions. In *sterilized water*, from whatever source, living cholera-vibrios can be demonstrated after the lapse of months. In *unsterilized water*, on the other hand, and especially in *bacterial mixtures*, in which the comma-

bacilli enter into contest with other microbes, the conditions vary in accordance with the external temperature and the amount of sodium chlorid present in the fluid in question. The high temperature of summer and increase in the amount of sodium chlorid favor the development of cholera-bacilli, while at low atmospheric temperatures, or in the presence of a small amount of sodium chlorid, they are quickly over-grown by the associated microorganisms. If cholera-dejections gain entrance into streams, the bacilli mostly adhere to the particles of mucus, which they utilize as a nutrient medium. They are thus often removed from the influence of the current and the competition of other bacteria, and they not rarely maintain their vitality in streams for a long time in spite of the unfavorable temperature and the process of self-purification.

The Occurrence of Cholera-vibrios.—Comma-bacilli are found constantly in all cases of Asiatic cholera, and in innumerable amount; in part, in pure culture in the liquid intestinal contents; further, in the intestinal walls of patients; and only exceptionally in other organs. The vibrios can be demonstrated *in the feces* on an average to the tenth day after the inception of the disease; not rarely, however, for a longer time, and sometimes after the termination of the disease (from the forty-sixth to the forty-eighth day of convalescence). Comma-bacilli have never been demonstrated in association with other diseases. During recent epidemics true cholera-bacilli were found repeatedly in the diarrheal stools of patients whose disease clinically pursued a mild course and apparently bore no relation to Asiatic cholera. It is possible, however, that these cases represented the mildest grades of cholera, caused by bacteria of low degrees of virulence. Further, comma-bacilli were found in some cases in the solid stools of healthy persons subjected to bacteriologic examination only because their relations brought them into the environment of cholera-patients, or because they had drunk suspected water, but who presented no symptoms of disease whatever. It is probable that in these cases there existed a natural immunity that permitted the passage of the vibrios through the intestinal canal without injury.

Outside the human body the cholera-bacillus of Koch has been found in stagnant water in India, where cholera is epidemic. In recent epidemics of cholera comma-bacilli

were repeatedly demonstrated in the water-supply through conduits, in the water of improperly situated irrigation-fields (Nietleben), in the water of streams, and in the bilge-water of ships. The waters in question all bore some close relation to cholera-foci, having been contaminated by the dejections of the first cases of the disease introduced, so that in this way they were capable of constituting the source for the further spread of the disease. These facts demonstrate that the cholera-bacillus may pass a saprophytic existence. Forms of comma-bacilli have, further, been cultivated from the Spree, the Elbe, the Danube, and the Seine, that, in their microscopic and cultural appearances, closely agreed with the vibrio of Koch, but whose relation to existing cases of cholera was not so easily demonstrable. These water-vibrios are in part to be distinguished from the bacillus of Koch by the absence of pathogenicity ; others, however, are toxic for animals. Thus, virulent vibrios were found almost regularly in the contents of Paris sewers in the summer of 1892, although at the time there was not a single case of cholera in Paris. All of these bacteria, however, exhibit differences as compared with the vibrio of Koch, even though they are often slight and difficult of demonstration. A bacterium absolutely identical with the cholera-bacillus, but in its source without any relation to cholera, has never been found.

There are two methods of making with certainty the differential diagnosis between the true exciting agent of cholera and the vibrios resembling it. These are the reactions of Pfeiffer and Gruber (pp. 62 and 63). In the performance of Pfeiffer's test the blood-serum of guinea-pigs or of other animals that have been highly immunized to cholera is diluted with ordinary bouillon in the proportion of 1 : 100. To one cubic centimeter of this mixture are added about two milligrams of the vibrios to be examined, caught up with a platinum loop, and the whole is injected into the abdominal cavity of a young guinea-pig weighing about 200 grams. By means of delicate capillary glass tubes specimens of the peritoneal exudate that at once forms are removed at intervals of five minutes and examined, both stained and unstained. If the suspected organisms are true cholera-vibrios, the bacilli are shortly seen to become immobile, later collected in small spherules, and finally, within twenty minutes, completely dissolved. If, however,

this phenomenon does not take place, the vibrio in question
is not the cholera-organism. One source of error must,
however, always be borne in mind, if Pfeiffer's reaction
proves positive : It is possible that the organisms present are
attenuated, to a certain degree saprophytic, vibrios, that
are dissolved in consequence of the normal bactericidal
activity of the organism of the guinea-pig, in the absence
of any specific serum. To avoid this error, a platinum
loopful of the culture in question added to one cubic centi-
meter of normal serum-bouillon mixture (1 : 100) is in-
jected into the peritoneal cavity of a control guinea-pig. If,
after the lapse of twenty minutes, the vibrios are found
living and motile in the control-animal, whereas those
treated with cholera-serum are destroyed, then the diagnosis
of Asiatic cholera may be made with certainty.

The second test, that of Gruber, is more readily per-
formed, as experiment on an animal is not required. The
suspected vibrios may be added to the serum of an
animal rendered immune to cholera in the proportions
of 1 to 50, 1 to 100, and above ; and the mixture is studied
at once with high powers of the microscope. If the
vibrios lose their motility; if they collect in groups ;
if, thus, agglutination takes place—then the suspected
microorganisms are true cholera-vibrios. One of the
macroscopic agglutination-tests (p. 64) may be made—*e. g.*,
bouillon is inoculated with the suspected vibrios and the
serum of an animal immune to cholera is added in the pro-
portion named. If, after the lapse of from sixteen to
twenty-four hours, the vibrios have formed a flocculent pre-
cipitate at the bottom of the test-tube, while the overlying
fluid has become clear, then again the suspected organisms
are true cholera-bacilli. Gruber's reaction has the advan-
tage of being independent of the virulence of the micro-
organisms, and, besides, it renders unnecessary the induc-
tion of a high degree of immunity in the animals yielding
the blood-serum.

The Development of Cholera.—Infection takes place
invariably by way of the *mouth*, the bacilli being taken up
with articles of food, and principally with drinking-water.
Infection through the *air* is possible only in the immediate
neighborhood of the source of infection. The rapid death
of the germs on drying renders improbable the transmission
of the disease for long distances by means of dust, etc.

The inhaled bacilli must also be restrained in the mouth, as infection does not take place through the lungs. From the mouth the vibrios gain entrance into the stomach. If the hydrochloric-acid content of the stomach is normal, the vibrios succumb to it. If infection takes place, it may be assumed that either large amounts of the infected water have been drunk, so that some of the bacteria escape the action of the hydrochloric acid, in consequence of the marked dilution of the gastric contents, or that the function of the stomach was impaired from some cause, and the hydrochloric-acid content was subnormal. Having gained entrance into the intestine, the bacteria multiply and give rise to the production of *toxins*. Mere multiplication of the bacteria in the intestine does not constitute cholera. Such an effect was observed in the well-known experiments of Pettenkofer, without the development of actual cholera. Only when sufficient toxin has been produced to cause *injury of the intestinal mucous membrane* and when the *toxin is absorbed* does the disease develop. While the tissues and the blood become impoverished in water, the profuse rice-water stools take place, and with them innumerable bacilli are evacuated.

The *toxic action* is manifested especially in the constitutional symptoms (feebleness of heart, decline of temperature, etc.). Cholera-typhoid also is now generally looked upon as an intoxication, and the renal disease complicating cholera likewise depends in part upon the toxic activity of the comma-bacilli, being caused in part by the ischemia resulting in consequence of the withdrawal of water.

Brieger and Fränkel have demonstrated the presence of a toxalbumin in cholera-cultures, but greater significance in experiments on animals has been attached to the poison contained within the bodies of the bacilli themselves, and whose effects have been studied, especially by R. Pfeiffer. If a young agar streak-culture, from twenty to twenty-four hours old, is destroyed by exposure for ten minutes to the action of chloroform, and ten milligrams of the bodies of the vibrios thus destroyed are injected into the peritoneal cavity of a guinea-pig, the animal will die. After the lapse of two hours it becomes relaxed, its temperature falls below 30° C. (86° F.), and death takes place in the course of eight or ten hours, usually amid violent clonic convulsions. The poison that thus adheres to the bodies of the

bacteria is of most evanescent nature.* It does not withstand exposure to temperatures above 60° C. (140° F.), drying and the like. If the cultures are boiled for several hours, according to the view of R. Pfeiffer secondary toxic substances are formed that manifest their activity only in much larger amounts. The intoxication, however, presents the clinical features described. This intoxication with the intracellular cholera-poison is remarkable for the rapidity with which it appears, and for the entire absence of any period of incubation, such as is commonly observed in connection with the diphtheria-toxin and the tetanus-toxin. The severity of the intoxication depends upon the amount of toxin and the rapidity of absorption. The effects are most quickly manifested when the poison is introduced directly into the blood-stream.

Epidemics of cholera arise, according to Koch, from the first imported case, through whose dejections, containing the bacilli, the disease is spread. The type of distribution may be of two kinds. The disease may spread in *foci :* a member in the family of the patient first attacked is seized, then others, then another family in the same house, a neighbor, a stranger accidentally present in the house, the laundress to whom the linen from the first case is sent ; in this way each disease-focus gives rise to another, and all together form a *closed chain.* In every instance infection has taken place through contamination with the dejections of an earlier case. Often enough the connection of the individual cases with one another is not demonstrable. Thus, for instance, insects may carry the disease-germs great distances and deposit them upon articles of food that seemingly have in no way come in contact with a focus of disease ; or apparently healthy individuals from the neighborhood of the person first attacked may disseminate the germs through their infected dejecta.

On the other hand, the outbreak of the disease may take place in an *explosive* manner, large numbers of cases appearing equably and simultaneously throughout a town or a city. This occurs when the water, whether delivered through conduits or obtained from streams, is infected by cholera-dejections, and the germ is thus capable of being equably spread over the entire city, and carried into every

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* The poison can also be expressed from the bodies of the bacteria according to the method of E. Buchner. (See foot-note, p. 179.)

household. The cholera-bacillus has never been demonstrated in the air or in the soil, which likewise might be considered as sources of such explosive outbreaks of cholera. The bacillus has been found during recent epidemics in river-water and in conduits.

Epidemics of cholera do not always conform strictly to one or the other of the two types described by Koch; one may be combined with, or pass over into, the other, etc. Individual predisposition, the quantity and quality of food, the density of population, individual and civic cleanliness, distinctly influence the distribution of the disease.

The foregoing theories, representing essentially Koch's views, are, in fact, capable of explaining almost all of the manifestations of recent cholera-epidemics. One point only, however, is obscure: namely, the question why in some instances an epidemic does not occur. Thus, Paris escaped in 1892, although the bacilli were present in the sewers; and only a few cases occurred in Berlin in the year 1893, although the waterways were known to be infected. It is possible that the energetic intervention on the part of the authorities, the filtration of the water, as well as the careful observance of all hygienic regulations, prevented the irruption of an epidemic. The thought, however, can not be entirely put aside that possibly the "local predisposition" was wanting. According to Pettenkofer, for the occurrence of an epidemic *a local and a temporal predisposition* are necessary. "The cholera-germ (x) forms upon the basis of the local and the temporal predisposition of the soil (y) the cholera-poison (z)." According to this view, the disease can never be transmitted from one human being to another, but the germ must first mature in the earth, and then the poison is taken up by the lungs. In view of the facts mentioned this theory of Pettenkofer's can scarcely be maintained any longer as against the bacillus of Koch. A number of epidemiologic facts, however, indicate that, in addition to the comma-bacillus, some other not yet sufficiently determined influences are necessary for the occurrence of an epidemic.

Experiments upon Animals and Human Beings.—Even though some uncertainty exists with regard to the last point, there can, however, be no further doubt with regard to the etiologic significance of the comma-bacillus as the exciting agent of cholera. The final evidence for this was

furnished by the experiments upon human beings that were undertaken in part unintentionally (accidental laboratory-infection), in part intentionally. The introduction of the bacilli into the stomach may be unattended with any effect; at times it gives rise to more or less intense diarrhea (auto-infection of Pettenkofer and Emmerich); in other cases—as, for instance, in one reported by Metschnikoff—however, it induces true dangerous cholera, with all its clinical symptoms. The sad fate of a young Hamburg physician is well known, who died of typical cholera after a drop of peritoneal exudate containing vibrios had entered his mouth in the performance of Pfeiffer's test. Subcutaneous inoculation with cholera-bacilli causes in human beings only moderate local symptoms and fever of brief duration. The blood under these circumstances acquires immunizing properties (G. Klemperer).

A disease resembling cholera can be induced in guinea-pigs by direct introduction of the vibrios into the duodenum, by avoidance of the stomach, after ligation of the choledoch duct; or by introduction of the bacilli into the stomach after previous alkalinization of the gastric contents by means of soda-solution and injection of two or three cubic centimeters of tincture of opium into the peritoneal cavity. The object of ligating the choledoch duct, as well as of the injection of the opium, is to inhibit the peristaltic activity of the intestine. Similar morbid manifestations—cholera-like stools, decline of temperature, etc.—can be induced in guinea-pigs and rabbits by intravenous introduction of the comma-bacilli, especially after previous intoxication with alcohol, or by simultaneous injection of comma-bacilli and metabolic products of varieties of proteus.

Intraperitoneal injection of virulent cholera-vibrios is followed in guinea-pigs (a platinum loopful containing about 2.5 mg. of bacterial colonies scraped from the surface of agar-agar for a guinea-pig weighing from 300 to 500 grams) by death, preceded by paralytic phenomena and rapid decline of temperature. There takes place under these conditions, as has already been stated, an intoxication with the poisons contained within the bodies of the comma-bacillus (p. 189). In the digestive tract of guinea-pigs destroyed by this means special changes are usually not to be observed. At times, the large bowel is markedly injected and the small intestine is not rarely filled with grayish fluid

containing large numbers of comma-bacilli. On the whole, however, it is scarcely possible to insist upon a complete analogy between the intoxication of guinea-pigs and cholera as it appears in human beings.

Bacteriologic Diagnosis of Cholera.—If an attack of disease characterized by the occurrence of profuse, rice-water diarrhea, vomiting, decline of temperature, cramps in the calves, etc., arouse suspicion of cholera—a suspicion that must be considered and investigated in every instance, especially in travelers during the summer season, and particularly when cholera exists—bacteriologic examination of the stools is an absolute duty. The procedure in a case suspected to be one of cholera is as follows :

1. Microscopic Examination.—Cover-slip preparations are made with a flake of mucus from the feces, and stained with dilute carbol-fuchsin solution. The diagnosis of Asiatic cholera is rendered in the highest degree probable if the vibrios form masses " in which the individual bacilli all point in the same direction, so that an appearance is created as if a small swarm of them, somewhat like fish in a slowly flowing stream, are following one another " ; or if, " in addition to isolated bacteria presenting the appearance of cholera-bacteria, only the bacterium coli is found. " The probable diagnosis of cholera can be made microscopically in about 50 per cent. of suspected cases, but in every instance it should be confirmed by cultural investigation.

2. Cultural Investigation.—(*a*) *Gelatin-plate Method.*—Three gelatin-inoculations are prepared, in the usual manner, from the feces, if possible from a flake of mucus, and the inoculated culture-media are poured into three Petri dishes ; these are kept at a temperature of from 22° C. (71.6° F.) to 26° C. (78.8° F.). If upon these plates colonies are found after from fourteen to thirty or forty-eight hours, with uneven, rough borders, strewn with bright granules resembling fragments of glass, and presenting an area of liquefaction, and if these colonies prove to be constituted of commas, a high degree of probability is given the diagnosis, and it is scarcely possible that the disease under consideration is any other than Asiatic cholera. So far as is yet known, there occur in the human intestine no vibrios other than the comma-bacilli that give rise to colonies presenting the characteristics that have been described.

13

(*b*) *Peptone-culture (Fertilizing Method, Schottelius, Koch)*.—The plate-method, which yields admirable results in the diagnosis of marked cases of Asiatic cholera, does not suffice, however, for other cases in which the dejections contain only a small number of comma-bacilli. This small number of vibrios are overrun on plates by the fecal bacteria, and do not develop at all. It is, therefore, necessary in every suspected case to adopt, in addition to the original plate-method, a further method of investigation : namely, the preparation of peptone-cultures. It has previously been pointed out (p. 184) that the cholera-vibrios thrive especially well in a simple alkaline solution of peptone and sodium chlorid (one per cent. peptone and one-half per cent. sodium chlorid). It may be added that the vibrios, by reason of their motility and their great need of oxygen, tend toward the surface of the liquid culture-medium, and there undergo enormous multiplication. Upon both of these facts is based the method of peptone-culture, which has the further great advantage that it leads to the desired result much more rapidly than the plate-method. A platinum loopful of the suspected feces, or, if this be obtainable, a flake of mucus, is introduced into a test-tube or an Erlenmeyer flask containing the solution described, and the vessel is exposed in the thermostat to a temperature of 37° C. (98.6° F.). As soon as the fluid exhibits the slightest traces of turbidity, which usually occurs in the course of from six to ten or twelve hours, a specimen is taken from the surface and is examined in hanging drop and in dry cover-slip preparations. If the examination discloses the presence of a pure culture of cholera-vibrios, the diagnosis is almost certain. In most cases, however, the procedure is not quite so simple. In the superficial layer of the peptone-solution the vibrios are usually intermixed with other microorganisms, and most frequently with the bacterium coli commune. There then remains nothing but to make plates from the material on the surface. These plates, however, are made under much more favorable conditions.

There is now no longer any danger that the small number of cholera-bacilli that were present originally in the feces will be overrun in their growth. Through the intermediation of the peptone-culture the vibrios have undergone enormous multiplication, and the Petri dishes

now exhibit, in consequence, numerous characteristic colonies.

From the gelatin-plates, finally, pure cultures are made, with which the cholera-red, and Gruber's and Pfeiffer's reactions are obtained, and experiments on animals are undertaken. When all these yield positive results, then the diagnosis of cholera-bacilli is final and certain.

Instead of gelatin-plates, agar-plates frequently are made. These have the advantage that they can be kept in the thermostat at a temperature of 37° C. (98.6° F.), and can be examined after from eight to ten hours. It has already been pointed out that only the superficial colonies on agar present an approximately characteristic, light grayish-brown appearance. It is, therefore, useful, in order to obtain only such superficial colonies, to have in readiness agar-agar poured in Petri dishes, upon the surface of which the inoculating material (the fertilized peptone-culture) is smeared by means of a platinum loop. As, however, the agar-agar always expresses a certain amount of water of condensation, which renders impossible the growth of isolated colonies, it is necessary to place dishes into which this culture-medium is poured in the thermostat before they are used, until the fluid has evaporated, or they are preserved in an inverted position. It is not sufficient in making a diagnosis to find light grayish-brown, transparent colonies, but by means of preparations it must be decided whether the bacteria constituting the colonies correspond also morphologically with cholera-vibrios.

Examination of Water for Cholera-bacilli.—The conditions are somewhat different if water is to be examined for cholera-bacilli. Formerly it was the custom to dilute freely the usually much polluted water, and to make plates with a portion of a drop. Under these circumstances it was largely a matter of chance whether cholera-germs were obtained or not. It would be necessary for them to be present in the infected water in considerable number, in order to be demonstrated in this way. These difficulties may be avoided by taking large amounts of water for examination. To this end from 100 to 1000 cu. cm. of the suspected water are placed in sterilized flasks, and to each specimen one per cent. of alkaline peptone (preferably the peptone of Witte) and ½ of one per cent. of sodium chlorid are added. The peptone and

sodium chlorid are kept in readiness in sterilized solutions, and, for instance, 5 cu. cm. and 2.5 cu. cm. of 20 per cent. solutions are added respectively to 100 cu. cm. of water. After testing its alkalinity, the mixture is placed in the thermostat, and it is then treated in exactly the same way as has been described for the peptone-culture. On micro-scopic examination of the surface of the peptone after eight, ten, fifteen, and twenty hours, curved bacteria that closely resemble the comma-bacilli are found in many samples of water. It can not be too strongly emphasized, however, that such a discovery is alone not demonstrative, if the vibrios are derived directly from the feces of a sick human being. In examinations of water it is still further an unavoidable postulate that plates of gelatin or of agar be made with the fertilized material from the surface. If upon these are found colonies that correspond in appearance with cholera-colonies, it is not yet demonstrated that they are true comma-bacilli and not merely similar microbes, of which a number have already been described. It is then necessary to make pure cultures, and these are to be identified by means of the indol-reaction, by experiments on animals, and especially by means of Pfeiffer's and Gruber's reactions (pp. 62, 63, 187, 188).

Disinfection and Prophylaxis.—Every cholera-patient must be isolated at once. Feces and vomited matters, as well as all materials soiled therewith, should be most thoroughly disinfected. The details of disinfection are given in the Appendix. Those who come in contact with the patient should, further, be watched with care, and their dejections should be examined for comma-bacilli. Personal prophylaxis extends to the maintenance of the digestive tract in a state of health (avoidance of all dietetic errors), and the protection against contamination of all articles of food (only boiled water should be drunk, etc.). The general prophylaxis concerns itself with the purification of the water-supply by means of suitable filtration, with the protection against contamination of waterways, and, above all, with the prevention of importation of the disease from abroad. The pilgrimages to Mecca are under the scrutiny of the International Sanitary Commission at Alexandria. Cholera prevails frequently among the pilgrims, and upon the slightest suspicion the pilgrim-ships must be subjected to quarantine before being permitted to pass through the Suez Canal.

Immunity.—A large number of persons—according to Koch almost half—are naturally immune to cholera. Recovery from one attack of the disease confers immunity : at least, second or more attacks have rarely been observed. Places that in one year suffer from a severe visitation of cholera generally remain exempt from the succeeding epidemic. The blood-serum of convalescents from cholera has, in some instances, exhibited a surprising degree of protective influence for guinea-pigs. Thus it was possible to immunize guinea-pigs against otherwise fatal inoculation with cholera-vibrios, in one case by means of 0.01 cu. cm., and in another case by means of 0.025 cu. cm. of serum from human beings that had recovered from cholera (G. Klemperer, Lazarus). As Metschnikoff has shown, not the serum of all convalescents from cholera possesses such immunizing properties ; while, on the other hand, these may be observed occasionally also in the blood of persons that have died of cholera.

Guinea-pigs, rabbits, and goats can be readily immunized to intraperitoneal and subcutaneous infection with comma-bacilli by means of cultures that have been heated to a temperature of from 54° C. (129.2° F.) to 60° C. (140° F.), or have been attenuated by any other means. All of the methods of protective inoculation hitherto employed have, however, been efficient only with relation to intraperitoneal or subcutaneous inoculation, not affording certain protection against introduction of the vibrios by the mouth. The blood-serum of immunized animals is, in turn, capable of conferring immunity. In the process of preliminary treatment the bodily fluids of the animals acquire specific bactericidal (lysogenic) properties, and the immunity to infection with cholera-bacilli appears to depend exclusively upon these bactericidal influences (p. 62).

According to the view of R. Pfeiffer, the blood-serum of convalescents from cholera likewise contains no antitoxin, but, on the other hand, the same lysogenic substances as the serum of animals immunized artificially. R. Pfeiffer, in consequence, maintains the view also for Asiatic cholera in human beings that the immunity depends upon specific bactericidal influences (lysogenicity). It is possible in human beings to stimulate artificially the formation of the lysogenic cholera anti-bodies in the blood. It is only necessary to vaccinate the individuals in question accord-

ing to the method first described by Haffkine. This observer injects first $\frac{1}{12}$ of an agar-culture, twenty-four hours old, carefully devitalized by chloroform, and five days later $\frac{1}{12}$ of a living virulent culture, and, again, after the lapse of five days, ⅛ of the last. Each inoculation is followed by an insignificant, slight, painful infiltration and mild fever, which, however, soon recede. The serum of persons thus inoculated acquires, as Kolle has shown, lysogenic properties at least equal to those of the serum of convalescents from cholera. This bactericidal activity begins on the fifth day, attains its maximum on the twentieth day, and then gradually diminishes. It is, however, still demonstrable after the lapse of a year. Kolle has shown, further, by experiments on human beings, that a single introduction of living or carefully devitalized vibrios is attended with the same success, so that repeated inoculation is not at all necessary to attain marked bactericidal activity. Little of a positive nature can yet be said with regard to the results of Haffkine's protective inoculations ; but they are at least quite encouraging.

CHOLERA NOSTRAS AND SUMMER DIARRHEA.

Cholera nostras includes all of those cases of severe diarrhea presenting symptoms similar to those of Asiatic cholera, but with an absence of the comma-bacilli of Koch from the feces, and without epidemic distribution. In by far the larger number of cases the bacterium coli commune is found in the stools and in the vomited matters. Under these conditions the bacterium coli manifests a considerable degree of virulence ; and in cultures from a case of cholera nostras it proves far more malignant in experiments on animals than in cultures from normal feces. In severe cases of cholera nostras the bacterium coli is often present in the dejections in pure culture. In some cases streptococci have been found in the feces in overwhelming number, or in pure culture, and in isolated instances also vibrios presenting a superficial resemblance to Koch's bacilli, without, however, agreeing perfectly with these—as, for instance, the vibrio Lisbon, in Appendix.

As the necessity for bacteriologic examination in cases of cholera nostras arises only in the presence of suspicious

conditions, when the point to be determined is whether the disease in question is true Asiatic cholera or not, the mode of procedure must in every instance be precisely that which has been described in the preceding chapter for Asiatic cholera.

Closely related to cholera nostras is the so-called summer diarrhea of children. The probable cause of this condition is thought to be the so-called bacteria of bitter milk, which are known under the collective name of bacillus lactis Flügge. Flügge has isolated twelve varieties of this organism, all of which belong to the group of the hay-bacillus, and with which they have many points of resemblance in common. (See Hay-bacillus and Potato-bacillus in Appendix.) These microorganisms cause peptonization of the casein of milk—hence the designation peptonizing milk-bacteria—in consequence of which the milk acquires a bitter taste. Some of them give rise to toxic metabolic products, which, when fed to young dogs, cause diarrhea, muscular weakness, and decline of temperature. The whole group of these milk-bacteria is characterized by the formation of highly resistant spores that withstand boiling for several hours without injury. It is on this account that the sterilization of milk is attended with so much difficulty ; even the method of Soxhlet is incapable of destroying the peptonizing microorganisms. If such an insufficiently or, to use the common expression, partially sterilized milk is preserved at a moderate temperature—as, for instance, 22° C. (71.6° F.) or above—the spores develop, and the poisons mentioned are generated. For this reason great care must be taken that milk prepared by the Soxhlet method is kept in a cool place until shortly before it is used, and in summer best in a refrigerator. The custom of sending with children a supply of milk for a considerable time, heated and kept in special warming bags, should be abandoned, as in the presence of numerous spores germination and beginning multiplication may set in speedily (in the course of one or two hours). These, however, are to be considered as dangerous, as, according to the investigations of Lübbert, "the active principle of the decomposed milk is to be looked for in the bodies of the bacilli." The anaerobic microorganisms to be found in milk—the bacillus butyricus Botkin and that of Flügge—probably play no part in the etiology of the intestinal catarrh of infants.

They occur only in small number and change the appearance and the odor of the milk to such a degree that it will probably not be used.

PLAGUE.

The exciting agent of plague was discovered by Kitasato and Yersin during an epidemic of cholera in China in the year 1894.

Morphology.—The plague-bacillus is a small, nonmotile rod, with rounded extremities. It is believed to possess a capsule, which, however, is not readily demonstrated. Plague-bacilli vary extraordinarily in form, both in cultures and in the products of the disease in human beings and animals. At
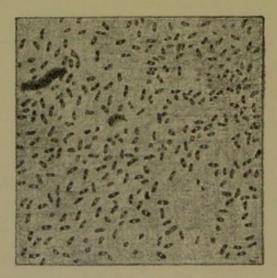
Fig. 50.—Bacillus of bubonic plague (Yersin).

times they appear as short, compact bacteria, at other times they are suggestive of diplococci, distinct rods, short, sharply bent threads, and, finally, in older cultures, degeneration-products occur in the form of swollen spheres and clubs. The plague-bacillus does not stain by Gram's method, but, on the other hand, readily with all aniline dyes, and most strikingly with methylene-blue. Under these circumstances it exhibits distinct polar staining. The extremities are more deeply stained than the central portion, which after feeble action of the staining solution appears to be a deficiency.

Spores are not present.

The temperature-optimum is that of the body, although the plague-bacillus thrives well at room-temperature.

Appearance in Culture.—On *gelatin-plates* the colonies appear as finely granular bodies of brownish color, with a smooth border. The superficial colonies possess a delicate marginal zone. The culture-medium is not liquefied.

In *gelatin stab-cultures* slow, uniform growth takes place along the line of inoculation, while a smooth deposit forms upon the surface.

In *gelatin streak-cultures* a light yellowish deposit forms.

On *agar-plates*, after twenty-four hours, delicate, dewdrop-like colonies form that appear, after the lapse of forty-eight hours, as grayish points, whose border is slightly iridescent. At times a number of large colonies are seen among the smaller.

In *agar stab-cultures* a tough, mucoid coating develops, the water of condensation is rendered turbid, but no membrane forms.

In *Löffler's blood-serum* the same development takes place as in agar streak-cultures.

Bouillon is rendered diffusely turbid. If, however, it be inoculated with a coherent bacterial mass from an agar-culture, the bacilli develop at the bottom of the tube, while the overlying fluid remains clear. In this way a growth is obtained that is suggestive of streptococci.

Milk is a poor culture-medium, and is not coagulated.

Upon *potatoes* a scanty, whitish-gray coating forms at a temperature of 37° C. (98.6° F.).

In culture-media containing *sugar* the plague-bacillus does not generate gas. It does not form indol either in bouillon or in peptone-water.

Wladimiroff and Kressling believe a neutral reaction of the culture-medium most favorable for growth. Addition of glycerin to the nutrient medium is rather disadvantageous.

Vital Capability of Plague-bacilli.—Exposure for ten minutes to a temperature of 55° C. (131° F.) or for five minutes to a temperature of 80° C. (176° F.) suffices to cause the death of the bacilli. They are destroyed at once by a 1 : 1000 solution of mercuric chlorid, and in ten minutes by one per cent. carbolic acid or one per cent. lysol. Mineral acids are very active : Sulphuric acid, 1 : 2000, destroys the bacilli within five minutes ; hydrochloric acid, 1 : 1000, within thirty minutes. When material containing plague-bacilli was transferred to linen, wool, earth, etc., the longest period for which life was preserved was eight days. The same observation was made with regard to preserved portions of organs. The sputum from cases of pneumonia complicating plague, kept in tubes closed with cotton, was no longer infective after sixteen days. In ordinary tap-water the bacilli die in three days, in sterilized water in eight days, in sterilized bilge-water after five days (German Plague-commission).

Portals of Infection for, and Mode of Distribution of, Plague-bacilli.—The plague-bacillus gains entrance into the human organism most frequently by way of slight injuries, scratch-wounds, etc. Infection through the skin may take place simultaneously at several different points of the body in the same case; it has not yet been decided whether it can occur through the intermediation of insects. The related lymph-glands nearest to the portal of infection become swollen, most frequently those of the inguinal and axillary regions (primary plague-boils). Sometimes the glands of the first degree remain free or are but slightly irritated, and only those of the second and third degrees are seriously affected. Sometimes the entire chain of glands from the nearest to the most remote are intensely inflamed. At the site of infection a pustule or a carbuncle is not rarely found, and between this and the related lymphatic enlargement frequently a distinct lymphangitis can be traced. In the milder cases the buboes disappear. If, however, the gland-filter is broken through, the plague-bacilli gain entrance through the glands into the blood and the internal organs, and the condition of plague-septicemia, which almost always terminates fatally, is established. If the buboes undergo suppuration, the plague-bacilli usually die quickly. Sometimes, however, secondary infection with streptococci is superadded, and this then constitutes a serious menace to the patient. In the septicemic form of the disease the bacilli gain entrance into the feces and the urine in consequence of the hemorrhages that occur so frequently.

A second and less common portal of infection is constituted by the lungs. Bronchopneumonic foci occur, in which the exciting agents of plague are found in pure culture or in association with diplococci and streptococci. The sputum of such patients contains the plague-bacillus. Finally, cases of primary infection through the tonsils have been observed, rapidly giving rise to general infection.

The German Commission that went to Bombay in the beginning of the year 1897 for the study of plague, and from whose report the foregoing statements are taken, emphasized that the incredible filth amid which the natives live, their crowding together in small dwellings, the frequent small injuries, especially of the bare feet, the constant scratching induced by vermin, are quite sufficient to explain

the frightful frequency with which plague prevails among the lower classes in Bombay.

Plague adheres obstinately to human habitations. It does not extend in an explosive manner over large portions of the same city, but passes from house to house. In addition to human intercourse, an important part is played by the remarkable relations that exist between rats and similar vermin and bubonic plague, as was already recognized in the middle ages. From numerous sources the German Plague-commission was informed that the outbreak of the epidemic was preceded by a pestilential disease among rats, with an enormous mortality. The natives believe so firmly in the connection between the plague of rats and that of human beings that some of them will at once leave their houses if they find a dead rat present. After great effort the German Plague-commission succeeded in obtaining the fresh cadaver of a rat that had been infected, and in it they found numerous plague-bacilli.* Yersin has made the noteworthy statement that he found the plague-bacillus in the dust and the filth of plague-houses.

Experiments on Animals.—The animal most susceptible to plague is the rat. Minimal amounts of a culture suffice on cutaneous inoculation to cause death regularly. The same result may be attained by application of the plague-bacilli to the mucous membrane of the eye or of the nose. Rats die after eating quite small amounts of plague-infected food, or after gnawing the bodies of other rats dead of plague. The latter circumstance especially is of great importance. It explains the incredibly rapid spread of rat-plague, and also renders it probable that rats are responsible for the extension of the disease from house to house. Next to the rat in susceptibility to the plague is the gray ape. Plague-bacilli are pathogenic for the usual laboratory-animals in general; pigeons alone form an exception. At the autopsy a mucous, at times hemorrhagic, exudate is found at the point of injection. The nearest glands are most enlarged, and they undergo suppuration but seldom. The blood and the internal organs contain the bacilli, the former, however, in but small amount. Kolle calls attention to a more protracted course for the disease, which takes place if rats and guinea-pigs are inoculated with cul-

* It is believed that squirrels and monkeys also may be infected by plague and act as disseminators of the disease.—A. A. E.

tures that have already been carried on artificially for several years. The animals then die in the course of the second week, and one or more glands, up to the size of a walnut, are found, which contain creamy pus in their central portions, in which plague-bacilli are present in large numbers. An involuntary experiment in the human being is also on record : A member of the German Plague-commission suffered infection in making an autopsy of a body dead of plague. Two days later a small pustule formed on the right hand, followed shortly by a lymphangitis, and a swollen gland in the axilla. The discharge from the pustule contained plague-bacilli. In spite of the alarming character of the disease at first, recovery took place.

Etiologic Relations of the Bacilli to Plague.—The constant presence in all cases of plague and the positive outcome of experiments on animals indicate with certainty that the plague-bacillus is the specific exciting agent of plague. In accordance with what has been said, the disease is to be looked upon rather as an infectious disease ; but a toxic element is by no means wanting. The hyperemia and the ecchymoses of the stomach and the bowel are considered by the German Plague-commission as purely toxic effects. As an evidence of the toxic action, the case is reported of a fetus born on the third day of the disease in the mother, in which the characteristic hemorrhages were found, although all portions of the body were free from germs. Certain sequelæ also are to be attributed to the toxic effects : paralysis of the recurrent laryngeal nerve, amaurosis, aphasia, deafness, paraplegia, etc. The poison of the plague-bacilli is probably contained within the bodies of the bacteria. The German Commission dried cultures carefully, mixed them with water, and heated them to a temperature of 65° C. (149° F.). As much as eighty milligrams of the dry residue were introduced into the peritoneal cavity of brown apes, which exhibited only mild manifestations of disease, slight decline of temperature, want of energy, and anorexia. In brown apes, which are by no means so susceptible to plague as gray apes, this toxic effect is, therefore, but little marked.

Bacteriologic Diagnosis of Plague.—The suppurating plague-buboes contain the characteristic bacteria in enormous number. These are readily recognized by their polar staining when treated with methylene-blue. A similar

phenomenon is exhibited among the pathogenic micro-organisms only by the bacilli of chicken-cholera. These, however, are larger than the plague-bacilli, and are of absolutely no significance with relation to human beings. If suppuration has not yet taken place, the diagnosis is not so simple, although it appears most necessary just under these conditions. Puncture of the buboes for this purpose was considered by the Commission in its first communication as not unattended with danger on account of the possibility of opening a blood-vessel. It has, however, since been shown that English physicians in plague-hospitals made long incisions into the diseased glands, with subsequent antiseptic treatment, for therapeutic purposes. In connec-tion with this procedure fluid from the gland is readily obtained for the purpose of making cover-slip preparations, plates, and cultures.

Microscopic examination of the blood yields successful results only in cases of general infection. Cultural investi-gation of the blood, however, yields better results. In order to cultivate plague-bacilli from sputum, and, in general, from bacterial mixtures, it is best to make gelatin streak-cultures. The suspected material is spread in several streaks upon the surface of a solidified gelatin-plate. The plague-bacilli grow well at a temperature as low as from 22° C. (71.6° F.) to 25° C. (77° F.) ; while the associated bacteria—the diplococcus lanceolatus and the streptococcus —grow but feebly if at all. From a diagnostic point of view it is further of the greatest significance that the blood-serum of human beings and of animals that have recovered from infection with plague possesses agglutinating activity (German Commission). It is said that the serum does not cause agglutination before the second week, and that this is most marked in the second and third weeks.

In cases of mixed infection, which occur especially in conjunction with suppuration of the buboes, streptococci appear, not only in the glands, but also in the blood.

The prophylaxis of plague consists in disinfection of all products of the disease that contain the specific bacillus. In the first place, general hygienic precautions should be observed, with especial regard to cleanliness and ventilation of dwellings, care of the skin, etc. In general, plague attacks preferably the lower classes of society, ex-posed to want, filth, and misery. Isolation of plague-

patients and observation of those by whom they are sur-
rounded are naturally necessary.

Immunity and Protective Inoculation.—Plague-bac-
teria retain their virulence until shortly before death. It is,
therefore, impossible to immunize susceptible animals with
old cultures (German Commission). Slightly susceptible
animals can be readily immunized by increasing doses of ·
living bacilli. Highly susceptible animals must, however,
be treated carefully with dead cultures. For this purpose
it is best to employ cultures that have been exposed for an
hour to a temperature of 65° C. (149° F.). In subjecting
a brown ape to protective inoculation the Commission em-
ployed a heated agar streak-culture. Seven days later the
animal was immune to subcutaneous infection, but only after
it had recovered from this was it protected against intra-
peritoneal inoculation. Filtered bouillon-cultures possess
only slight immunizing properties.

Haffkine has employed his method of protective inocu-
lation, which he first recommended for cholera, also in the
treatment of plague. A fluid preparation, made from care-
fully devitalized plague-bacilli, is injected in doses of from ½
to 2½ cu. cm., in accordance with the age of the individuals.
Slight reaction follows, and the injection is repeated in a
somewhat larger dose after the lapse of eight or ten days.
The results obtained with Haffkine's method are not un-
favorable, but a definite decision as to its value must be de-
ferred for the present; at any rate, the protection conferred
is not absolute.

A plague-serum is prepared in the Pasteur Institute for
therapeutic purposes from the blood of highly immunized
horses. In the observations of the German Commission,
three cubic centimeters of this serum sufficed to protect
brown apes against subsequent subcutaneous infection. As
much as ten cubic centimeters, however, did not suffice to
protect susceptible gray apes. The serum exhibits, also,
an undoubtedly curative action (after twelve hours) in pre-
viously inoculated brown apes. With regard to its efficacy
in cases of plague, nothing definite can as yet be stated.

DIPHTHERIA.

The **diphtheria-bacillus** was grown in pure culture in 1884 by Löffler.

The *diphtheria-bacillus* is a rather plump rod of varying size, from 1 to 6 μ long and from 0.5 to 1 μ thick. Its form is subject to great variations in different cultures. At times it appears as a small, wedge-shaped body; at other times as a rather long body, with a bulbous thickening at one extremity— a so-called club; and at still other times as a double club, or a dumb-bell. Slender forms, occasionally curved slightly, are also observed, especially in membranes. Not rarely bifurcations are encountered, and upon the basis of this observation diphtheria-
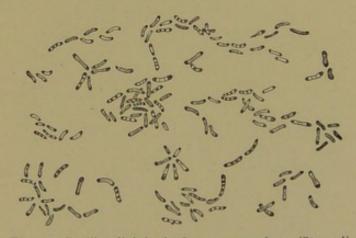
Fig. 51.—Bacillus diphtheriæ from a pure culture (Stengel).

bacilli have been placed in relation with streptothrices, and even with hyphomycetes. The diphtheria-bacillus is incapable of independent movement.

Spore-formation is wanting.

Staining Properties.—The diphtheria-bacillus is best stained with methylene-blue or dilute carbolfuchsin. Gentian-violet overstains and conceals the more delicate structural relations. The organism is stained by Gram's method. The small, blunt clubs stain equably, while the longer specimens exhibit unstained areas, so that the rods appear in places to consist of several segments. Flügge has described a method of double staining, devised by M. Neisser, which is considered to possess essential differential diagnostic significance. The preparation is placed for from three to five seconds in an acetic-acid solution of methylene-blue (methylene-blue 1, alcohol 20, distilled water 950, glacial acetic acid 50), rinsed in water, and counterstained for from three to five seconds in vesuvin (vesuvin 2,

dissolved in boiling distilled water 1000, and filtered). The bodies of the bacilli appear brown, and, as a rule, they contain two blue granules, which have at once been intensely stained by the first aniline dye, and have not yielded their color to the vesuvin subsequently employed. These are the so-called Babes-Ernst bodies (p. 19). The form, arrangement, and situation of these bodies are considered as characteristic of the diphtheria-bacillus under the following conditions: The preparations must be made only from cultures that have grown upon Löffler's blood-serum (solidified at 100° C.—212° F.) at a temperature of 34° C. (93.2° F.) or 35° C. (95° F.), and never above 36° C. (96.8° F.). The cultures must not be less than nine
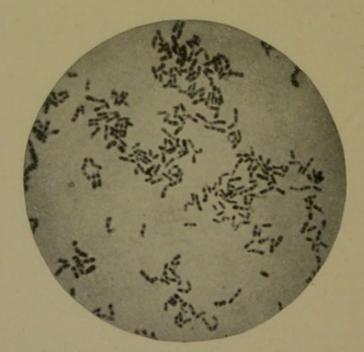
Fig. 52.—Bacillus diphtheriæ, from a culture upon blood-serum; × 1000 (Fränkel and Pfeiffer).

hours, and not more than from twenty to twenty-four hours, old. At one end, more frequently at both ends, of the brown rod a blue granule is then to be seen, and not rarely a third is visible in the middle. These granules are oval in shape, and possess a greater diameter than the bacillus itself, which, however, if the whole appearance is to be considered of diagnostic significance, must be distinctly visible in its entire length and form.

It is further said to be characteristic of the diphtheria-bacillus that the individual bacteria are arranged side by side like palisades. As the most distinctive feature, M. Neisser considers the appearance of impression-preparations from a serum-plate six hours old that has developed at a temperature of from 34° C. (93.2° F.) to 36° C. (96.8° F.). In these there are

visible "moderate-sized free masses in which the slender, rather long, slightly curved bacilli lie in characteristic irregular arrangement—an appearance that is to some degree represented by placing the extended fingers of one hand in varying combinations over or by the side of those of the other."

Cultural Properties.—The diphtheria-bacillus thrives only at temperatures between 20° C. (68° F.) and 40° C. (104° F.) upon all slightly alkaline culture-media. Its temperature-optimum is that of the body.

Upon *gelatin-plates* round, whitish colonies form that remain small. Microscopically these appear yellowish brown and granular, with an irregular border.

In *gelatin stab-cultures* similar small, whitish, spherical colonies, not exceeding a certain size, form along the line of inoculation. At a temperature of 24° C. (75.2° F.) superficial growth takes place, with indications of nail-culture. The gelatin is not liquefied.

Upon *agar*, and better upon *glycerin-agar* plates, after from twenty-four to forty-eight hours, small, grayish-white, glistening colonies form that often exhibit microscopically concentric lamination ; with low powers of the microscope they appear peculiarly granular, with an irregular border.

In *agar streak-cultures*, after twenty-four hours, small, translucent, slightly raised colonies appear. Further growth is inconsiderable, and scarcely extends beyond the line of inoculation.

In *agar stab-cultures* the colonies develop along the line of inoculation, and there is slight growth upon the surface.

Upon *Löffler's serum*, after twenty-four hours, fairly large, whitish, opaque colonies of firm consistence appear. In the succeeding days these colonies increase but little in size. Löffler's blood-serum (p. 84) constitutes, all in all, the best culture-medium for diphtheria-bacilli. It is, therefore, always employed for purposes of differential diagnosis.

In *bouillon*, after twenty-four hours some precipitate has formed, after two days slight turbidity, which increases to the fifth day, then to grow less, until finally the fluid overlying the crumbling, flocculent precipitate is completely clear. Not rarely a thin, fragile coating appears upon the surface of the bouillon.

A delicate coating forms upon the surface of *potato* rendered alkaline.

Milk constitutes a favorable nutritive medium, but is not coagulated.

In boiled and unboiled *egg*, both white as well as yolk, the diphtheria-bacillus grows well; upon coagulated egg-albumin it not rarely exhibits branching.

In slightly alkaline Löffler's *meat-infusion bouillon* the diph-

14

theria-bacillus generates acid. The increase in acidity is distinctly appreciable within twenty-four hours ; it augments from the second day, then to subside. Later, after two or three weeks, the bouillon becomes again alkaline. On addition of litmus to the culture-medium these variations can be distinctly followed by the changes in color.

Tenacity of the Diphtheria-bacilli.—A solution of mercuric chlorid, 1 : 1000, destroys cultures in a thick layer within twenty seconds ; and five per cent. potassium permanganate, five per cent. aqueous solution of carbolic acid, three per cent. solution of carbolic acid in thirty per cent. alcohol, four per cent. solution of kresol in forty per cent. alcohol, within the same time. Five per cent. potassium chlorate is still ineffective after sixty seconds (Löffler). Pure lemon-juice likewise destroys the bacilli speedily. They are destroyed by exposure for ten minutes to a temperature of 60° C. (140° F.), although in a somewhat thicker layer they withstand drying for months. In the form of dust, however, they rapidly die. They withstand cold well, although in the refrigerator the diphtheria-bacilli rapidly lose their property of generating toxins. Abel found diphtheria-bacilli on building-blocks with which a child suffering from diphtheria had played six months previously. Diphtheria-bacilli have been found, further, upon soiled bed-linen, on the rim of a drinking-glass, in the hair and on the shoes of nurses, etc. In gelatin-cultures they may survive, according to Löffler, for three hundred and thirty-one days. Diphtheric membranes dried and preserved in the dark yield cultures even after the lapse of months.

Pathogenic Properties of the Diphtheria-bacillus for Animals.—Diphtheria does not naturally occur in animals. So-called spontaneous diphtheria of fowl, of pigeons, etc., are etiologically different diseases.

Diphtheria-bacilli give rise to the formation of true diphtheric *pseudomembrane*, with multiplication of the bacilli, in the previously injured vagina or conjunctiva of guinea-pigs, in the trachea of guinea-pigs and rabbits after tracheotomy. Most birds, especially pigeons and chickens, then young dogs, rabbits, and especially guinea-pigs, are susceptible to the diphtheria-bacillus. Subcutaneous introduction of the bacilli is first followed by purely local alterations ; more or less extensive hemorrhagic edema of the subcuta-

neous connective tissue. Then, in the presence of fever, there develop pleuritic effusions, swelling and redness of the adrenal glands, hemorrhages into the structure of the lymphatic glands, and death, preceded by decline of the temperature, occurs in the course of from twenty-four to forty-eight hours. If the virulence of the bacillus is slight, the disease is protracted : it may last three, four, or five days, and even several weeks. In the less susceptible rabbit the slow course is the rule. Under these circumstances there occur true diphtheric *palsies*, at first involving the posterior extremities of the animal, then the anterior, and finally the muscles of the neck. Death takes place after marked emaciation. At the autopsy fatty degeneration of the liver and the kidneys is found, and also inflammatory changes in the spinal cord and the nerves. If the virulence is still further diminished, the constitutional symptoms (fever) and the remote manifestations (pleuritis, palsies) are less conspicuous. After recovery from the local inflammatory process, which terminates in necrosis of the skin, the animal may be restored to health.

Physiology of the Disease in Animals.—Whether their virulence be great or slight, the bacilli remain at the site of introduction, and, as a rule, they do not penetrate more deeply into the organism. Multiplication of the bacilli at the point of injection takes place only to a small extent, and only at the beginning—in the first six or eight hours ; later, the number of bacteria rather diminishes. They may, however, survive for a long time, and they have been found alive after weeks among the necrotic portions of skin at the point of injection. The constitutional disease of animals results exclusively from the poison generated by the bacteria : the diphtheria-toxin. The disease developed in animals by diphtheria-bacilli is, thus, a purely *toxic* one. If the poison alone, without the bacilli, is introduced into the bodies of animals, there result the same morbid manifestations, with the exception of the false membrane, and especially the palsies, just as after inoculation with the bacilli themselves. In experimental intoxication of animals a bouillon-culture, several weeks old, is employed for inoculation whose reaction, originally alkaline, has become acid and is again rendered alkaline, and which has been freed of bacteria by filtration through a porcelain filter (Chamberland filter in which the fluid con-

taining the bacteria is under positive pressure, or Kitasato's porcelain cylinders in which suction of the filtrate takes place). Instead of filtering them, the bacilli in the culture may be destroyed by addition of carbolic acid in proportion of 0.5 per cent.

Reference has been made to the efforts directed to obtaining the poison chemically pure from the toxin-containing bouillon-filtrate (p. 29). Brieger and Boer have made the greatest advances in the preparation of the diphtheria-toxin. To the toxin-containing filtrate they add twice as much of a one per cent. solution of zinc chlorid. The resulting precipitate is agitated with from a three to a six per cent. solution of ammonium carbonate, and enough ammonium phosphate is added to effect complete solution; then a delicate white turbidity results on addition of zinc phosphate. To free the solution of metallic precipitate, it is passed through a hardened filter, is well washed, and the filtrate is saturated with ammonium sulphate. In this way a precipitate is obtained that contains the diphtheria-toxin. A portion of the peptone adherent to the toxin is separated by solution in water and precipitation with sodium sulphate, but the albumin can not be completely eliminated, even by repeated washings.

Brieger and Boer have, therefore, cultivated the diphtheria-bacillus upon dialyzed urine of human beings, and have prepared from this albumin-free culture-medium by the methods just described a toxin free from peptone and albumin. This toxin is destructive to animals, and the conditions found after death are characteristic. It does not yield the usual reactions of albumin, and is uninfluenced by reducing substances, but it is almost immediately destroyed by oxidizing substances. Acids also destroy the purified toxin of Brieger and Boer, while feeble alkalies do not.

By means of a concentrated solution of ammonium chlorid it is possible, as Brieger and Boer, further, have shown, to free the bodies of the bacteria completely of the specific poison. If the bacteria thus treated are ground to powder, and small amounts of this suspended in water are injected into guinea-pigs, the animals die with necrosis and suppuration at the point of infection. The bodies of the bacilli are, therefore, possessed, further, of a necrotic poison.

Relations of the Bacilli to Diphtheria in Human Beings.—The diphtheria-bacillus may be found in all cases of diphtheria in the diphtheric membrane, and in cases of diphtheria of the tonsils throughout the cavity of the mouth. It lies superficially, usually in large number ; it rarely penetrates deeply. In the bodies of patients suffering from diphtheria the bacillus has almost never been found at any other place than in the false membrane, and especially never in the blood. Only in the bodies of patients dead of diphtheria has it been detected on several occasions also in the blood and in the viscera. Diphtheria in human beings must thus be viewed as a *toxic infectious disease*. The bacillus is responsible only for the local process ; the constitutional manifestations (fever, palsy, etc.) being dependent in human beings also upon absorption of the toxic metabolic products of the bacillus. The local action of the diphtheria-bacillus consists in necrosis of the epithelium and of the uppermost layer of the mucosa, which are thus converted into diphtheric membrane. This true diphtheric inflammatory process does not occur—apart from the action of certain poisons, as, for instance, diphtheria of the large intestine in conjunction with mercurial poisoning—in the absence of diphtheria-bacilli. The diphtheria-bacillus, however, by no means always gives rise to such a necrotic inflammatory process, when present in the body. The organism has been found repeatedly also in cases of fibrinous rhinitis and of croupous conjunctivitis, the latter being clinically quite different from diphtheria of the conjunctiva. Under these conditions the diphtheria-bacilli have simply given rise to a croupous exudation without necrosis. Finally, Löffler has found virulent diphtheria-bacilli in the mouth of a healthy child, an observation that has since been repeated by others. The diphtheria-bacillus may, thus, under certain conditions, vegetate upon mucous membranes in a virulent state, without occasioning disease of those structures.

Mixed Infection in Human Beings.—The diphtheria-bacillus is rarely found alone in the membranes, being usually associated with streptococci, also with staphylococci, pneumococci, and coli commune. It is almost certain that the severe purulent and septic manifestations observed in some cases of diphtheria are to be attributed to such mixed infection with especially virulent streptococci. It is a matter

of interest in this connection that Roux and Yersin have shown that attenuated diphtheria-bacilli can be rendered again virulent by simultaneous inoculation with virulent streptococci.

Saprophytic Occurrence of Diphtheria-bacilli outside the human body has not yet been observed.

Pseudo-diphtheria-bacilli.—Hoffmann Wellenhof, and independently of him Löffler, cultivated as early as 1887 a microorganism bearing an extraordinary resemblance to the specific diphtheria-bacillus, and which Löffler designated pseudo-diphtheria-bacillus. Since then, reports of observations of this or quite similar bacteria have multiplied, so that a whole series of pseudo-diphtheria-bacilli obtained from cases of angina, rhinitis, from the healthy mucous membrane of the mouth, pharynx, and nose, and from the skin and its diseases is now known. The confusion became still greater when the xerosis-bacilli were included in this group, and when it was later shown that these bacilli are present upon the normal conjunctiva.

The pseudo-diphtheria-bacillus exhibits the same variations in form as the true diphtheria-bacillus. The short bacillus is said to occur more commonly, but this is by no means always the case. According to M. Neisser the pseudo-diphtheria-bacilli exhibit negative manifestations when double staining with methylene-blue acetate and vesuvin is employed. Only rare examples of certain varieties of xerosis-bacilli take the stain. In applying this staining reaction the conditions laid down (pp. 207, 208) must be strictly observed, as this means of differentiation does not suffice for cultures several days old. Further, impression-preparations from serum-cultures six hours old (p. 208), according to Neisser, constitute a serviceable aid in differential diagnosis. The pseudo-diphtheria-bacilli are not arranged in a typical manner, and after the lapse of the short interval of time mentioned they do not exhibit the uniformly slender, rather long form. The xerosis-bacilli develop but slightly in the course of six hours ; they adhere so firmly to the nutrient medium that typical accumulation does not appear in the impression-preparation. The bacilli exhibit thickenings, swellings— in short, appear older than diphtheria-bacilli cultivated for six hours upon serum usually do. In gelatin stab-cultures the pseudo-diphtheria-bacilli form small colonies that spread upon the surface, and after two days quickly grow larger.

In agar streak-cultures large, grayish-white colonies form, that soon give rise to extensive elevated deposits; whereas, in the case of the diphtheria-bacillus, development extends only a short distance from the line of inoculation. On serum the colonies soon become larger, brighter, and softer than those of the diphtheria-bacillus. In bouillon they form a precipitate, clarification taking place after the lapse of three weeks. Upon alkaline potato slight growth takes place, and in milk and egg a good growth. Milk is not coagulated. The xerosis-bacilli develop but slightly upon all culture-media.

The pseudo-diphtheria-bacilli usually generate no acid in their development in ordinary bouillon; individual varieties form minimal amounts, and only one culture of xerosis-bacilli is mentioned by Neisser that exhibited as great an increase in acidity as is observed in the case of diphtheria-bacilli.

On the whole, the pseudo-diphtheria-bacilli and the xerosis-bacilli are not pathogenic for guinea-pigs, although, according to Spronck and C. Fränkel, there are some varieties that exhibit a certain degree of virulence for these animals (causing edema, and after inoculation of three cubic centimeters loss of weight, etc.). The guinea-pigs could not be protected against the action of these pseudo-bacilli by previous injection of diphtheria-antitoxin. The observers named, therefore, reached the conclusion that diphtheria-bacilli and pseudo-diphtheria-bacilli are not identical. Roux and Yersin, however, take the position that the pseudo-diphtheria-bacillus represents an attenuated or temporarily nonvirulent diphtheria-bacillus. This view still has adherents, who support their contention with the fact that in every case of true severe diphtheria nonvirulent as well as virulent diphtheria-bacilli are always observed. The diphtheria-bacillus would, accordingly, be somewhat analogous to the pneumococcus, which, likewise, is a common inhabitant of the healthy mouth.

The Susceptibility of Human Beings to Diphtheria can not be considered as great, in view of the possibility of the presence of diphtheria-bacilli upon the mucous membrane of healthy persons. It would appear that a special predisposition on the part of the mucous membranes or special virulence on the part of the bacilli is necessary for the development of the disease. The first years of life have

distinctly a greater susceptibility than adults, but this grows less after the fifth or sixth year.

The portal of infection for the diphtheria-bacillus may be constituted by any mucous membrane, as that of the nose, the pharynx, and the larynx, as well as that of the vagina, the conjunctiva, etc., and, further, every wound-surface. The larger number of cases of diphtheria are attributable to direct association with diphtheria-patients. The diphtheria-bacilli may, under such conditions, gain entrance into the pharynx, where infection takes place most commonly; as well as with the food and by way of the air-passages. The diphtheria-bacillus is probably not carried great distances through the air. It has never been found in the air, and it is rather sensitive to drying in a thin layer. In thicker layers the bacilli may survive for four months, and if the drying be incomplete, for seven months. Probably the bacilli are conveyed directly through the agency of the portions of membrane coughed up by the patients. In some cases eating-utensils and drinking-utensils, handkerchiefs, toys, etc., have been the means of conveying the disease-germs from children suffering from diphtheria, as well as convalescents. Children may also be infected by adults through kissing, if these have suffered from a harmless angina clinically free from diphtheric characteristics. In other cases direct infection can absolutely not be demonstrated, and at times such an occurrence can be actually excluded—as, for instance, when the first case of diphtheria occurs in a village cut off from all communication with the external world. Under such circumstances it must be concluded that the diphtheria-bacilli have been derived from a case of diphtheria that pursued the clinical course of a benign angina and gave rise to no suspicion of diphtheria; or from a case already cured, in which the bacilli may be present for a long time after the disappearance of all symptoms (p. 230); or the conjunction of special circumstances has, in accordance with the theory of Roux and Yersin, endowed with virulence pseudo-diphtheria-bacilli that have been present in the mouth of the infected individual. What conditions may bring about this circumstance, or whether it is at all possible, is not yet known. On the other hand, it is known, from an observation in which virulent bacilli were found in the mouth of a healthy child, that a certain predisposition, a lesion of the mucous

membrane, or the like, must be present for diphtheria to result from infection with true diphtheria-bacilli from a case of diphtheria.

Method and Significance of the Bacteriologic Diagnosis of Diphtheria.—Every case of inflammation of the throat should be examined with regard to the presence of diphtheria-bacilli, and, if these be found, the case should be treated from both the medical and the hygienic standpoint as one of diphtheria (just as every case of diarrhea with comma-bacilli in the stools should be treated as one of cholera), as the mildest case of diphtheric angina may give rise to severe infection.

For diagnostic purposes, simple microscopic examination of the mucus or of the membrane in stained cover-glass preparations will suffice in some cases. Double staining, by the method of M. Neisser, which is so characteristic for preparations made from cultures, has not proved entirely reliable in the study of original preparations. If the preparations, stained single or double, are not entirely convincing, cultural investigation must be additionally undertaken.

A small portion of the diphtheric membrane is removed with forceps, sterilized by heat, or, directly, with a strong platinum loop ; or a sterile swab of cotton or a sterile bit of sponge is rubbed upon the suspected surface to be examined, and six or eight strokes are made upon a plate of Löffler's blood-serum. Should this not be available, from three to five tubes of blood-serum or glycerin-agar, solidified in slants, are successively inoculated. Plates and tubes are placed in the thermostat at a temperature of 37° C. (98.6° F.)—according to M. Neisser best at a temperature of 34° C. (93.2° F.) or 35° C. (95° F.). In the first streak or in the first tube inoculated the colonies are too dense, and they coalesce ; in the last they are isolated, and these are then examined further. It is recommended also that the membranes, before being smeared, be rinsed for several minutes in two per cent. solution of boric acid, whereby a considerable number of the saprophytic bacteria, accidentally present, are removed, so that a separation of the individual colonies is brought about in the first inoculation-smears. After six or eight hours impression-preparations are made from the serum-plate. If these display the typical collections described (p. 208), then a positive diagnosis can be made. A negative diag-

nosis, however, should be made only with great reserve
after the lapse of only six hours (M. Neisser). After
eighteen or twenty hours smear-preparations are made, and
these are treated by the method of double staining already
described (p. 207). Typical granules in typical bacilli may
be considered as conclusive, although Neisser himself issues
a warning against dependence upon double staining alone
until his observations have been amply confirmed by others.
If, in accordance with older methods, dependence is placed
upon simple staining of an ordinary cover-slip preparation
from a plate or a test-tube of Löffler's serum, the possi-
bility of confusion with pseudo-diphtheria-bacilli must con-
stantly be borne in mind. It is then necessary to scrutinize
carefully the peculiarities that have been described as in-
dicating either diphtheria or pseudo-diphtheria. No single
feature should be accepted as conclusive in the diagnosis ;
but the conjunction of all should be required. Frequently,
experiments on animals must be resorted to in order to
determine whether the bacilli are virulent or not. If they
prove virulent, the organisms are probably without question
true diphtheria-bacilli.*

Immunity and Specific Therapy.—Diphtheria may
attack the same child on several occasions. It is not one
of those diseases that leave behind them permanent immu-
nity, but rather one of those that predispose to recurrence.
Nevertheless, it is certain that after recovery from diph-
theria some degree of immunity exists temporarily. This
is demonstrated by the observations of Escherich, Kle-
mensiewicz, and others, who showed that the blood-
serum of children convalescent from diphtheria exhibits
immunizing properties.

Experimental animals are readily immunized to diph-
theria. Behring and, later, Roux conferred such immunity
on a large scale upon horses by preliminary treatment with
diphtheria-toxin whose toxicity was attenuated by addition
of iodin trichlorid or solution of iodin and potassium iodid.
By the introduction of gradually increasing amounts of
diphtheria-toxin the immunity of these animals was in-
creased to a high degree.

* The method commonly practised in public laboratories consists in inocula-
tion of a tube of Löffler's serum with a previously sterilized swab applied to the
suspicious membrane, cultivation in the incubator overnight, and microscopic
examination of cover-slip preparations stained with Löffler's solution.—A. A. E.

The following instance, taken from the text-book of Macé, will illustrate the immunization of a horse by the method of Roux. For the better comprehension of the description it should be mentioned, as has already been pointed out in the consideration of the physiology of the disease (p. 211), that the toxin is the equivalent of the filtrate of a bouillon-culture, or of a culture in which the bacilli have been destroyed by addition of carbolic acid in a proportion of 0.5 per cent. :

DAY.	INJECTION OF	TOXIN WITH ADDITION OF IODIN-POTASSIUM IODID.		
1	¼ cu. cm.	1 : 10	No reaction.	
2	½ cu. cm.	1 : 10	"	
4, 6, 8	½ cu. cm.	1 : 10	"	
13, 14	1 cu. cm.	1 : 10	"	
17	¼ cu. cm.	Pure toxin	Slight edema, without fever.	"
22	1 cu. cm.	"	"	"
23	2 cu. cm.	"	"	"
25	3 cu. cm.	"	"	"
28	5 cu. cm.	"	"	"
30, 32, 36 . . .	5 cu. cm.	"	"	"
39, 41	10 cu. cm.	"	"	"
43, 46, 48, 50 .	30 cu. cm.	"	Marked edema, disappearing in the course of twenty-four hours.	
53	60 cu. cm.	"	"	"
57, 63, 65, 67 .	60 cu. cm.	"	"	"
72	90 cu. cm.	"	"	"
80	250 cu. cm.	"	"	"

Small doses of pure toxin may also be employed in the first inoculations. Some horses bear from the beginning 1 cu. cm. of a toxin of which $\frac{1}{10}$ cu. cm. is sufficient to destroy within forty-eight hours a guinea-pig weighing 300 grams. The immunity can be looked upon as well established after the animal has received from 60 to 70 cu. cm. of this toxin. It is then possible to inject much larger amounts without injury. In order to obtain diphtheria-antitoxin it is best to employ horses, in the first place because they are very readily immunized, and in the second place because they can be bled innumerable times.

In immunizing the animals a normal toxin-solution must be available to begin with, and which affords a standard of comparison. Behring designates as a *diphtheria normal toxin* that diphtheria-solution of which 1 cu. cm. represents the minimal lethal dose for 100 guinea-pigs each weighing 250 grams. To indicate this normal toxin, he has intro-

duced the abbreviation DTN[1], and he designates a toxin of 10 times this strength as DTN[10] and one of $\frac{1}{10}$ this strength as $\frac{DTN}{10}$. As guinea-pigs weighing 250 grams are not always obtainable, the important minimal lethal dose is not estimated with regard to the entire experimental animal, but upon the basis of each gram of living bodily weight. In order to express this relation also in simple symbols Behring designates the whole animal with an m,* and a gram of the living bodily weight with an M. The weight of the guinea-pig in each individual instance is added besides, and above the letter m (thus, for instance, m^{280} stands for an animal weighing 280 grams). The minimal lethal dose for 1 gram of body-weight—thus, for 1 M—is indicated by prefixing the symbol +. +1500M expresses that amount of toxin that just suffices to destroy 6 guinea-pigs each weighing 250 grams. One cubic centimeter of DTN[1] causes the death of 100 m^{250}, and is thus equal to +25,000 M—that is, it represents 25,000 minimal lethal doses for each gram of living guinea-pig by weight. Of diphtheria-toxin 10 times the normal strength, DTN[10], 1 cu. cm. = +250,000 M ; 1 cu. cm. of diphtheria-toxin one-tenth the normal strength, $\frac{DTN}{10}$, = + 2500 M.

In order to obtain an active diphtheria-toxin it is obviously necessary to employ highly virulent diphtheria-bacilli in preparing the bouillon-cultures. As a consequence it is frequently necessary to fortify the virulence of the diphtheria-bacilli. A diphtheria-bacillus that has completely lost its property of generating toxin can not be rendered virulent again by any method thus far known. If, however, a trace of virulence remain, this may be augmented by repeated passage through animals. Six or eight hours after the injection a specimen is obtained from the area of local edema, and with it a culture is made, which then serves for the inoculation of a new animal. Roux and Yersin augmented and accelerated the production of toxin on the part of the diphtheria-bacilli by cultivating these in a shallow layer of bouillon through which a current of moist air was constantly passed. The diphtheria-bacillus thrives vigorously in nutrient media containing sugar, although it generates considerably less toxin.

* In the original, the German capital letter 𝔐 is employed, but for convenience sake the English small letter m is here substituted.—A. A. E.

When the horses have been rendered sufficiently immune, five or six liters of blood are withdrawn from the jugular vein by means of a trocar, eight or ten days after the last injection. The blood, after standing in the refrigerator, yields a clear serum, which exhibits its maximum activity eight or ten days after the injection of toxin. If the blood is removed earlier, the serum is considerably weaker. After the period of maximum activity, which persists for several days, a gradual reduction takes place, which may lead to complete disappearance of the antitoxin, unless meanwhile new injections of toxin are made. For purposes of preservation carbolic acid, in the proportion of 0.5 per cent., is added to the antitoxin (Behring, Höchst serum), or 0.4 per cent. trikresol (Aronsohn, Schering serum), or a bit of camphor (Pasteur Institute).

It has been pointed out in the general section that toxin and antitoxin, admixed in a test-tube, mutually neutralize the activity of each other, so that when injected simultaneously into guinea-pigs, no manifestations of disease follow. In this mutual interaction a regular gradation is observable. In estimating the immunizing value of the curative serum Behring and Ehrlich did not proceed from the simple lethal dose, but from ten times that amount. They designate as normal serum that of which 0.1 cu. cm. suffices to neutralize ten times the lethal toxic dose, so that a guinea-pig weighing 250 grams withstands the injection without injury. One cubic centimeter of this serum represents a *normal antitoxin-unit*. The further calculation can be readily made upon this basis. A serum, for instance, of which 0.01 cu. cm. neutralizes 10 times the lethal dose represents 10 times the strength of normal serum—that is, 1 cu. cm. contains 10 normal antitoxin-units. Should 0.001 cu. cm. of a serum suffice for this purpose, it represents a strength 100 times that of normal serum, and 1 cu. cm. contains 100 normal antitoxin-units.

Behring subsequently introduced a new method of calculation. He designates as diphtheria normal antitoxin, DAN[1], that serum of which 1 cu. cm. neutralizes 1 cu. cm. of diphtheria normal toxin, DTN[1], $= + 25,000$ M. These figures he converts directly into weight of guinea-pigs, in grams, M, by simply prefixing the minus sign. DAN[1] thus equals $-25,000$ M : that is, 1 cu. cm. of diphtheria normal antitoxin is capable of neutralizing the lethal dose

for 25,000 grams in weight of guinea-pig. As may be readily seen, this mode of calculation agrees entirely with the first described. According to that, 0.1 cu. cm. of serum was capable of neutralizing 10 times the minimal lethal dose; while according to this, 1 cu. cm. of serum is capable of neutralizing 100 times the minimal lethal dose in the typical guinea-pig weighing 250 grams.

Roux estimates the immunizing power in quite another manner. He determines how much serum must be injected from twelve to twenty-four hours previously, in order to protect a guinea-pig against the lethal dose of diphtheria-bacilli or of diphtheria-toxin that otherwise would have destroyed the animal in not more than thirty hours. He fixes as the power of the serum that figure which expresses the relation between the amount of serum and the body-weight of the guinea-pig. An immunizing power of 20,000 indicates thus that of this serum $\frac{1}{20000}$ part of the body-weight of a guinea-pig must be injected in order to protect the animal against the amount of diphtheria-culture or diphtheria-toxin that would cause death in thirty hours.

Spronck has proposed a special formula for converting the immunity-unit of Roux into that of Behring. $B = \frac{R}{500}$ —that is, it is only necessary to divide Roux's figures by 500 in order to obtain the number of Behring units.

In a communication upon the estimation of the strength of diphtheria-antitoxin and its theoretic basis Ehrlich starts with a dry diphtheria-antitoxin prepared in the Höchst works in order to obtain a sufficiently constant standard and a unit of measure for the serum that is protected from the destructive influence of water, oxygen, light, and heat. This contains 1700 immunization-units or normal antitoxin-units to the gram. It is preserved in a small apparatus consisting of two communicating glass tubes, of which one contains the powder and the other phosphoric anhydrid as a dehydrating agent. The apparatus is most carefully exhausted of air, and kept in a dark, cool place. Each tube contains 2 grams of antitoxin-powder, which before being used is dissolved in 200 cu. cm. of a solution of sodium chlorid (10 per cent.) and glycerin (from 50 to 80 per cent.). One cubic centimeter of this solution diluted 17 times represents the immunity-unit or normal antitoxin-unit. If to this immunity-unit are added increasing quantities of diphtheria-toxin, it is possible to fix two limits (L), which

are of great importance for the characterization of the toxin. The first (L_0) represents that amount of toxin that is neutralized by the serum, so that injection of the mixture is borne by the guinea-pig without injury. The second limit (L_+) represents the amount on injection of which such a marked excess of toxin is rendered active in spite of the presence of the anti-body that death of the guinea-pig takes place within four days. According to the foregoing considerations, L_0 represents about 100 lethal doses ; for, as has been explained, if the antitoxin-unit DAN [1] neutralizes the toxin-unit DTN [1], that is 1 cu. cm. of that toxin of which 0.01 cu. cm. destroys a guinea-pig weighing 250 grams ; and the difference (D) between the two limits (L_0 and L_+) should be equal to the minimal lethal dose. These assumptions are, however, not justified, as Ehrlich has shown as the result of most painstaking experiment. L_0 varies between 27 and 109 toxin-doses, and D between 1, 7, and 28. Ehrlich, upon the basis of experiments with tetanus, had already come to the conclusion that by the action of carbon disulphid tetanus-toxin could be transformed into an innocuous modification, but which is still capable, both in the test-tube and in the animal body, of combining with the anti-bodies. The spontaneous attenuation of the diphtheria-toxin so frequently encountered, without the slightest diminution in its neutralizing activity, is attributed by Ehrlich to a similar transformation of a portion of the toxin into such toxin-modifications, and for these he proposes the name of *toxoids.*

Ehrlich divides the toxoids into three groups : (1) Protoxoids, which unite with the antitoxin more easily than the toxin.; (2) syntoxoids, which exhibit for the antitoxin the same affinity as the toxin ; and (3) epitoxoids, which possess a lesser attraction for the antitoxin. As the protoxoids possess a greater affinity for the anti-bodies than, and the syntoxoids the same affinity as, the toxin, they can not be displaced by the toxin from their combination with the anti-toxin. If, therefore, the neutral toxin-antitoxin-mixture (L_0) consists of toxins, protoxoids, and syntoxoids, then L_+ is produced by the addition of the simple lethal dose, or, what amounts to the same thing, D, the difference between L_0 and L_+ is equal to this lethal dose. The conditions are, however, quite different when epitoxoids are present. These must, in the first place, be displaced by the

toxin added, and then there must be present, besides, a simple toxin-unit in order to attain L_+ and to cause the death of the animal. D corresponds here to the epitoxoid-units plus 1 toxin-unit. Every normal toxin-bouillon contains the three toxoids. Their relations may be represented as follows: Diphtheria-bouillon = x-toxoids (protoxoids and syntoxoids) + y-toxin + z-epitoxoid. L_o, then, = x-toxoid saturated + y-toxin saturated + z-epitoxoid saturated. To determine the equivalent of a in toxin, it is only necessary to know the number of lethal doses present in L_o. L_+ then corresponds with the formula: x-toxoid saturated + (y + z) toxin saturated + 1 toxin free + z-epitoxoid free. The number of epitoxoids β equals, as has been seen, D — 1, but only in the event of the bouillon being constituted exclusively of toxin and epitoxoid. If additional toxoids are present, then β expresses a relative value, and is, therefore, designated by Ehrlich as a function of β, thus, $F(\beta)$. The formula for the toxin-bouillon may, therefore, be expressed as follows: x-toxoids + a toxin + $F(\beta)$ epitoxoid.

For the majority of diphtheria-poisons it may be shown by addition of the simple immunity-unit that they originally contained the required 100 doses of toxin. The attenuation is effected gradually according to the principle of thirds or halves. Of three toxin-molecules two are converted into toxoids, or 1 toxin is converted into equal parts of toxoids and toxin. It appears that in this process of decomposition in the cold no epitoxoid—which always occurs in cultures maintained at a temperature of 37° C. (98.6° F.)—is formed, but only protoxoids and syntoxoids. In the study of an especially active toxin immediately after its acquisition Ehrlich obtained the following figures: $L_+ =$ 100 doses of toxin; $L_o =$ 50 doses of toxin. The toxin, thus, was of half strength, and the figures obtained had, therefore, to be multiplied by 2. The value of L_+, then, equaled 200, and the formula for the bouillon was as follows: 50 toxoids + 50 toxin + 100 epitoxoids. As a result of this demonstration that the antitoxin-unit saturates 200 toxin-equivalents, that the poison itself is attenuated dichotomously, it was possible without difficulty to explain the previously mysterious manifestation that with freshly prepared toxins frequently just 100 toxin-equivalents are neutralized by the immunity-unit.

The total of toxoids, toxins, and epitoxoids, equals, according to Ehrlich, 200 ; a toxin possessing the α equivalent of toxin and z-epitoxoid should yield the following formula : $(200 - \alpha - z)$ toxoids $+ \alpha$ toxin $+$ z-epitoxoids. To this the immunity-unit is added, and in this way is obtained the value $L_0 = (200 - \alpha - z)$ toxoid-antitoxin $+ \alpha$ toxin-antitoxin $+$ z-epitoxoid-antitoxin. The value of L_+ is obtained by adding to the neutral mixture so much of the original material that the z-epitoxoid-antitoxin is decomposed by the mixture of toxoid $+$ toxin. As is evident, this addition must represent $\alpha.\frac{z}{200-z}$ toxin-units. The epitoxoid toxin-units have just been found to be $\beta = D - 1$. It results that $\beta = \alpha.\frac{z}{200-z}$ and $z = \frac{200.\beta}{\alpha+\beta}$. If the amount of epitoxoid is estimated according to this formula, figures are obtained that stand in the simplest relation to that found for the number of immunity-units, 200—namely, ½, ¼, ⅛, or ⅓, or ⅙ thereof.

As the outcome of these experiences, Ehrlich has suggested the following alterations in the directions for testing the diphtheria-antitoxin, and these have been confirmed by a decree dated March 29, 1897 :

I. As a standard for the estimation of the antitoxin an antitoxin-powder of accurately determined strength, protected against the influence of oxygen and water, is employed. This is contained in carefully measured quantities in specially prepared vacuum-tubes. The apparatus at the time present in the laboratory are filled each with 2 grams of a dry antitoxin 1700 times the normal strength.

II. To secure the greatest possible degree of permanence the antitoxin should be dissolved in a mixture of equal parts of 10 per cent. solution of sodium chlorid and glycerin. A tube is to be opened every three months and a new solution prepared. Of the dry antitoxin at the time preserved in the laboratory the contents of a tube are dissolved in 200 cu. cm. of the mixture described, and thus a test antitoxin-solution 17 times the normal strength is prepared.

III. The present test-dose of toxin is determined with the aid of an immunity-unit, such as is contained, for instance, in 1 cu. cm. of a $\frac{1}{17}$ dilution of the test-antitoxin 17 times the normal strength. To this amount of antitoxin increasing amounts of toxin are added, and by means of

15

most careful experimental observations the limit is determined at which just that excess of toxin becomes manifest which causes death of the animal in the first four days. The amount of toxin thus obtained represents the immediate test-dose. By means of the same dose of serum, for the more exact characterization of the toxin, the determination of a second limit is made, for the purpose of learning the dose of toxin that is just neutralized by admixture with the amount of serum named.

IV. The determination of the strength of a diphtheria-antitoxin is made by means of the test-dose of toxin (see paragraph III) as follows: The test-dose of toxin in question —for instance, 0.355 cu. cm. of tested toxin at the time present in the laboratory—is mixed with 4 cu. cm. of anti-toxin corresponding to the test-figures given. As the test-dose of toxin is estimated for 1 cu. cm. of antitoxin of normal strength, or for 4 cu. cm. of antitoxin $\frac{1}{4}$ the normal strength, an antitoxin of x-strength will have to be diluted $\frac{1}{4}$ x, and in testing an antitoxin 100 times the normal strength, $\frac{1}{400}$.

V. The mixture obtained is injected unmodified sub-cutaneously into guinea-pigs weighing from 250 to 300 grams. If the animals die in the test-experiments made by two observers in the laboratory within the first four days, the antitoxin does not possess the required strength. Should death occur within five or six days, the antitoxin is close to the required strength, and in order to avoid the early withdrawal to be anticipated an improvement of from 5 to 10 per cent. is recommended the manufacturers. Indurations that occur in the animals experimented upon do not, however, constitute sufficient ground for objection. In the case of the dead animals an autopsy should be held, and careful attention directed to complications with previously existing disease (tuberculosis, pseudo-tuberculosis, and pneumonia) that may induce undue susceptibility on the part of the test-animals.

VI. Both liquid and solid toxins may be employed for test-purposes, if the limits defined in paragraph III can be accurately estimated, and the difference between them does not exceed fifteen simple lethal doses. If liquid toxins preserved in toluol are employed, this should be done only if as a result of preliminary investigation the permanency of the test-constants is demonstrated, if the test-

dose does not exceed 1 cu. cm. The examination with regard to the qualities of the test-toxins should be continued.

VII. The test-toxins, if liquid, are to be examined monthly with regard to their sterility by means of culture-methods.

VIII. The test-poison is to be redetermined at intervals of six weeks by means of the test-dose of serum, the test-dose and the net-valuation being estimated anew. If, on reexamination, any considerable deviation in the test-dose should be detected, the toxin must be considered to be in process of decomposition, and it should be replaced by fresh toxin.

IX. The manufacturers are to be informed that the test-toxin in small amounts decomposes readily, and that even brief exposure to light may induce considerable attenuation. It is therefore to be recommended that a new supply of toxin be obtained from the laboratory every three weeks.*

Diphtheria-antitoxin is prepared in Germany in four establishments—namely, the Höchst Works, the Factory of Schering, in Berlin, the Pasteur Institute in Stuttgart, and the Factory of Sthamer, Noack & Company in Hamburg.

The Höchst Works manufacture the following preparations :

ANTITOXIN OF 250 TIMES THE NORMAL STRENGTH.

Number 0, yellow, . . 0.8 cu. cm. contain 200 immunity-units.
Number 1, green, . . 2.4 " " 600 "
Number 2, white, . . 4 " " 1,000 "
Number 3, red, . . . 6 " " 1,500 "

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* Dr. Jos. McFarland and Dr. Chas. T. McClintock, to whom these regulations were submitted, kindly describe, as follows, the method of testing pursued by the largest manufacturers of diphtheria-antitoxin in the United States :

"In order to secure uniformity in the toxin the same culture of the diphtheria-bacillus is always employed. This is grown for seven days at 37° C. (98.6° F.) in an accurately prepared alkaline two per cent. peptone-bouillon. The same degree of alkalinity is always secured (phenolphthalein being used as the indicator in titration). After addition of 0.4 per cent. trikresol, this toxic bouillon is filtered through unglazed porcelain and stored in a dark, cold place. The amount of this toxin that will kill a guinea-pig weighing 250 grams on or before the sixth day is considered the minimum fatal dose. Should this toxin deteriorate ten per cent. from its original strength, it must be discarded.

"In testing antitoxin, a series of guinea-pigs, weighing from 240 to 270 grams, are injected with ten times the minimum fatal dose of toxin previously mixed with varying amounts of antitoxin. Those pigs are considered protected that do not die or lose more than 20 per cent. of their original weight in seven days."—A. A. E.

STRONG ANTITOXIN OF 500 TIMES THE NORMAL STRENGTH.

Number 0 D, yellow,	. 1 cu. cm. contains	500	immunity-units.
Number 2 D, white,	. 2 "	contain	1,000	"
Number 3 D, red,	. . 3 "	"	1,500	"
Number 4 D, violet,	. 4 "	"	2,000	"
Number 6 D, blue,	. . 6 "	"	3,000	"

ANTITOXIN OF 600 TIMES THE NORMAL STRENGTH.

Number 6 E, blue, . . 5 cu. cm. contain 3000 immunity-units.

The Pasteur Institute, of Stuttgart, manufactures only one preparation of 50,000 Roux immunity-units.

The Schering factory produces the following antitoxins :

A, 100 immunity-units per cu. cm.
B, 200 " " " (in vials of 5 and 10 cu. cm.).
Strong antitoxin of 500 times the normal strength, 500 immunity-units per cu. cm. (in vials of 2 and 4 cu. cm.).

The Hamburg establishment dispenses diphtheria-antitoxin prepared by Ruete and Enoch and containing 300 immunity-units per cu. cm.

In injecting the serum the rubber-ball syringe of Koch may be employed, and this is easily manipulated by one who is experienced. (Fig. 36, 2.) The antitoxin-syringe of Roux is to be recommended as especially practical, and it can be sterilized with the greatest ease. (Fig. 36, 1.) It is either boiled in a one per cent. solution of soda, or it is cleansed with a five per cent. solution of carbolic acid, after which it is rinsed with a 0.5 per cent. solution in order that the antitoxin may not be injured in consequence of too great concentration of the antiseptic.

The number of immunity-units to be injected in the individual case will depend entirely upon its severity, and upon the day of the disease that the patient comes under treatment. In a mild case, coming under observation on the first day, 600 immunity-units will suffice. Severe cases, seen as late as the third day, will require 1000 immunity-units. In still more severe cases, seen at a late stage, 1500 immunity-units and more are employed.

The results that have thus far been obtained in the treatment of diphtheria with the antitoxin have been exceedingly favorable. The mortality from diphtheria has been reduced about half under the influence of the antitoxin. In support of this statement we may quote the results of

the collective investigation with regard to the diphtheria-antitoxin for the period from April, 1895, to March, 1896, conducted by Dieudonné in the Imperial Health-office. There were treated with antitoxin in hospitals 9581 cases, among which death occurred in 1589—15.5 per cent. If the cases still under treatment at the conclusion of the report be excluded, the proportion of deaths to recoveries was 3 to 16. In the eleven years preceding the introduction of the antitoxin (from 1883 to 1893) the proportion of deaths to recoveries averaged 6 to 16. Nearly one-half of the cases included in this study were designated in the reports as severe. The mortality among children under two years old (1189) equaled 39.1 per cent., and that of those subjected to tracheotomy (2744) 32.3 per cent.*

In the cases submitted to treatment with antitoxin the disease pursued, in general, a milder and more favorable course. Existing manifestations of stenosis improved in a large number of cases, so that tracheotomy was avoided. Serious sequelæ with certainty attributable to the antitoxin have thus far not been observed. In rare cases unpleasant *complications* of a transient character occur—viz., infiltration at the point of injection, pains in the joints and extremities, urticaria, exanthemata, possibly also albuminuria. These symptoms are not at all dependent upon the antitoxin itself, but are to be attributed exclusively to the serum of the horse. Like amounts of antitoxic serum induce the same results in this respect, whether they contain a larger or a smaller number of immunity-units. For this reason it is desirable to obtain as highly concentrated an antitoxin as possible in order that as small amounts of serum as possible need be injected. Behring succeeded in preparing an antitoxin of 1200 times the normal strength, but, unfortunately, it was soon found that such highly concentrated solutions could not be preserved for as long a time as less concentrated solutions, and that in the course of time the immunizing activity diminished considerably.

Behring was, therefore, led to the preservation of serums of greater than 500-strength by converting them into a dry form by a special procedure. The dry powder, containing

* These results have been amply confirmed not only on the Continent of Europe, but also by observations in England and in America, with reference to both nonoperative and operative cases.—A. A. E.

sodium chlorid and albumin, can be preserved for an indefinite time without any addition, and it is readily soluble in water. One gram contains at least 5000 immunity-units, and in some preparations as much as 10,000 immunity-units. From $\frac{1}{16}$ to $\frac{1}{8}$ gram thus represents a simple curative dose; from $\frac{1}{8}$ to $\frac{1}{4}$ gram, twice as much, for severe cases; and from $\frac{1}{4}$ to $\frac{1}{2}$ gram, four times as much, for advanced cases. Behring considers it desirable that the preparation of these solutions should be undertaken by pharmacists, and he is hopeful that the dried serum may in due time be incorporated into the Pharmacopeia.

Prophylaxis.—It is important to know that after complete disappearance of the membrane virulent diphtheria-bacilli may persist in the mouth and pharynx of children until the fifth week, and sometimes even much longer. The children must, therefore, be isolated for from five to six weeks after recovery from the disease, and, above all, be kept from school during that time. Those who surround the patient should also be kept under medical observation. Careful disinfection of the sick-room is a matter of importance, as it is known that diphtheria-bacilli will persist for a long time and most tenaciously in a house in which there has been a case of diphtheria.

With regard to personal prophylaxis by means of *injections of antitoxin*, this has not yet been employed upon a large scale. An injection of 250 normal antitoxin-units confers protection lasting three or four weeks. This will suffice in most cases, but if the danger of infection continues for a longer time, a second prophylactic injection will become necessary. The main objection that has hitherto stood in the way of this form of diphtheria-prophylaxis was the fear of disagreeable complications due to the serum. Such fear would, however, be removed by the use of a highly concentrated antitoxin in powder-form, as 0.025 gram of the powder with a strength of 10,000 contain the requisite 250 immunity-units.

TETANUS.

The **exciting agent of tetanus** was recognized by Nicolaier, of Göttingen, in 1885, as a bristle-like rod with a terminal bulbous spore, but it was first isolated by Kitasato in 1889 from the foreign bacteria always associated with it

in infectious earth and in pus from a case of tetanus and grown in pure culture.

The *tetanus-bacillus* is a long, slender rod, from 0.3 to 0.5 μ thick and from 3 to 5 μ long, with rounded extremities and with feeble, but distinct, motility, which, however, ceases immediately in the presence of oxygen. It frequently grows in filaments, the individual elements of which are not always distinctly differentiable from one another. The temperature-optimum is from 36° C. (96.8° F.) to 38° C. (100.4° F.), although the bacillus thrives also at room-temperature. It does not develop

Fig. 53.—Bacillus tetani; × 1000 (Fränkel and Pfeiffer).

below a temperature of 14° C. (57.2° F.), while at 42° C. (107.6° F.) or 43° C. (109.4° F.) it presents distinct involution-forms, and at 60° C. (140° F.) it is destroyed quite rapidly. The tetanus-bacillus is an anaerobic organism, although it still grows in the presence of small amounts of oxygen. Protected from air and light the tetanus-spores retain their vitality and their virulence in cultures after the lapse of a year.

Spore-formation.—At a temperature of 37° C. (98.6° F.) after the lapse of thirty hours, and at room-temperature within a week, the tetanus-bacillus forms spherical spores always situated at one extremity. The bacillus swells at one extremity like a drumstick, and there results the characteristic head-bearing bacillus (pin-shaped or note-shaped). The organism is always

nonmotile. The diameter of the spores measures from 1 to 1.5 μ. They are extremely resistant to the action of heat. They are not injured by exposure for an hour to a temperature of 80° C. (176° F.), and they are not destroyed by live steam with a temperature of 100° C. (212° F.) before the lapse of five or eight minutes. The spores are also rather resistant to chemic disinfectants. They are not destroyed in five per cent. carbolic acid before the lapse of fifteen hours; and they die in one per cent. solution of mercuric chlorid within three hours.

Staining.—The tetanus-bacilli stain readily with the usual dyes, and they can be stained also by Gram's method. The spores can be demonstrated by the usual methods for staining spores.

Pure Cultures.—The tetanus-bacillus is encountered in nature (garden-soil, dust, animal excrement) and also in the pus from wounds infected with tetanus always in association with numerous other bacteria, some of which are anaerobic and others aerobic. On account of the difficulty of isolating the tetanus-bacillus from its associates, the attempt to obtain it in pure culture long failed. Kitasato overcame this difficulty by availing himself of the great resistance of the tetanus-spores. He inoculated tetanus-pus upon agar-tubes; after exposure for two days in the thermostat, in addition to the other bacteria there were found also numerous characteristic bristle-like rods with bulbous heads. The mixed culture was now heated in the water-bath at temperature of 80° C. (176° F.) for an hour, when all of the bacteria, including the tetanus-bacilli, were destroyed, and only the tetanus-spores survived. These could now be grown in pure culture without difficulty by the various culture-methods employed for other anaerobic bacteria. It ought, however, not occasion surprise if a pure culture should not succeed even by this method, if, as happens rarely, other resistant spores are present in the material submitted to examination. Under these circumstances anaerobic plates must be made, by means of which, further, it is possible to isolate the tetanus-bacilli in the discharge (pus).

Cultural Properties of the Tetanus-bacillus.—The tetanus-bacillus grows upon all the customary culture-media with exclusion of oxygen, and to which grape-sugar (two per cent.) may be advantageously added. All tetanus-cultures possess in common a peculiar, rather disagreeable odor of burned material.

On *gelatin-plates* at room-temperature there appear about on the fifth day, slowly growing, small colonies, with radiate processes that give to the whole a feathery or thistle-like appearance. Microscopic examination discloses in the center a dense, yellowish mass, with numerous delicate, ciliary fibers and pro-

cesses arranged radially at the brighter margin. The gelatin is slowly liquefied.

In *high gelatin stab-cultures* growth confined to the lower portion of the line of inoculation appears after about a week. From the grayish-white bacterial mass innumerable small, pointed processes extend in every direction into the gelatin, giving the culture a characteristic appearance suggestive of a large-branched fir-tree. In the second week liquefaction sets in and obliterates this appearance. The process advances slowly, until gradually the entire culture is converted into a turbid, grayish-white, viscid mass, of which the upper portion subsequently becomes clear, while the bacilli sink to the bottom as a cloudy gray mass.

On *agar-plates* delicate colonies develop, which with low powers of the microscope appear to consist of a network of fine threads such as is not usually seen in anaerobic cultures.

On *agar stab-cultures* the growth is similar to that upon gelatin, although not so pronounced. It takes place much more rapidly at the temperature of the body. Stab-cultures in a high layer of grape-sugar agar develop within from twenty-four to forty-eight hours close to the surface. They exhibit the characteristic odor, and are usually attended with abundant gas-formation.

In *glucose-bouillon* growth at 37° C. (98.6° F.) is very energetic. On account of the abundant formation of gas it is well not to close the bouillon-flasks too tightly. The bouillon is at first rendered quite turbid; after standing for weeks the bacterial masses settle to the bottom as a grayish-white layer, so that on careful suction of the overlying clear fluid a solution of toxin free from bacteria is obtained. This may be secured with greater certainty by filtering the bouillon-cultures through porcelain cylinders.

The tetanus-bacillus develops in *milk* without causing any change.

Upon *potatoes* a moist invisible deposit forms similar to that caused by typhoid-bacilli (Vaillard and Vincent).

Tetanus in Animals.—Tetanus occurs under natural conditions in horses, sheep, and neat cattle; it has not been observed in dogs, and especially not in fowl. The mouse is the animal best adapted for the experimental development of tetanus. Of an old bouillon-culture (freed of bacilli by filtration, or by careful decanting) 0.001 cu. cm., and frequently still much smaller amounts, suffice on subcutaneous injection to cause death in white mice (from twelve to fifteen grams in weight) within twenty-four hours. The guinea-pig is almost equally susceptible. The rabbit is much less susceptible: not less than from 0.5 to 1 cu. cm.

of the culture mentioned being required to cause tetanus in an animal weighing about 1000 grams, with death after the lapse of several days. The dog may be looked upon as being naturally immune to tetanus, being affected only on in-oculation with large doses of toxin : from 5 to 10 cu. cm. and more. Birds are highly immune, withstanding inoculations of from 10 to 20 cu. cm. of highly toxic tetanus-bouillon ; still larger amounts of toxin, naturally, give rise to fatal tetanus both in the hen and in the pigeon. Frogs also may suffer from tetanus if after inoculation they are kept permanently in a heated room ; but relatively large doses of toxin are necessary, and the disease develops only after two or three weeks.

Introduction of the tetanus-virus into a wound or injec-tion into the large cavities of the body or directly into the veins acts in the same way as subcutaneous inoculation. Experimental infection can not be induced by way of the digestive or the respiratory tract.

The *period of incubation* varies in accordance with the susceptibility of the animal and the virulence and amount of toxin inoculated : between one and two days, in the mouse ; and between eight and fourteen days, in the rabbit.

Tetanus in animals manifests itself in the form of extensor spasm, which gives rise to a clinical picture entirely analo-gous to that exhibited by tetanus in human beings. The spasm appears first in the neighborhood of the site of inocu-lation ; thus, when the root of the tail is inoculated, in the hind extremities and also in the tail ; when the nape of the neck is inoculated, in the fore extremities and the muscles of the neck. In the further development of the disease the entire muscular system is involved in the spasm, which quickly leads to death. After intraperitoneal and intra-venous inoculation the spasm is general from the outset. The longer the period of incubation, the slower generally is the course of the disease, and the shorter the period of incubation, the more unfavorable is the prognosis. After the disease has existed for some time recovery not rarely takes place. This ensues always but slowly, the rigid extremities relaxing in the course of weeks or months. On postmortem examination, only slight changes are found at the site of in-oculation when a pure culture has been employed in the inoculation—viz., slight infiltration or hemorrhage, and nothing else. If the inoculation has induced no extensive

injury, the site of inoculation may escape observation at autopsy. If a mixed culture was inoculated—*e. g.*, tetanus-infected earth, or pus or fragments of tissue from a wound in a case of tetanus—a focus of suppuration will be found at the site of inoculation. No changes are demonstrable in the remaining organs of animals dead of tetanus ; above all, tetanus-bacilli have never been found present, and, likewise, not in the blood. The bacilli have been demonstrated only at the site of inoculation, and in rare instances in the nearest adjacent lymph-glands ; but even in these places they are present only in small number, and they usually can be found only with great difficulty.

The Nature of Tetanus.—The postmortem findings just described are explained by the fact that tetanus is an exquisitely toxic-infectious disease. The convulsions and death are due to a poison generated by the tetanus-bacilli and rapidly absorbed from the site of inoculation. The evidence in favor of this view has been derived from various sources. In the first place, exactly the same clinical picture can be developed with the germ-free filtrate of a tetanus bouillon-culture, which thus contains only the dissolved chemic tetanus-toxin, as by means of the bacilli themselves. In the next place, Kitasato has inoculated mice with tetanus-bacilli at the root of the tail, and has excised and cauterized the site of inoculation throughout a large extent after one-half, one, and one and a half hours, and longer, so that there was no chance for the bacilli, which do not penetrate beyond the site of inoculation, to be retained within the body, and only the toxin absorbed could in any way be responsible for the further development of the disease. Only those animals operated on half an hour after inoculation remained well, while all of the others were attacked with tetanus. This means that as early as an hour after the inoculation so much toxin was absorbed that the bacilli were no longer necessary to the development of the attack of tetanus.

The subordinate position taken by the bacilli in their significance for the clinical picture, as compared with the toxins to which they give rise, led Vaillard and Vincent to the conclusion that the bacilli, through themselves and their spores, are not alone capable of causing the disease, and that in a pure state they are entirely inactive ; and only the layer of toxin which adheres to them externally gives rise to the

outbreak of tetanus. If this is removed, for instance by washing with large amounts of water or by exposure to a temperature of 65° C. (149° F.) or by growth of the cultures at a temperature of 20° C. (68° F.) or 22° C. (71.6° F.)—under which conditions the bacilli generate no poison for the first six days—such toxin-free bacilli will be incapable of inducing tetanus. These views have in general been confirmed. The introduction of toxin-free bacilli does not, as a rule, give rise to infection; but the spores freed of toxin reacquire their virulence, and 'tetanus will develop with certainty if with them there are introduced other bacteria not giving rise to tetanus, or if simultaneously traumatism is inflicted, or a splinter of wood is forced beneath the skin, or, finally, a chemic agent—for instance, lactic acid, trimethylamin, etc.—is injected at the same time. These experimental data are of the highest importance for a comprehension of tetanus developing spontaneously. From them the probable conclusion may be drawn that under natural conditions tetanus is not induced by the tetanus-bacilli alone, but that the presence of favoring bacteria—thus a mixed infection—is necessary, or that a severe traumatism, or the like, must attend the infection.

The question has been studied experimentally as to how the extensor spasm arises in cases of tetanus, and whether the tetanus-toxin has a central or a peripheral action. Tizzoni and Vaillard divided all the nerves of an extremity in an animal before inoculation with tetanus. This member remained relaxed, whereas the remainder of the muscular system became rigid. Buschke curarized a tetanized frog, and the tetanus ceased at once. The toxin, thus, can not act upon the muscles themselves or upon the peripheral nerves. After removal of the brain the tetanized frog remained rigid. A direct influence of the poison upon the motor centers through application to the cerebral cortex was without effect upon rabbits. It thus appears that the brain also is not the part attacked by the toxin. Gradual destruction of the spinal cord in tetanized animals causes disappearance of the rigidity in the parts corresponding to the respective sections of the cord. Accordingly, the activity of the toxins appears to be localized in the spinal cord, as is the case also with strychnin.

The Tetanus-toxin.—The germ-free filtrate of a tetanus-culture from two to four weeks old that has been

grown upon feebly alkaline glucose-bouillon contains the specific tetanus-toxin in an exceedingly active state. Often as little as 0.000005 cu. cm. of such a filtrate will suffice to cause death in a white mouse. The toxin is destroyed in solution by exposure to a temperature of 65° C. (149° F.) for five minutes, and it is gradually enfeebled in the thermostat. In a cold compartment (refrigerator) and protected from light it retains its toxicity unchanged for months. For the preservation of such a toxic filtrate it is advisable to add glycerin in equal amount, or carbolic acid in proportion of 0.5 per cent., and it may then be employed for months in the same way as the solution of a chemic poison of known strength.

Efforts have not been wanting to isolate the toxin in a pure state. Brieger obtained from cultures two basic substances—tetanin and tetanotoxin—both of which caused death in animals, preceded by manifestations like those of tetanus. Neither, however, can have any important bearing upon the occurrence of the disease, as relatively large amounts were required in order to induce disease in animals. The actual tetanus-toxin, however, must, in accordance with what is known regarding the toxicity of the bouillon-filtrate, be active in minimal amounts. Brieger and Fränkel have, according to their method, by precipitation with alcohol, obtained a toxalbumin that is much more active. Even this albuminous powder does not represent the tetanus-toxin chemically pure; the toxic substance is merely adherent to it.

The attempts to obtain the virus in its chemic purity have been pursued unremittingly, and it has been obtained in a quite concentrated form. Reference was made in the general section (p. 30) to the investigations of Brieger and Boer, from which it appears that the toxin of the tetanus-bacillus is not an albuminous substance at all. These investigators precipitated the tetanus-toxin by the original toxalbumin-method of Brieger and Fränkel with the aid of ammonium sulphate, and then by means of a weak solution of mercuric chlorid (from 10 to 20 cu. cm. of a 0.05 per cent. solution to 10 cu. cm. of tetanus-toxin fluid) precipitated the redissolved toxin. The carefully washed precipitate is well rinsed with water, and decomposed by successive treatment with ammonium carbonate, ammonium phosphate, and ammonium sulphate. Brieger

and Boer conclude the description of this rather complicated procedure with the following statement: If the readily soluble specific toxin has been cleansed as thoroughly as possible and deposited upon the filter, it will be completely absorbed by hardened filters, and there will be nothing to suggest its presence except a little salt, or the yellowish discoloration of the filter, which is often quite considerable, especially in the case of the tetanus-toxin. The activity of these substances will, therefore, not be expressed by the sum of decimals, but to a certain degree only as integers. For quantitative studies of the tetanus-toxin the bouillon-filtrate appears for the present, therefore, to be the best suited.

Tetanus-infection in Human Beings.—The portal of entry for tetanus-bacilli in human beings may be constituted by any lesion of the external integument. The tetanus-bacillus has been repeatedly demonstrated in the earth, especially in that which has been manured, and more particularly in the uppermost layers; in the dust of the crevices of floors; in manure from horses and cows. If a certain amount of any of these substances be introduced subcutaneously in guinea-pigs, there result, usually, malignant edema and, rarely, tetanus. This result depends upon the fact that the bacilli of malignant edema, frequently present in earth and in manure, overrun and suppress the tetanus-bacilli. In order to determine whether tetanus-bacilli are present in suspected material (earth, etc.), bouillon mixed cultures may be made, according to the suggestion of Sanfelice, and kept for a considerable length of time in the thermostat, and then filtered through porcelain cylinders. The results of injection of the filtrate will decide whether the material examined contains tetanus-bacilli or not.

Tetanus in human beings is a toxic disease, just as is tetanus in animals. In human beings also the bacilli never enter the blood or the viscera, but remain confined to the site of original infection. On the other hand, the presence of the tetanus-poison in the blood in cases of tetanus has been demonstrated by successful intoxication of mice with the blood-serum from such cases. Likewise, a substance has been obtained from the liver, the spleen, and the spinal cord of a patient dead of tetanus by precipitation with alcohol, and which, dissolved in water, is capable of destroying

small animals immediately, after the development of tetanic manifestations. Tetanus-toxin has been found on several occasions in the urine of tetanus-patients, but never in the sweat or in the saliva.

The *portal of infection* is ascertainable in the majority of cases. Often it is a wound of considerable extent, which can not escape observation—as in puerperal tetanus the wounded surface of the uterus, in tetanus of the new-born usually the umbilical wound, in cephalic tetanus (hydrophobic tetanus) an injury of the head. The *source of infection*, also, may often be discovered without difficulty. Not rarely a relation between the patient and horses can be elicited, as these animals, according to Verneuil, occupy a central position in the etiology of tetanus and they may convey the tetanus-bacillus to the ground with their manure ; or the patient may have introduced beneath the skin a splinter to which the bacilli were adherent ; or in working about a garden he may have contaminated an already existing wound with earth ; and the like.

It should here be mentioned that tetanus-virus retains its virulence for an exceedingly long time. An observation has been recorded in which a splinter of wood that had already given rise to infection again caused tetanus after the lapse of eleven years. Bandages and dressings from tetanus-wounds likewise preserve their infectivity for a long period, and tetanus-cadavers also retain for a considerable time their capability of causing tetanus.

Direct contagion has also been observed, tetanus having been transmitted in a hospital-ward from one patient to his neighbor, or several patients occupying successively the same bed each developing tetanus. In some cases, however, the portal and the mode of infection are ascertainable with difficulty. Cases have been reported in which operation-wounds, after antiseptic treatment, healed by primary union and without complication, and later tetanus developed (*cicatrix-tetanus*). Further, there occur cases of so-called *idiopathic* or *rheumatic tetanus* in which apparently no lesion exists anywhere, and also at autopsy no suppuration can be found. The information gained from experiments on animals furnishes a complete explanation for these cases. The portal of entrance may be the smallest abrasion or fissure of the skin, which will escape observation, and which perhaps has already healed some time before the

convulsions occur. If the infection is single—that is, if pyogenic cocci do not gain entrance into the wound at the same time as the tetanus-bacilli—there will be no focus of suppuration at autopsy to indicate the portal of infection. In an animal experiment recorded by Vaillard spores healed without reaction in the wound induced artificially, and only some time later, when the affected member was irritated, did tetanus occur. The cases of tetanus that occur in connection with wounds treated antiseptically are susceptible of a similar interpretation. The tetanus-bacillus may long remain latent ; the antiseptic employed not being powerful enough to destroy the tetanus-spores with which the wound is infected, and these are included in the healing wound, to proliferate and to induce the disease later in consequence of some predisposing influence. The period of incubation of tetanus in human beings varies from one to twenty-two days ; in the case of an injury induced in the laboratory with tetanus-toxin it was four days. The course of the disease is the more violent, and the prognosis the more unfavorable, the shorter the period of time that has intervened between the infliction of the injury (infection) and the outbreak of the disease. Recovery took place in only slightly more than three per cent. of cases with an incubation-period of from one to ten days ; in twenty-five per cent. of those with an incubation-period of from ten to twenty-two days ; and in as high as fifty per cent. of those with longer periods of incubation. The susceptibility of human beings to the toxin of tetanus must, therefore, be looked upon as pronounced.

Bacteriologic Diagnosis.—If it is desired to demonstrate the bacilli in the pus, or in the granulation-tissue of the wound in a case of tetanus, or if a specimen of earth or a splinter of wood is to be examined for the presence of tetanus-bacilli in order to discover the source of infection, the suspected material may be directly introduced into a pocket of skin at the root of the tail of a mouse. If the animal dies of tetanus in the course of a few days, the pus at the site of inoculation is treated in the manner described for the purpose of obtaining a pure culture (p. 232). The material to be examined may also be introduced into bouillon, through which a current of hydrogen is passed, and which is then placed in the thermostat for from three to five days. The mixed culture that has

developed in the bouillon is exposed over a water-bath for an hour at a temperature of 80° C. (176° F.), and from it a new anaerobic bouillon-culture is made at once. If after several days' development this culture still contains foreign bacteria, anaerobic plates are prepared.

Immunity and Cure of Tetanus in Animals.—The laws of immunity and immunization have been more thoroughly studied in tetanus than in any other disease. The reason for this resides, in the first place, in the exceedingly sharp and certain reaction of animals to tetanus-infection, and, in the second place, in the possibility of testing quantitatively the degree of immunity by means of treatment with the toxic bouillon-filtrate.

Immunity to a toxic-infectious disease is indicative of proof to intoxication: an animal is protected from infection with tetanus if the tetanus-toxin is incapable of manifesting its toxic activity within the body of the animal. Animals slightly susceptible (dog, hen) can be further immunized simply by injection of gradually increasing amounts of tetanus-toxin. Their serum, in the hen, likewise the egg-yolk, also acquires thereby immunizing properties. From the serum of dogs subjected to such preliminary treatment Tizzoni and Cattani have precipitated their so-called antitoxin by means of alcohol.

Behring's method of immunization by means of tetanus bouillon-cultures attenuated with iodin trichlorid is applicable to the immunization of susceptible animals (mice, rabbits, horses, sheep). The animals are treated first with a bouillon-culture containing 0.25 per cent. iodin trichlorid, and, finally, with undiluted bouillon, of which the animals—smaller at intervals of from three to five days, and larger at intervals of eight days—then receive steadily increasing doses, until, finally, they bear quite large amounts of pure toxin.

A fluid suitable for immunization may be secured also by warming the cultures (Vaillard heats the filtrate at first to 60° C.—140° F., later to 55° C.—131° F., and, finally, to 50° C.—122° F.); further, by addition of iodin-water or of lactic acid to the cultures, by cultivation of the bacilli in thymus-bouillon, etc.

The serum of immunized animals transmits immunity to animals not previously treated; the higher the original degree of immunity, the more active is the blood-serum.

16

This acts by rendering the animal toxin-proof ; it contains an antitoxin that enters into innocuous combination with the tetanus-toxin.

Also after infection, and even after the development of symptoms of tetanus, the serum of the animal may afford protection ; it thus exerts a curative action. To effect cure in animals, however, a much larger amount of serum, or a much more highly active serum, is required than for pro-phylactic immunization ; and the amount of serum required becomes the greater the longer the period of time that has intervened between the intoxication and the employment of the serum. According to observations of Dönitz, little more serum is required for the protection of the animal four minutes after the intoxication than in the test-tube for the neutralization of the amount of toxin employed (in the test-tube a serum-dilution of $1 : 2000$, in the body after four minutes a dilution of $1 : 1200$) ; after the lapse of eight minutes six times the amount of serum will be required ($1 : 200$) ; after the lapse of fifteen minutes twelve times the amount ($1 : 100$) ; and after an hour twenty-four times the amount of serum.

Serum-therapy in Human Beings.—It is obvious that the outlook for serum-therapy in the case of tetanus in human beings is from the outset less promising than it is in that of diphtheria. As it can not be determined from inspec-tion that a wound is infected with tetanus-bacilli, the disease comes under medical observation only after the toxin has already invaded the central nervous system, and has induced more or less extensive and in part perhaps irreparable injury. The cases of tetanus, therefore, thus far treated with serum have not, on the whole, yielded good results. The expla-nation for this may be found in the weakness of the serum ; in view of the lateness of application of the treatment the serum should be of especial strength. Behring has, there-fore, and also in conjunction with Knorr, sought mainly to increase the activity of the serum. To obtain tetanus-toxin he employs horses, which are immunized in the same way as to diphtheria-toxin (p. 218). The tetanus normal toxin, Tet N T[1], with which Behring starts out, is so strong that one gram contains 1,000,000,000 lethal minimal doses for one gram by weight of guinea-pig (+ 1,000,000,000 M), 150,000,000 lethal doses for one gram by weight of mice (+ 150,000,000 Ms), 1,000,000 lethal minimal doses for

one gram by weight of rabbit ($+$ 1,000,000 K). That serum serves provisionally as tetanus normal antitoxin, Tet AN[1], whose curative activity Behring and Knorr demonstrated at a meeting of the Physiologic Society in Berlin, on January 13, 1893. This serum, injected on several days successively in doses of 0.04 cu. cm., cured mice that had been infected from twenty-four to twenty-eight hours previously with the lethal minimum dose, and that already presented distinct symptoms of tetanus. One cubic centimeter of this serum contains one normal anti-toxin-unit. For the future it is, however, probable that that serum will be designated as normal antitoxin of which 1 cu. cm. will render innocuous 1 cu. cm. of tetanus normal toxin.

The Höchst Works have placed two preparations on the market. The first occurs in the form of a dry powder packed in 5-gram vials. This antitoxin has 100 times the strength of the normal (Tet AN[100]); each vial thus contains 500 tetanus normal antitoxin-units. The dried serum keeps well, without addition of an antiseptic, in well-closed bottles, and remains of uniform strength. The 500 anti-toxin-units constitute a curative dose for human beings and horses. They are dissolved in 45 cu. cm. of sterile, luke-warm water, not above 40° C. (104° F.), and are injected, in the case of horses, directly into a vein. By introduction into the blood-stream a much more energetic action is obtained, and this, in addition, appears twenty-four hours earlier than after subcutaneous inoculation. For this reason, Behring and Knorr recommend also in human beings, in urgent cases, that the injection be made into a vein. Recovery may be expected after subcutaneous injection in acute cases only when the serum is employed before the lapse of the first thirty-six hours after the symptoms of tetanus have set in. The time lost is less readily compensated for by increase of the dose in the case of tetanus than in that of diphtheria.

The second preparation dispensed by the Höchst Works is in solution in vials of 5 cu. cm.; 1 cu. cm. contains 5 normal antitoxin-units—the equivalent of five times the normal antitoxin (Tet AN[5]). As in the case of the diph-theria-antitoxin, 0.5 per cent. of carbolic acid is added to prevent decomposition. This dissolved antitoxin may be employed prophylactically in human beings and animals

after such injuries as experience has shown may be followed by tetanus. The size of the dose (from 0.5 to 5 cu. cm.) is governed by the period of time that has elapsed since the infliction of the injury. If it be desired to make an injection of antitoxin in advance of an operation that is frequently followed by tetanus in animals—as, for instance, preceding castration—0.2 cu. cm. will suffice.

Recent observations have afforded noteworthy support for the justification and the utility of serum-therapy with relation to tetanus. Dönitz established the fact in the Institute for Serum-therapy that the tetanus-toxin, as soon as it enters the blood, is taken up by the tissues of the body, and that, in cases of severe intoxication, the simple lethal dose is bound within from four to eight minutes. The serum, however, separates the toxin from its combination with the tissues, and neutralizes it. ·The dissolution of the toxin-combination is effected with the greater difficulty the severer the intoxication and the longer the period of time that has elapsed before the serum is employed. Of especial significance is the discovery by Goldscheider and Flatau that certain degenerative processes affecting nerve-cells that are caused by tetanus-intoxication recede under the influence of the serum. We have thus anatomic evidence for the curative activity of the serum.*

As to the results of tetanus-therapy, a definite opinion can not yet be given, as the new preparations have been generally available for too short a time. As bearing upon the value of *prophylactic inoculations* in veterinary medicine, we have the statistics of Nocard, covering the period from August 1, 1895, to June 1, 1897. Altogether, 2707 animals were vaccinated. Of this number, 2300 received an injection of serum immediately after the operation (castration, amputation of the tail, etc.). Not one of these animals developed tetanus. The remainder were inoculated from one to four days after the operation or after the infliction of a traumatism. Only one horse exhibited symptoms of tetanus, but these rapidly subsided. Nocard considers these results as quite remarkable, as the animals were derived from stables in which tetanus had prevailed shortly before. On the other hand, the sixty-three veterinary surgeons who

* The results appear, further, to be more promising when the serum is introduced beneath the dura mater than after any other method of introduction. —A. A. E.

had furnished Nocard the material in question, observed 259 cases of tetanus in the same time in animals that had not been inoculated.

BOTULISM.

In spite of numerous investigations with regard to the exciting agent of *meat-poisoning* this whole question was involved in considerable doubt until recently, when, through the discovery by Van Ermengem, in an epidemic at Elle-zelles (Belgium), of a specific anaerobic microbe, the *bacillus botulinus*, renewed attention was directed to this important subject.

Under the designation of meat-poisoning are included two entirely distinct symptom-complexes, which should properly be rigidly differentiated from each other. The one variety, which is most appropriately designated the *gastro-intestinal*, simulates the clinical picture of cholera nostras—a simple or hemorrhagic gastro-enteritis. Among associated manifestations may be mentioned fever, albumin-uria, and cutaneous exanthemata of most varied kind and in-tensity. These gastro-intestinal lesions arise after the inges-tion of decomposed meat or of meat derived from diseased animals, the diseases especially concerned being pyemia, septicemia, and puerperal fever. In most cases of this kind the exciting agents are bacteria of the colon-group, and in other cases, occurring less commonly, bacteria of the proteus-group.

The second variety is identical with so-called *sausage-poisoning*. It is characterized especially by nervous symp-toms of central origin, secretory and motor disturbances, cessation of salivary secretion, dryness and redness of the mucous membrane of the mouth and pharynx, dysphagia, hoarseness, barking cough, paralysis of accommodation, mydriasis, ptosis, diplopia, etc. For this form of the dis-ease the designation meat-poisoning should no longer be employed, and it has been given the name of *botulism*.

Botulism may arise after the ingestion of special kinds of sausage—as, for instance, blood-sausage or liver-sausage —which are prepared particularly in certain parts of Wür-temberg and Baden. It is further caused by decomposed salt fish, by smoked meat, ham, preserved meat, venison, old roasts, and the like. These are articles of food that

are intended for consumption after a considerable length of time, and that, according to Van Ermengem, are, by reason of their mode of preparation, exposed to the danger of undergoing anaerobic fermentative processes. Van Ermengem believes the actual cause to be the anaerobic bacillus botulinus, which he isolated in the epidemic at Ellezelles from a piece of ham that gave rise to typical cases of intoxication. As opportunity has not been afforded since Van Ermengem made his communication for investigating cases of botulism, the following description will closely follow his statements.
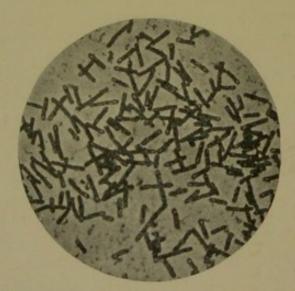
Fig. 54.—Bacillus botulinus; eight-day culture in glucose-gelatin; × 1000 (Van Ermengem).

Morphology of the Bacillus Botulinus (Fig. 54).—The bacillus is a large, slightly motile rod, from 4 to 6 μ long and from 0.9 to 1.2 μ thick, with slightly rounded extremities, and possessing from 4 to 8 flagella. The formation of filaments is but rarely observed ; more frequently involution-forms are present, the bacilli becoming smaller, presenting deficiencies, and being at times arranged in filaments.. Both in cultures and in the body the bacillus botulinus generates spores of oval form, generally polar, rarely central, and exceeding the thickness of the bacillus. The bacillus is readily stained, and also by Gram's method, but the exposure to alcohol must not be too protracted.

Cultural Peculiarities.—The temperature-optimum is between 20° C. (68° F.) and 30° C. (86° F.). Below a temperature of 16° C. (60.8° F.) the bacillus botulinus grows but slowly, while above 35° C. (95° F.) it no longer generates spores, does not itself thrive so well, and exhibits involution-

forms. The organism is strictly anaerobic, so that, in order to cultivate it, anaerobic methods of investigation must be pursued. The culture-media must always be distinctly alkaline, and the growth is favored by the addition of two per cent. of grape-sugar.

Gelatin-plates exhibit between the fourth and the sixth day round, transparent, brownish-yellow colonies, composed of thick, glistening granules, in constant movement. The colonies are surrounded by a slight zone of liquefaction. Later, the margin becomes irregularly radiate, and, finally, processes of the most varied form extend out from it.

High gelatin stab-cultures exhibit no peculiarity. Round, whitish masses develop along the line of puncture, at times sending processes out into the adjacent gelatin. The gelatin is not liquefied, but active gas-formation takes place.

In *agar stab-culture* the growth, apart from liquefaction, is quite similar.

Glucose-bouillon is rendered deeply turbid.

In *milk* slight development takes place, without alteration in the nutrient medium.

On *potato* no growth takes place.

All of the cultures emit an odor of butyric acid. In addition the bacillus generates in nutrient media containing glucose still other fatty acids, butyl alcohol, hydrogen, carbon dioxid, and methane.

Tenacity of the Bacillus Botulinus.—The cultures retain their capability of development for more than a year if they are kept at a temperature below 30° C. (86° F.). At temperatures above 35° C. (95° F.) they die in the course of a few weeks. The spores possess relatively little resistance. They are destroyed at a temperature approximating 85° C. (185° F.) within a quarter of an hour, and at a temperature of 80° C. (176° F.) with certainty within an hour. Five per cent. carbolic acid causes their destruction in less than twenty-four hours. Dry spores exposed to diffuse daylight exhibit capability of germination after three months. The growing forms in emulsion with distilled water die under these conditions within three or four weeks.

Pathogenic Properties.—The bacillus botulinus proves pathogenic when fed to guinea-pigs, mice, and monkeys. One or two drops of a liquefied gelatin-culture placed upon a bit of bread or in milk suffice to cause the death of the animal within one or two days, after paretic manifestations,

mydriasis, aphonia, dysphagia, and blepharoptosis. Cats withstand the action of considerable amounts of the culture without injury when introduced by the stomach ; but subcutaneous injection, on the other hand, leads to death amid such typical manifestations that the cat may be considered as the physiologic reagent for the bacillus botulinus. After considerable doses (of from five to ten cubic centimeters) cats die within one or two days, but after smaller doses (of from one to two cubic centimeters), only after from eight to twelve days. After an incubation-period generally of thirty-six hours the animal becomes depressed, no longer moves about, and refuses nourishment. On the third day it exhibits a peculiar physiognomy. The facial expression is dull, and the blinking and licking movements that healthy cats never fail to exhibit are abolished ; the eyes are almost completely immobile, and the pupils widely dilated. The dilatation of the pupils becomes, in the course of a few days, quite enormous. The tongue hangs out of the mouth and can scarcely be drawn in again. Besides, aphonia sets in and, further, dysphagia, which finally may increase to total aphagia. Urine and feces are retained. Death occurs usually in consequence of paralysis of respiration and circulation. Small doses of the bacilli give rise to a form of marasmus, as a result of which the cats die after the lapse of several weeks amid paralytic manifestations and with degeneration of the parenchymatous organs. Rabbits, guinea-pigs, and mice die in consequence of subcutaneous injection of minimal amounts, amid paretic manifestations, salivation, dysphagia, etc. After injections of one or two cubic centimeters of the culture pigeons exhibit at first paresis of the wings, and then general paralysis. Intravenous injection leads to the same results as subcutaneous inoculation, while intraperitoneal injection is followed by less marked consequences.

The pathologic-anatomic alterations found at autopsy consist in more or less marked hyperemia of most of the viscera ; in an acute, sometimes interstitial, sometimes parenchymatous, hepatitis, with fatty degeneration ; in desquamative parenchymatous nephritis ; in fatty degeneration of the muscular fibers of the heart and of the ocular muscles. Of especial interest are the degenerative changes in the central nervous system, which are especially marked in the spinal cord, and less so in the medulla oblongata.

These are entirely wanting in the central nerves, and almost entirely in the brain. In the spinal cord the gray substance is involved almost exclusively, and particularly the anterior horns; in the medulla oblongata the nuclei of the hypoglossus, the dorsal nucleus of the vagus, the middle smallcell nucleus of the oculomotor nerve—in short, the nuclei of the cerebral nerves affected.

Physiology of Botulism.—Microscopic examination of the organs and of the blood of animals dead of botulism fails to disclose the presence of the bacilli anywhere; even at the site of injection they are to be found in only small numbers after the lapse of three or four hours, and then distinctly in process of degeneration. After intravenous injection, cultures prepared from the fresh organs exhibit, as early as twelve hours, only a limited number of colonies. If, however, these organs are placed in the thermostat at a temperature of 30° C. (86° F.), numerous microorganisms can be cultivated from them after the lapse of from twelve to twenty-four hours. Botulism, therefore, represents essentially an intoxication and not a true infection. If the cultures are freed by filtration of the bodies of the bacilli, and if animals are inoculated with the toxic solution thus obtained, precisely the same morbid manifestations are induced as follow inoculation of the bacilli themselves. Van Ermengem attempted, in the same way as Vaillard and Rouget in the case of tetanus, to free the spores of the layer of toxin surrounding them by washing them in water, and he found that the spores thus treated were much less active. He was unable, however, to free the spores entirely of the toxin, and he, therefore, came to the conclusion that the protoplasm of the microbes must retain a certain amount of toxin.

Alkalies, and even sodium bicarbonate, destroy the toxin of botulism. If spores are permitted to macerate for a day in an alkaline solution that is so feeble as not to destroy their germinating capability—as, for instance, a saturated solution of sodium bicarbonate—and are then exposed for two hours to a temperature of 50° C. (122° F.), and animals are inoculated therewith, the latter continue to live. The spores, whether introduced under the skin or into the stomach, are incapable of multiplying and generating toxin; outside the living body, however, the spores retain the capability of generating their toxin in ordinary strength.

Cultures made with them also prove equally toxic with those from spores not exposed to the action of an alkaline solution and of heat. The influence to which is due the fact that the bacillus botulinus is incapable both of multiplying and of generating toxic metabolic products within the animal body is not yet thoroughly understood. Perhaps it is due to a high degree of saprophytism, which does not permit the microbe to adapt itself to the animal body ; and, perhaps, further, to the fact that the bodily temperature, which is above 35° C. (95° F.), is not favorable to its development. It has been shown experimentally that, at a temperature of 37° C. (98.6° F.) and above, involution-forms occur, and no toxin is generated. The bacillus botulinus thus occupies a position of its own. It is pathogenic for human beings only by reason of its toxin, which it forms outside the body upon dead substances (articles of food). Van Ermengem proposes that it be designated as toxicogenic.

The toxin of botulism is to be placed upon the same plane as that of diphtheria and that of tetanus. It can be obtained by precipitation with alcohol, tannic acid, and neutral salts. It was further separated by Brieger and Kempner by treating the toxin-containing filtrate of the culture according to the method proposed by Brieger and Boer for diphtheria and tetanus.

Occurrence of the Bacillus Botulinus.—The organism has hitherto never been observed apart from its discovery by Van Ermengem in ham and in the body of a patient dead of botulism.

Mixed Infection.—The associated bacteria that were found together with the bacillus botulinus appear neither to favor nor to inhibit the generation of toxin.

Bacteriologic Diagnosis.—Articles of food that have given rise to botulism are examined by means of culture (plate-procedure) for the presence of anaerobic bacteria. In the case of Van Ermengem the spores of the bacillus botulinus could be demonstrated in the ham microscopically. These were present principally in the red portion of the ham, and in smaller number in the fat. They were irregularly distributed, and were entirely absent in some places. In addition, it is well to inoculate animals with an aqueous maceration (four parts of chopped ham and five parts of water). In the bacteriologic examination of the

bodies of individuals dead of botulism or of animals that have succumbed to injection of the maceration, it is to be borne in mind that the viscera and the blood contain the bacilli only in small numbers. It is, therefore, necessary to examine large quantities, or to place entire organs in the thermostat at a temperature of from 20° C. (68° F.) to 30° C. (86° F.), in order to bring about fertilization. Van Ermengem has succeeded in isolating the bacillus from the spleen and from the gastric and intestinal contents from the body of a patient dead of botulism.

Immunity and Specific Therapy.—Immunization of animals to botulism was effected by Kempner with the filtrate of a bouillon-culture in the same way as in the case of diphtheria and of tetanus. The serum of immunized animals is highly antitoxic—a further evidence of the fact that the toxin of botulism is closely related to that of diphtheria and that of tetanus. When Kempner injected his serum in doses of from one to five cubic centimeters within from three to twenty-four hours after inoculation of guinea-pigs, the animals survived. As investigations of Kempner and Pollack show, changes in the central nervous system have already taken place after the intoxication has existed for twenty-four hours. These changes in the nerve-cells, as the anatomic evidence seems to show, are neutralized by the serum.

TUBERCULOSIS.

The exciting agent of all tuberculous processes is the *tubercle-bacillus* discovered and cultivated by Robert Koch in the year 1882.

The first evidence of the infectious nature of tuberculosis was furnished in 1865, by Villemin, who rendered healthy animals tuberculous by inoculation of tuberculous material. These observations were confirmed by Cohnheim, and they were supplemented by inoculation into the anterior chamber of the eye. Cohnheim, upon the basis of his experiments, formulated the doctrine of the specific etiology of tuberculosis, and this opinion was crystallized into absolute certainty by the classic bacteriologic investigations of Koch ("Arbeiten aus dem Reichsgesundheitsamt," II).

Morphology and Staining of Tubercle-bacilli.—The tubercle-bacilli are delicate, slender rods, from 0.2 to 0.4 μ

thick, and, on the average, from 3 to 4 μ long. They are slightly curved, nonmotile, and as a rule lie singly, but in cultures at times in small chains of from 4 to 6. In rare cases they exhibit bulbous terminal enlargement and bifurcation, in consequence of which a certain relationship with the actinomyces-group (p. 25) is suggested. Tubercle-bacilli are distinguished from all other bacteria by the fact that they stain with extreme difficulty, but, having once taken up the stain, they retain it with great tenacity. The simple solutions of aniline dyes ordinarily employed for staining other bacteria do not suffice for tubercle-bacilli unless the exposure be very prolonged. For this reason tubercle-bacilli are stained with aniline-water staining solutions (Koch-Ehrlich stain) or with the more commonly employed carbolfuchsin (Ziehl stain).

Upon cover-slip preparations, made in the usual way from pure cultures and fixed, freshly prepared aniline-water fuchsin
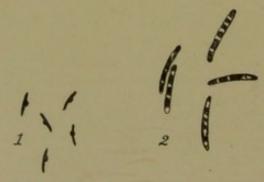
Fig. 55.—Tubercle-bacilli: 1, Forms suggesting sporulation; 2, forms described as beaded (the open spaces in the fragmented rods are sometimes mistaken for spores).

(or aniline-water gentian-violet or aniline-water methyl-violet), or carbolfuchsin is dropped; then heat is applied by means of a small gas-flame or spirit-flame until vapor of steam arises, and after a minute the excess of stain is washed off with water. The tubercle-bacilli are now stained, and if the specimen is treated for several seconds with dilute acid—for instance, fifteen or twenty per cent. nitric acid—and alcohol—from 60 to 70 per cent.—they will not yield up their color. Stained in this way the tubercle-bacilli frequently exhibit bright deficiencies that have not taken the stain. These were at first looked upon as spores, and, later, as degenerative manifestations, but both views are incorrect.

Originally, the tubercle-bacilli were stained in cold solutions, in which they were permitted to remain for several hours; but by application of heat the duration of the exposure can be reduced to a few minutes.

The cause of this characteristic behavior in staining was con-

sidered by Ehrlich to depend upon the fact that the tubercle-bacilli possess an especially resistant *cell-membrane*, which permits the stain to enter the body of the cell only with the aid of mordants (aniline water, carbolic acid, etc.). This membrane, later in the process of decolorization, prevents the entrance of acids into the interior of the bacillus, so that the microorganisms do not give up their stain. The tubercle-bacillus can be stained by Gram's method.

According to recent investigations by Robert Koch, the tubercle-bacilli contain two unsaturated fatty acids, one of which is soluble in dilute alcohol, and is saponified by sodium hydroxid; whereas the other is not saponifiable, and is soluble only in boiling absolute alcohol and ether. Both fatty acids take the specific stain of the tubercle-bacilli; but as one of them is soluble in alcohol, only the other remains after decolorization, and this fixes the stain and must be looked upon as the medium of the color-reaction. By means of hot sodium hydroxid the fatty acids may be slowly driven out of the bodies of the bacilli and their escape in the form of tingible drops uniting into larger drops can be observed under the microscope. According to Koch, these fatty acids form a coherent layer within the bodies of the bacilli, to which they afford protection against external influences.

Culture of Tubercle-bacilli.—Pure culture of tubercle-bacilli is difficult, and mainly because the microorganisms develop very slowly and require a temperature of 37° C. (98.6° F.) for their growth. Their temperature-minimum is 29° C. (84.2° F.), their temperature-maximum 41° C. (105.8° F.), and their temperature-optimum 37° C. (98.6° F.) or 38° C. (100.4° F.). Tubercle-bacilli thrive well upon blood-serum, upon from four to six per cent. glycerin-agar, and upon glycerin-bouillon. The preparation of glycerin-agar plates for the purpose of isolating tubercle-bacilli from the mixture of bacteria in tuberculous sputum is scarcely possible, as the tubercle-bacilli develop so slowly that they are overgrown and suppressed by the colonies of the other bacteria. Their cultivation will, therefore, be successful only if the material examined is uncontaminated. To this end the following plan of procedure is pursued: Several guinea-pigs—animals that are extremely susceptible to tuberculosis—are inoculated with material containing tubercle-bacilli. After the lapse of about four weeks the first of the inoculated animals will die, the autopsy revealing marked tuberculosis of the abdominal viscera. One of the remaining guinea-pigs is now killed, its skin cleansed most carefully by means of hot water and 1 : 1000 solution of mercuric chlorid, the skin turned back with a knife sterilized in the flame, the peritoneum opened with another instrument similarly treated,

and the spleen is drawn forward with forceps sterilized in the flame, as this organ appears to be involved in greatest degree in this mode of infection. A bit of the spleen containing a tuberculous nodule is excised with sterilized scissors, the nodule is compressed between two aseptic scalpels or glass slides, in order to set the tubercle-bacilli free, and the material thus obtained is transferred by means of a strong platinum wire to the surface of blood-serum tubes. The whole procedure must be carried out with the utmost celerity and with the most scrupulous cleanliness, for should a foreign microorganism gain entrance into the serumtube, it will soon overgrow the tubercle-bacilli. For the sake of greater security, several tubes are always treated in the manner described. As the tubes must be kept for a long time in the thermostat at a temperature of 37.5° C. (99.5° F.), they are closed with rubber caps that have been sterilized in mercuricchlorid solution. After the lapse of fourteen days, if the culture be successful, the first signs of growth are observable in the serum-tubes. In the neighborhood of the expressed material there form gray, dry, small scales, which, with low powers of the microscope, appear to be made up of delicate curved lines. Development then progresses slowly, and after the lapse of from four to six weeks it is possible to continue inoculations from this culture. For further culture blood-serum tubes likewise are used. Also in this second generation growth is first observed distinctly only after the lapse of two weeks.

Subsequent generations, usually after the fifth or sixth, grow more vigorously and more rapidly; and, particularly when a special thermostat is employed and is saturated with steam, so that the rubber caps can be dispensed with, the entire surface of the serum, after from seven to fourteen days, is found strewn with the characteristic dry scales. From the fifth serum-generation transference to glycerin nutrient media may be readily effected.

Upon *glycerin-agar* development is much more abundant than upon blood-serum. The bacilli form upon this a grayish, dry deposit of coarse particles, with the same wavy, slightly raised outline. This coating extends downward, and envelops the water of condensation present, without, however, rendering it turbid; and if the culture is kept long enough in the thermostat, the deposit grows even upon the free surface of the test-tube, where no nutrient medium is present, and for a considerable distance upward.

As a fluid culture-medium *veal-bouillon* with six per cent. glycerin is preferably selected, and this is poured into Erlenmeyer flasks. In inoculating bouillon the dry scales must be introduced into the nutrient fluid in such a way that they float upon its surface. The tubercle-bacilli have a strong avidity for oxygen, and they develop luxuriantly only where air has suffi-

cient access. Upon veal glycerin-bouillon the tubercle-bacilli grow in the form of a superficial membrane, which exhibits the same characters as the deposit upon glycerin-agar. In this also, after the lapse of weeks—under favorable conditions, after ten days—they reach the walls of the vessel, and likewise grow for a certain distance upward upon the glass. The underlying bouillon remains perfectly clear—a feature that is characteristic of the growth of tubercle-bacilli.

Upon *potatoes* whose lower extremity projects into a solution of glycerin (5 per cent.) and sodium chlorid (0.5 per cent.), the tubercle-bacilli grow exceedingly well. Upon the surface of the potato they form a dense deposit. The glycerin-solution remains clear, and it is covered by the well-known coating. In the preparation of such cultures it is best to employ the cylinders of potato recommended by Roux.

In the year 1892 Kitasato described a procedure suggested by Koch for obtaining tubercle-bacilli directly from the sputum of tuberculous patients. The patient should rinse his mouth carefully with an antiseptic gargle, and then expectorate into a sterilized double dish. One of the masses of sputum ejected is washed in several sterilized dishes containing sterile water, and is thus freed from the bacteria adherent to its surface. From the central portion of such a mass a flake is removed, by means of a platinum wire, and smeared upon blood-serum in tubes. The cultures that develop under these conditions exhibit quite a different appearance than do those cultivated from the animal body. There develop round, and rather whitish and translucent colonies, which in subsequent generations grow in precisely the same manner as the bacilli obtained by the method first described.

Resistance of the Tubercle-bacilli.—Tubercle-bacilli are destroyed only after exposure for ten minutes to a temperature of 70° C. (158° F.); for one minute, at a temperature of 95° C. (203° F.); for an hour, at a temperature of 60° C. (140° F.); and for four hours, at a temperature of 55° C. (131° F.). They are, thus, more resistant to heat than most other varieties of vegetative bacteria, which are generally destroyed by brief exposure to temperatures between 54° C. (129.2° F.) and 60° C. (140° F.). Tubercle-bacilli resist for only a relatively short time the influence of direct sunlight—in accordance with the density of the bacterial mass, from several minutes to several hours. Diffuse daylight causes death after the lapse of a week. Tubercle-bacilli can be cultivated for many years through a series of generations, without loss of vitality, although they gradually become somewhat less virulent.

Spontaneous and Experimental Tuberculosis in Animals.—Spontaneous tuberculosis occurs in animals, with frequency only among cattle and in monkeys living in captivity, although there is scarcely a domestic animal that is immune to tuberculosis. Among laboratory-animals the guinea-pig is the most susceptible, a few tubercle-bacilli sufficing to induce infection in them. Next in susceptibility is the rabbit, and then the field-mouse. Less susceptible, though by no means immune, are white mice and dogs. Young animals exhibit a much greater predisposition to tuberculosis than old animals. Typical tuberculous disease is induced experimentally in animals (guinea-pigs, rabbits, field-mice) by means of subcutaneous injection, by inoculation of the anterior chamber of the eye, by intrapleural, intraperitoneal, and intravenous injection, or by inhalation of moist tubercle-bacilli in the form of dust. The last remaining portal of entry for tubercle-bacilli into the organism, the gastro-intestinal tract, also was utilized successfully by Koch in experiments on animals. Susceptible animals that are given tubercle-bacilli in considerable number with their food die of intestinal tuberculosis. The resulting tuberculous process is, apart from the results of intravenous injection, at first always *local*, restricted to the site of infection; it extends, however, steadily, but slowly, by way of the lymph-paths. The bacilli quickly gain entrance into the lymph-glands nearest the portal of infection. As early as three days after inoculation of tuberculous material into the anterior chamber of the eye Baumgarten found that the bacilli had advanced as far as the auricular lymph-glands. When the bacilli are injected into a vein, *general miliary tuberculosis* takes place at once.

Of great assistance in the comprehension of some tuberculous local processes in human beings are the observations of Schüller, who inoculated tuberculous material at some indifferent portion of the body of the animal and then inflicted traumatism in the neighborhood of the knee-joint, and found that the infection localized itself at this point.

The chronic cold abscesses, which upon bacteriologic examination are found free from pyogenic microbes, are certainly to be attributed to tubercle-bacilli. Dead tubercle-bacilli, destroyed by live steam or by other means, exhibit pyogenic activity experimentally. They are positively chemotactic : they attract leukocytes. The pyogenic sub-

stance contained within the bodies of the bacilli can be extracted only with great difficulty.

If dead tubercle-bacilli are inoculated into rabbits by intravenous injection, and the animals are subsequently killed (some of them die spontaneously), the lungs and the liver are found strewn with small nodules, consisting of round cells and epithelioid cells, giant-cells, which contain dead tubercle-bacilli, and which are thus indistinguishable from true tubercles. Prudden and Hodenpyl, who were the first to make these observations, believed the formation of the tubercle to be a result of the activity of a specific bacterial proteid contained within the protoplasmic body of the tubercle-bacilli. Baumgarten does not agree with this opinion, considering the formation of nodules by the dead bacteria as tuberculosis due to a foreign body (p. 276). The agency through which tubercle-bacilli in general give rise to the formation of tubercles, and whether a toxic influence is operative, are questions that also have not yet been determined with certainty with regard to the living bacilli. It is undoubted, however, that the tubercle is a direct result of the presence of the tubercle-bacillus, for this is found in every tubercle, and wherever it is introduced into animals, there tubercles develop with certainty. It is noteworthy that in the clinical picture of tuberculosis manifestations of general intoxication are not conspicuous so long as the process is in its incipiency, is localized, and mixed infection has not yet taken place.

Pathologic Anatomy of Tuberculosis in Animals.— In different varieties of animals the bacilli cause anatomic changes of a widely different character. Thus, in the liver and the spleen of guinea-pigs coagulation-necrosis occurs, without true caseation ; in monkeys rapid softening takes place, with the formation of a thin, diffluent purulent secretion ; in the tuberculosis of cows there is simultaneous calcification and caseation. The most common product of tubercle-bacilli in animals is, however, the tubercle, which is also always encountered in connection with the experimental development of tuberculosis, and which resembles in every respect the tuberculous nodule of human tuberculosis to be described shortly.

Portals of Entry for the Tubercle-bacillus in Human Beings.—The tubercle-bacillus most frequently gains entrance into the human body through the *respiration* by way

17

of the *lungs*. The frequency of occurrence of other forms of tuberculous disease falls far behind that of involvement of the lungs. The next most important portal of entry is the *digestive tract*, which plays an important rôle especially in the etiology of tuberculosis in early life. Under these conditions the infection gives rise, on the one hand, to swelling of the cervical lymph-glands, often with consequent suppuration (scrofulosis), and on the other hand— and more frequently without primary involvement of the intestine—to caseation of the mesenteric glands, or to chronic peritonitis. In adults the digestive tract is relatively seldom the portal of entrance for tubercle-bacilli, which in that event give rise to ulcerative processes in the intestine. Breaches in the continuity of the *skin* constitute a third and not at all uncommon medium of infection with tubercle-bacilli. Cases of cutaneous tuberculosis and of tuberculosis of wounds have in recent years been reported in considerable number. The so-called postmortem tubercle must be included in this category, as careful investigation has shown. Further, the relationship of lupus to tuberculosis is scarcely any longer a matter of serious doubt. Koch has succeeded in isolating tubercle-bacilli in pure culture from lupus-nodules.

The Pathogenic Activity of the Bacilli in the Human Body.—The most characteristic product of tubercle-bacilli is the *tubercle*. In this the bacilli lie mainly within the interior of the giant-cells, partly at the center, partly at the periphery ; but the bacilli are to be found also in and among the round cells constituting the tubercle. At times the rods within the giant-cells appear not to be distinctly stained, but disintegrated. Metschnikoff considers such appearances as the remains of tubercle-bacilli, and, in consequence, believes the giant-cells to be phagocytes. The specific nodules persist only for a short time, and soon disintegrate into cheesy-necrotic material.

Besides giving rise to the formation of nodules, the tubercle-bacilli exhibit varied other pathogenic activity. At times they give rise to serous, purulent, or hemorrhagic inflammation, and in rare cases even to fibrinous exudation. They may further cause cheesy-necrotic inflammation without the previous formation of nodules. In some cases the inflammation caused by the bacilli has from the beginning a tendency to the formation of connective tissue, so

that it soon leads to indurative cicatricial processes. This great variability in the anatomic alterations induced by the tubercle-bacilli explains the difference in the point of view taken by the pathologic anatomist and by the clinician in part with regard to tuberculosis. The anatomist, in describing the various processes, undertakes to separate them, in spite of their common etiology, while to the clinician the etiologic standpoint is alone productive, and therefore decisive ; and he considers all diseases tuberculous in whose products tubercle-bacilli can be demonstrated.

Susceptibility of Human Beings to Tuberculosis (Predisposition).—Tubercle-bacilli are widely distributed throughout the inhabited world. About one-seventh of mankind is attacked by tuberculosis. The susceptibility of human beings to the disease is, therefore, not especially great. This view receives strong support from the observation that has now been repeatedly made that the bronchial glands of apparently healthy persons dying suddenly by accident contain living and virulent tubercle-bacilli (Loomis, Pizzini). In these cases the bacilli have passed the lungs without giving rise to disease. From the anatomic-bacteriologic point of view such individuals in perfect health as harbor tubercle-bacilli in the bronchial glands or in some other part of their body may be designated tuberculous ; while from the clinical standpoint only those may be considered tuberculous that exhibit the clinical manifestations of tuberculosis.

From these observations the significance of the general *predisposition* to tuberculosis must be clear. It is evident that only a healthy and resistant body may harbor the bacilli without danger, whereas every debilitating influence facilitates the proliferation of the bacilli within the body. The time of invasion by the bacilli and the appearance of the disease do not always coincide. Only a poorly nourished organism enfeebled by grief and worry or by protracted disease will suffer immediately after the entrance of the bacilli. A special predisposition is conferred by the abundance of sugar in the tissues in cases of diabetes. Not rarely contusions of the lung determine the mobilization of previously latent tubercle-bacilli (traumatic tuberculosis). Resistance to invasion by the bacilli appears to be strengthened in human beings by elevated climates. Elevations of above 2000 meters (6500 feet) are measurably

free from tuberculosis. In the large cities of Mexico and Pueblo, situated between 2000 (6500 feet) and 2500 meters (8200 feet) above the level of the sea, tuberculosis is said to occur but rarely, in spite of the density of the population.

Localization of Tuberculosis in Human Beings.—In human beings tuberculosis usually remains localized to the lungs. Primary tuberculosis of other organs is rare in adults. From the lungs, in the course of the disease, propagation of the bacilli frequently takes place, partly, through contact, to the pleura or other organs, partly, through the intermediation of expectorated or swallowed sputum, to the larynx and the intestine. In these organs also the tuberculosis pursues the course of a localized disease. The bacilli are carried by way of the lymph-paths and the blood-stream to the membranes of the brain, the genito-urinary apparatus, the bones and the joints, where they also give rise to local disease.

Highly febrile and rapidly fatal *general tuberculosis* occurs when tubercle-bacilli gain entrance into the pulmonary veins in large number, and rapid dissemination through the whole body follows (*miliary tuberculosis*). Under these conditions tubercle-bacilli can be found in the blood.

Mixed Infection.—The symptom-complex of tuberculosis in human beings is at times quite materially changed by the presence and proliferation of other microorganisms in addition to the specific exciting agents. Especially in pulmonary cavities is this almost regularly the case. In the walls of such cavities sarcinæ, streptococci, staphylococci, tetragenus, the bacilli of blue pus, varieties of proteus, and others, may lodge. There thus result septic and pyemic disturbances that are foreign to the tuberculous process as such. Thus, the marked intermittent fever observed in so many cases of tuberculosis is probably dependent principally upon the activity of streptococci (streptococcus-curve).

Occurrence and Distribution of Tubercle-bacilli.—The tubercle-bacilli are found especially in the lungs and in the sputum in cases of pulmonary tuberculosis; further, in all tuberculous lesions, including lupus. The blood contains the bacilli only in cases of general miliary tuberculosis and then only in small number. Infection may take place

through all of the products of the disease. Most danger-
ous in this respect naturally is tuberculous sputum. Of
less importance are the feces in cases of intestinal tuber-
culosis, the urine in cases of genito-urinary tuberculosis,
and the pus in cases of bone-tuberculosis. By means of
the sputum of tuberculous patients who, instead of using
a spit-cup, expectorate upon the floor or into handker-
chiefs, the tubercle-bacillus is disseminated in the environ-
ment of the patient. The microorganism exhibits consid-
erable resistance to drying, and in dried sputum, for in-
stance, retains its vitality and infectivity for six months. The
dried and powdered sputum is readily carried as dust in
currents of air, and the tubercle-bacilli may in this way
enter the air-passages of other individuals and there give
rise to infection.*

The credit for pointing out this mode of distribution
for tubercle-bacilli belongs to Cornet, who was able to in-
fect guinea-pigs with tuberculosis by means of dust from
the walls, the floors, the furniture, etc., of hospital-wards
and dwelling-apartments occupied by tuberculous patients
careless with regard to the disposition of their sputum.
Dust from localities not invaded by tuberculous patients
invariably proved free from tubercle-bacilli.

Reference must, however, be made at this place to the
observations of Kitasato, who showed by culture-methods
that not rarely the tubercle-bacilli in the sputum of tuber-
culous patients have died. To what extent current views
as to the danger from tuberculous sputum must be quali-
fied by this fact can not yet be determined with certainty.

A further source of infection, whose importance must
not be underestimated, is constituted by the milk of tuber-
culous cows. Tuberculosis in cattle is a manifestation of
the activity of the tubercle-bacillus—a tuberculous process
that differs from human tuberculosis only in the occurrence
of calcification coincidently with caseation. The milk of
cows suffering from tuberculosis contains tubercle-bacilli

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* According to observations made by Flügge, the larger particles of sputum,
both in the fresh and in the dry state, are less dangerous. The main danger is
believed to reside in the fine particles of fluid that are ejected simultaneously
with the sputum, and which may float in the air for a long time and be inhaled
by those with whom the patient comes in contact. These small drops, as
Flügge has demonstrated experimentally, contain bacilli. To what extent
this fact should influence the attitude of the physician with regard to the
sputum from the prophylactic standpoint (p. 266) can not yet be defined.

with extraordinary frequency (in fifty per cent. of diseased animals), even when no tuberculous changes are appreciable in the udder.

If the frequency of tuberculosis in cows is considered—in some districts it occurs in from twenty to fifty per cent. of all the cows—the danger from the use of unboiled or insufficiently boiled milk, especially the mixed milk of large cities, and particularly for children, must be obvious. The hydrochloric-acid content of the stomach does not afford sufficient protection. The tubercle-bacilli pass the barrier interposed by the stomach, and gain entrance, at least in part, uninjured, into the intestine. A large proportion of the cases of tuberculosis of the intestine and the peritoneum, which is so common in childhood, may be

Fig. 56.—Tubercle-bacilli in sputum; Zeiss' homogeneous immersion $\frac{1}{12}$, Oc. 4; × about 1000 diameters.

attributed to the use of such infected milk. The use of meat from tuberculous cows probably gives rise to the development of intestinal tuberculosis only exceptionally. The parts that contain nodules are not permitted to be offered for sale, and the parts free from nodules contain no bacilli. Only in cases of acute general miliary tuberculosis could meat free from tubercles contain bacilli, carried to it through the blood.

The diagnostic demonstration of tubercle-bacilli is of the highest importance for the early recognition of tuberculosis. Owing to the specific behavior of tubercle-bacilli with relation to stains, the demonstration of the microorganisms can be made with the greatest certainty by means of the microscope.

(*a*) *Demonstration of the Bacilli in Sputum* (*Pus*).—For convenience of observation the sputum is spread upon a black plate or upon a glass dish with a black background (of paper). The well-known yellowish masses ("lentils") are sought for and one of these or some other purulent portion of the sputum is placed, by means of forceps, upon a cover-slip, and spread as uniformly as possible upon its surface. After the cover-slip preparation has dried in the air, it is passed, in the usual manner, with the aid of the forceps, three times through the flame; then a few drops of a freshly prepared aniline-water fuchsin-solution or of a carbol-fuchsin solution are added, and the preparation is heated over the flame until the vapor of steam distinctly escapes. After the lapse of a minute the cover-slip is moved to and fro

Fig. 57.—Tubercle-bacillus in sputum (Fränkel and Pfeiffer).

for several seconds in dilute nitric acid (from fifteen to twenty per cent.), for purposes of decolorization, and it is then introduced into alcohol (seventy per cent.) for the removal of the coloring-matter dissolved by the nitric acid. These two manipulations are repeated until the preparation appears scarcely stained. In the preparation everything has now been decolorized, and only the tubercle-bacilli, if these be present, have retained the stain. The alcohol is removed with distilled water. In order that the stained tubercle-bacilli may be more sharply differentiated from their surroundings, the preparation is counterstained by exposure for a short time to the action of a dilute aqueous solution of methylene-blue or vesuvin. It is then again rinsed with distilled water, and is finally mounted in the

usual way for examination. The microscopic examination is best made by means of an oil-immersion lens ; slightly curved bacilli stained red are looked for, and if these are found, they can be tubercle-bacilli only. At times the spores of mold-fungi and of bacilli, portions of hair, fragments of horny epithelial cells, cholesterin-plates, crystals of fatty acids, and similar elements, stained red, are observed, which have resisted decolorization ; but a little experience will prevent confusion of these with tubercle-bacilli.

Decolorization and counterstaining may be practised together (B. Fränkel-Gabbet) by adding the decolorizing acid to the solution employed in counterstaining. After staining with hot carbol-fuchsin solution the cover-slip preparation, rinsed in water, is placed in the following solution : Nitric acid 20, alcohol 30, water 50, methylene-blue to saturation. After washing with water the preparation is dried and mounted in the usual manner.

In order to find the tubercle-bacilli when these are present in small number, it is advisable, according to the suggestion of Biedert, to dilute the sputum in a test-tube with water, to add potassium or sodium hydroxid, and to continue the application of heat until the fluid assumes a homogeneous appearance. Sedimentation is then permitted to take place, and the bacilli, by reason of their weight, sink to the bottom of the tube. The sediment is then examined by one of the methods just described.

(*b*) *Examination of Feces.*—A flake of mucus or of pus is selected from the feces, and treated precisely in the manner described for sputum.

(*c*) *Demonstration of Tubercle-bacilli in Urine.*—The urine, which is usually turbid from the presence of pus, is permitted to settle in a conical glass, or it is centrifugated. The sediment is placed upon a cover-slip in a somewhat thicker layer than the sputum, but in other respects it is treated in exactly the same manner. In urine the tubercle-bacilli are prone to lie together in small masses (nests).

(*d*) *Pus from cold abscesses and fluid from pleural effusions* suspected to be tuberculous are often examined in vain for tubercle-bacilli. After intraperitoneal injection of such material (possibly after centrifugation) guinea-pigs not rarely die of experimental tuberculosis. In this way it is possible, although after the lapse of weeks, to make a diagnosis of the presence of tubercle-bacilli.

(*e*) *Staining of Tubercle-bacilli in Sections.*—The staining of sections in hot solutions is not practicable. The preparations are, therefore, kept in the aniline-water staining solution or the carbolfuchsin for from twelve to twenty-five hours at room-tem-

perature, or from one or two hours at 37° C.(98.6° F.) in the thermostat. They are decolorized in ten per cent. nitric acid for about two minutes until they appear greenish blue, then in seventy per cent. alcohol until they appear pale rose. Next, they are introduced into water, counterstained with a dilute aqueous solution of methylene-blue or vesuvin for two or three minutes, dehydrated in absolute alcohol, cleared in cedar-oil, and mounted in Canada balsam.

(*f*) *Examination of Milk for Tubercle-bacilli.*—The milk is advantageously first centrifugated. Before being stained the cover-slip preparations are placed for from four to six minutes in chloroform for extraction of the fat. On their removal the chloroform is permitted to evaporate.

Fig. 58.—Tubercle-bacilli in the urine; from a case of tuberculous cystitis (Jakob).

The demonstration of tubercle-bacilli in the sputum renders unequivocally certain the diagnosis of pulmonary tuberculosis. A negative result permits of a definite conclusion only when frequently repeated. The demonstration of tubercle-bacilli in the stools permits of a diagnosis of intestinal tuberculosis only in the absence of primary pulmonary tuberculosis. If this is present, it may be concluded that sputa have been swallowed, the bacilli of which appear in the feces unchanged. For this reason examination of the stools but rarely yields practical results. If urinary sediment is stained from a suspicion of genito-urinary tuberculosis, the possibility of confusion with smegma-bacilli (see Syphilis) must be considered, as these bacteria also, when exposed to acids, retain tenaciously the

stain taken up. Smegma-bacilli are, however, readily to be distinguished from tubercle-bacilli in that they are decolorized within a minute in absolute alcohol, whereas the tubercle-bacilli retain their stain under like conditions.

Prophylaxis.—The principal source of tuberculous infection is the sputum of tuberculous patients. If it were possible to render all *sputum* containing tubercle-bacilli innocuous, complete suppression of tuberculosis might be conceivable. The too zealous pursuit of this ideal aim would, however, lead to hardship on the part of tuberculous patients, so that in application the principle must be modified in accordance with humane considerations. Besides, a certain degree of moderation is permissible in this connection, as the liability to the disease may be diminished in not less degree by increasing the individual resistance—that is, by improving the general conditions of life—than by
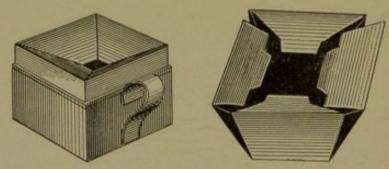
Fig. 59.—Pasteboard spit-cup for receiving infectious sputum. After being used, the pasteboard can be removed from the steel frame and burned.

destruction of the bacilli. In any event, however, the following principles should be enforced, or at least impressed upon the general public : The tuberculous patient should expectorate only into a *spit-cup* of glass or porcelain, filled with water, in order that there may be no opportunity for the sputum to dry and to be converted into dust. In this conversion into dust resides the greatest danger, and for this reason the addition to the cup of all substances capable of generating dust, such as sand, ashes, etc., should be avoided. Disinfection of the sputum can not be accomplished by means of antiseptic agents, as most of these substances cause coagulation of the albuminous constituents of the sputum and thus give rise to a dense membrane that surrounds the mass and shields the tubercle-bacilli in the interior from contact with the disinfectant. In general,

it will suffice to empty the sputum-cup into the water-closet, as the tubercle-bacilli are sure to die in putrid mixtures. In hospitals it will be best to sterilize the sputa, together with other excretions, by boiling. (See Disinfection, Appendix.) Especial attention should be paid to the tuberculous patient out of doors. Sputum expectorated indiscriminately upon the street is especially dangerous, because when reduced to powder, innumerable persons are exposed to the liability of infection. The use of the handkerchief to receive the expectoration is likewise not without risk, because here, also, there is danger of drying and conversion into dust. For this reason it has been wisely recommended that tuberculous patients carry with them small pocket-flasks for the reception of the expectorated matters —for instance, Dettweiler's spit-cup. It must, however, not be overlooked that certain insurmountable difficulties stand in the way of a general employment of such flasks, and that a careful use of the handkerchief may yet be permitted.

The *rooms* occupied by tuberculous patients should be cleansed quite frequently with moist materials, in order to prevent the conversion into dust of the sputum that has possibly become deposited on the floor or on the furniture. After the death of a tuberculous patient the rooms previously occupied and, besides, all articles with which he came in contact should be disinfected with the same care that is observed in other infectious diseases. In hospitals, prisons, etc., those suffering from tuberculosis should be separated from the others.

In cases of intestinal and genito-urinary tuberculosis and of tuberculous suppuration care should be taken that the *feces* or the *urine* or the *pus* is rendered innocuous. *Sexual intercourse* should be forbidden those suffering from genito-urinary tuberculosis, as the disease may be transmitted through the spermatic fluid.

Finally, it may be stated that *milk* should never be drunk unboiled, but should always be boiled before being used. It is best to forbid the use of *meat* from a tuberculous animal, or at least to permit its sale only when thoroughly cooked, if the disease is not strictly localized to a single organ.

Heredity of Tuberculosis.—A distinction is made between *direct* inheritance of tuberculosis—the disease being transmitted as such to the offspring—and *indirect*

inheritance—only the tendency or the predisposition to the disease being transmitted. With indirect inheritance the children of a tuberculous father or mother have inherited a feeble body, a small chest—in brief, the tuberculous habitus. They are not tuberculous, but are greatly predisposed to tuberculosis (sujets tuberculisables, Peter).

Cases of direct inheritance—that is, of *congenital* tuberculosis—are known both in human pathology and in veterinary medicine, but their number is extremely small in comparison with the wide distribution of the disease. The same statement is applicable to tuberculosis in the first few days of life. On the other hand, the mortality from tuberculosis in early infancy, especially in the first year of life, is quite enormous. Most observers assume that this high mortality is readily explained by the numerous sources of infection to which the child of tuberculous ancestry is exposed after birth. The kiss of a tuberculous father, the milk of a tuberculous mother, are equally dangerous, independently of the fact that with neglect in the treatment of the sputum on the part of the parents there is also afforded frequent opportunity for inhalation of the tubercle-bacilli. That the disease is so prevalent and so fatal in the first year of life is to be explained by the fact that at this period the children are more predisposed, and less resistant, to the tubercle-bacillus than later. The relation in these cases would thus be one of indirect inheritance and not one of true congenital tuberculosis.

Baumgarten, probably the most earnest advocate of the doctrine of direct inheritance of tuberculosis, takes the ground that the tubercle-bacilli are transmitted to the embryo during fetal life. The inherited germ, however, does not proliferate because the tissues of the new-born child, through their vital activity, offer considerable resistance. The bacilli thus to a certain degree remain latent in the lymph-glands, the bone-marrow, etc., later, under the favoring influence of traumatism, intercurrent disease, or diminution in the vital energy of the cells, to unfold their deleterious activity. This view is supported by the observations of Landouzy, Martin, Birch-Hirschfeld, Schmorl, and others, who found tubercle-bacilli in apparently healthy organs from fetuses born of tuberculous mothers. On the other hand, Gärtner lays emphasis upon the fact that the infantile cells do not exert an especially injurious influence upon

the bacilli. Tuberculosis pursues a more rapid course in youth than at a later age, and experimentally no difference can be observed between young and old animals. On the other hand, Gärtner is of the opinion that in the act of parturition, perhaps in consequence of tears in the placenta, the bacilli pass over from the mother to the child. The possibility of such a transfer of the tubercle-bacilli to the embryo Gärtner has himself demonstrated with certainty in his experiments upon mice, canary-birds, and rabbits. Fetal infection, according to this view, takes place late, and in all probability it is effected through only one or a few bacilli. Consequently, the disease can not be manifest at birth, but it becomes evident only after the child has lived for some time outside the uterus. Also, the cases of primary tuberculosis of the skin, the joints, the bones, the glands, the spleen, the liver, which are not uncommon in early childhood, indicate a hematogenous fetal infection. The objection that the frequency of pulmonary tuberculosis is opposed to the preponderant occurrence of hematogenous fetal transmission, is, according to Gärtner, justified; but it is to be borne in mind that, in spite of exhibiting the most conspicuous changes, the lungs are by no means always the seat of the primary tuberculous lesion. The lungs of human beings are, "by reason of their constitution, their situation, and their chemistry, especially adapted for the lodgment and development of tubercle-bacilli," which may gain entrance from any primary focus (perhaps of fetal origin). The question whether tuberculosis may be transmitted to the embryo from the father Gärtner answers in the negative. He rendered male rabbits and guinea-pigs tuberculous by injection of bacilli into the testicles, and he then brought them in contact with females, but the offspring were not tuberculous; whereas when the bacilli were present in the seminal fluid in considerable numbers, the females were infected.

Therapeutic Experiments.—Many efforts have been made to confer immunity to tuberculosis, and to effect the cure of tuberculosis by the same means. This end was sought by the use of the *blood-serum of animals* that are relatively refractory to the disease, such as dogs (Richet and Héricourt) and goats. Further, attempts have been made to establish immunity by means of attenuated or sterilized cultures of tubercle-bacilli. All of these endeavors, how-

ever, have thus far proved unsuccessful. Richet maintained for a time that he had succeeded in vaccinating dogs by inoculating them with the bacilli of fowl-tuberculosis, or with small amounts of mammalian tubercle-bacilli, but his observations have not been confirmed.

R. Koch endeavored to secure a direct and specific influence upon the tuberculous lesions by means of *tuberculin*. This product was obtained by concentrating mature bouillon-cultures of tubercle-bacilli (from six to eight weeks old) to one-tenth of their volume over the water-bath, and freeing the culture-fluid of the bodies of the dead bacilli by means of filtration through porcelain or gravel. Experimental observation showed that healthy guinea-pigs withstand without harm subcutaneous injections of as much as two cubic centimeters of tuberculin, whereas tuberculous animals, infected four weeks previously, succumb to a dose of 0.6 cu. cm. On autopsy in cases of animals dead after injections of tuberculin the following conditions were found : The site of tuberculous inoculation was markedly reddened, as well as the adjacent lymph-glands. In the viscera enormous dilatation of the capillaries surrounding the tuberculous foci was discernible microscopically. The hemorrhage-like spots on the surface of the liver were quite pathognomonic. In tuberculous guinea-pigs treated with small doses of tuberculin (at first 1 mg., increasing to 0.1 and 0.2 gram) a striking effect upon the morbid condition was observed. Improvement in the primary area of inoculation in the abdominal wall was observed, with diminution in size of the adjacent tumid lymph-glands, but complete recovery, as later reports from the Koch Institute have shown, occurred only exceptionally. The animals survived for a longer time than tuberculous control guinea-pigs not treated with tuberculin. Postmortem examination in the case of animals treated disclosed marked retrogression, in part cicatrization of the tuberculous lesions in the abdominal viscera, but eventually death resulted from pulmonary tuberculosis.

The *curative influence* of tuberculin is explained by Koch on the assumption that as a result of its action the necrotic substance, which is always present in the neighborhood of a tuberculous lesion, is increased in amount, so that coagulation-necrosis occurs throughout a wide extent, and this hinders the bacillus in its further growth, and at

times causes its death. Opposed to the original opinion of Koch is the fact that the action of tuberculin upon tuberculous tissues is, however, not specific, and not confined to it alone. As Römer was the first to show, the products derived from other microorganisms by boiling (proteins) induce precisely the same reaction.

The influence of tuberculin upon healthy and diseased human beings has been tested from both a diagnostic and a therapeutic standpoint in so large a number of cases that a definite opinion can now be expressed. Diagnostically, tuberculin has proved an exceedingly delicate reagent for the detection of tuberculous lesions. Subcutaneous injection of five milligrams is followed by no appreciable effect in healthy persons. In tuberculous individuals, however, this dose gives rise to fever of moderate degree, lasting for several hours, while at the same time a local reaction, manifested by redness and swelling, takes place in the tuberculous lesions in so far as these are accessible to examination. Initial doses of ten milligrams cause · in tuberculous subjects greater and more protracted elevation of temperature, together with headache, nausea, and vomiting. Similar doses cause also in healthy persons febrile disturbance and general manifestations.

The undoubted *diagnostic utility* of minimal doses of tuberculin is rendered practically unavailable from the fact that the injection is followed by febrile movement in all persons who harbor tubercle-bacilli, even when these are latent and encapsulated—as, for instance, in lymphatic or bronchial glands. It has been found, in numerous cases, that apparently healthy persons develop fever after injections of tuberculin in the same way as tuberculous patients. From what has been said with regard to the frequent occurrence of tubercle-bacilli in healthy persons, this manifestation should not occasion surprise; but a diagnosis of tuberculosis is not, in a clinical sense, justifiable when merely the presence of tubercle-bacilli within the organism has been demonstrated. The conditions are quite different in the diagnosis of tuberculosis than in that of diphtheria or of cholera. It is of the greatest sanitary importance to recognize the existence of diphtheria or of cholera in persons in whom, though apparently in perfect health, the exciting agents of those diseases are present in the saliva or in the dejections, because further cases of infection may

arise through them. In the case of tuberculosis, however, only those individuals are dangerous who present actual symptoms of disease—that is, those who throw off tubercle-bacilli with the sputum or other excretions. In the case of tuberculosis thus the diagnostic requirements on the part of both physician and sanitarian are identical ; whereas in the case of cholera and of diphtheria these diverge. From the foregoing considerations the employment of injections of tuberculin for diagnostic purposes should be restricted to cases of actual disease of obscure etiology. Frequently, however, under such conditions also this test has been abstained from because experience in some cases has demonstrated the possibility of the bacilli being disseminated from a previously circumscribed focus throughout the entire body as the result of an injection of tuberculin.

The *therapeutic employment* of tuberculin in tuberculous individuals has yielded the following results : Lupus is at times favorably influenced by tuberculin. Extensive areas of lupus undergo necrosis and are exfoliated, so that complete recovery may take place ; but no case is yet known in which recurrence has not taken place. Ulcers of the larynx clear in a most remarkable manner after injections of tuberculin, and undergo healing ; but in these cases also recurrence generally takes place. Intestinal and peritoneal tuberculosis appears to pursue a relatively favorable course when treated with injections of tuberculin. Under all of these conditions, however, the fact stands out that the results are not final, because tuberculin lacks immunizing properties. Tuberculous disease of bones and joints is not at all influenced by the treatment. With relation to pulmonary tuberculosis, it may be stated with certainty that in the presence of advanced infiltration, of cavity-formation, and of mixed infection success can not be attained with tuberculin-treatment ; and it is questionable, further, whether even incipient tuberculosis can be cured by the original method of Koch. A large number of unfavorable results are opposed to a small number of undoubted cases of incipient tuberculosis treated with favorable results. The objection may be raised that the results obtained in these cases are not attributable to the specific agent, but to the nutritive and general therapeutic measures employed simultaneously. Tuberculin has been almost entirely abandoned by physicians. In veterinary medicine, especially in France,

it is still employed on a large scale for diagnostic purposes, and, it appears, with great success.

The New Tuberculin-preparations, TO and TR.—After the failure of the original tuberculin, Koch applied himself unremittingly to the improvement of tuberculin-preparations. He found that immunity could not be conferred upon animals by subcutaneous injection of unchanged tubercle-bacilli. He, therefore, undertook to disintegrate the tubercle-bacilli mechanically, in order to render them the more easily absorbable. The cultures were dried in a vacuum and rubbed up in an agate mortar without addition until only a small number of bacilli were visible microscopically. The powder was mixed with distilled water, and centrifugated for from a half to three-quarters of an hour. In this way Koch effected a separation into two layers—an upper transparent, opalescent layer, which contained no bacilli, and a densely adherent lower layer. The latter was again dried, rubbed up, and centrifugated in the manner described, and the entire process was repeated until all the bacilli had practically been reduced to solution. Koch then determined that the solutions thus obtained were readily absorbable, and that they did not give rise to the formation of abscesses. It was found, however, that the fluid obtained from the first centrifugation exhibited reactions different from that obtained from the second and from subsequent centrifugations. Koch designated the first tuberculin O (*obere*, upper—TO); and all of the others, which were alike in their reaction, tuberculin R (residue— TR). TO contains the constituents of the tubercle-bacilli soluble, and TR those insoluble, in glycerin. It can be readily understood that the properties of TO are comparable, on the whole, with those of the original tuberculin. TR, however, according to Koch, exhibits distinct immunizing properties, and gives rise to reaction in tuberculous subjects only when used in large doses. Its action is entirely independent of the reactions that played so important a part when the original tuberculin was used. Koch states that in conferring immunity with TR the reactions may be entirely avoided, and, by carefully increasing the dose, tuberculous subjects may be habituated quite rapidly, without any reaction, to considerable amounts of the new remedy. When this has been accomplished, the organism may be considered as immune to the original

18

tuberculin and to TO—that is, to all of the bodily con-
stituents of the tubercle-bacilli.

The new preparations are put upon the market by the
Höchst Works. For purposes of preservation 20 per cent.
of glycerin is added. One cubic centimeter of the new
tuberculin TR contains 10 mg. of solid substance. After
appropriate dilution with sterile, physiologic solution of
sodium chlorid (if the daughter-solution is to be preserved
for some time, 20 per cent. of glycerin is added), the treat-
ment is begun by injecting $\frac{1}{500}$ mg. The next higher
dose is to be administered on the second day, and it should
be of such an amount that temperature-elevations of more
than $\frac{1}{2}°$ do not take place. Should these occur, the next
injection must be deferred until the temperature has again
become normal. As a rule, the treatment is suspended
when the dose reaches 20 mg., and, if no reaction follows,
the same amount is repeated at considerable intervals.

If it is desired to immunize healthy animals, as large a
quantity is injected at first as is well borne by them—for
instance, in the case of guinea-pigs, 2 or 3 mg. In this
way Koch was able to immunize a considerable number of
guinea-pigs to highly virulent tubercle-bacilli. The height
of the immunity is attained two or three weeks after ad-
ministration of the large dose. From this it appears that
in therapeutic experiments on artificially infected guinea-
pigs, which rapidly succumb to the disease, the treatment
must be instituted quite early—not later than two weeks
after the introduction of the virus.

Koch recommends that this new remedy be employed
only in recent, pure cases of tuberculosis, uncomplicated
by mixed infection. Patients who exhibit a temperature
above 38° C. (100.4° F.) are not adapted to the new
method of treatment. In cases of lupus Koch obtained
considerable improvement without noteworthy local re-
action. At the conclusion of his communication Koch
emphasizes the fact that perhaps combinations of TO and
TR with serum-preparations made from TO and TR may
more quickly lead to the desired results. The clinical
reports that have thus far been made with regard to the
treatment of tuberculosis with TR show the freedom from
danger that attends the use of the remedy, but little as to
its therapeutic utility. Personally, no noteworthy results
have been obtained in fifteen cases of pulmonary tubercu-

losis thus treated. Behring is of the opinion that tuber-
culin R is less well adapted for therapeutic employment in
the case of human beings than for the fundamental inocu-
lation of animals for the purpose of further immunization.
His own experiments, in connection with von Lingelsheim,
in the preparation of a serum for tuberculosis, according to
his statement read before the Fifteenth Congress for In-
ternal Medicine, appear promising. Behring and von Lin-
gelsheim in their experiments employed dry, highly viru-
lent, pure cultures of tubercle-bacilli. According to Beh-
ring, the tubercle-bacilli contain various substances, but
only one of the tuberculosis-toxins appears to possess im-
munizing properties. With the aid of this toxin it may be
hoped that a curative serum or an antitoxin will be pro-
duced, as in the case of diphtheria and of tetanus ; but ac-
cording to Behring, years may elapse before this serum will
prove sufficiently powerful to be introduced into general
practice.

FOWL-TUBERCULOSIS.

The **bacillus of fowl-tuberculosis** is closely related to the
bacillus of human and mammalian tuberculosis. It is somewhat
longer and thinner, and exhibits more frequently bulbous and
branched variations. It is more easily stained, but it retains the
stain with similar tenacity. It is not so fastidious with regard to
culture-media, and it develops upon ordinary agar and upon or-
dinary bouillon. The addition of glycerin, however, materially
favors its growth, which, on the whole, is more rapid than that
of the bacillus of mammalian tuberculosis. The cultures are
not so dry, but more moist, and on solid media they form a
coherent coating that bridges over the water of condensation.
All cultures constantly exhibit a yellowish discoloration. At
temperatures of 42° C. (107.6° F.), 43° C (109.4° F.) or 45°
C. (113° F.) the bacilli of fowl-tuberculosis thrive as luxur-
iantly as at a temperature of 37° C. (98.6° F.). This is their
most radical distinguishing feature as compared with the bacilli of
human tuberculosis, which will not develop at this temperature.
If the two varieties are considered identical, this difference
must be explained by the fact that by reason of their residence
in the body of birds, which naturally have a higher temperature
(41° C.—105.8° F., or 42° C.—107.6° F.), the bacilli have
adapted themselves to a higher temperature. The bacilli of
fowl-tuberculosis are even more resistant to heat than those of
human tuberculosis, being destroyed by exposure for fifteen
minutes to a temperature of 70° C. (158° F.).

Occurrence of the Bacilli.—The bacilli are present in the tuberculous lesions of fowl, which consist of dense, tumor-like masses containing calcareous deposits. Giant-cells are present in but small number. Fowl-tuberculosis has been observed in rare cases also in human beings and in mammals.

Experimental Development of Fowl-tuberculosis.— Most birds are highly susceptible, and infection may take place through all portals of entry. According to Baumgarten fowl-tuberculosis occurring spontaneously is congenital in almost all instances. Rabbits, also, succumb to infection with fowl-tuberculosis. Guinea-pigs and dogs prove rather refractory, without, however, being entirely immune. On the whole, the bacilli of fowl-tuberculosis thrive poorly in mammals, and mammalian tuberculosis, conversely, develops only with difficulty in birds.

Diagnosis.—The examination of tuberculous masses for bacilli is conducted in precisely the same manner as in the case of human tuberculosis.

PSEUDO-TUBERCULOSIS.

By *pseudo-tuberculosis* is understood certain pathologic processes that exhibit the external appearance of tubercles, but are dependent upon other causes than the tubercle-bacillus. The **etiology** of pseudo-tuberculosis is quite varied. Among the causative factors may be mentioned:

1. Inanimate foreign bodies.
2. Animal parasites.
3. Bacteria.
4. More highly organized vegetable parasites.

The *tuberculosis due to foreign bodies* may be developed experimentally with ease through the agency of all possible substances. It is, however, not transmissible from animal to animal.

Pseudo-tuberculosis due to animal parasites is observed almost exclusively in animals. Among the varieties that are well known is the tuberculosis of the cat, caused by ollulanus tricuspis; that of the sheep, due to pseudalius ovis pulmonalis; that of the calf, due to strongylus rufescens; that of the dog, due to strongylus vasorum. Miura alone has observed a single case in a human being.

This occurred in a man dying of beri-beri, in whose omentum were found fibrous tubercles caused by the ova of distoma.

Bacterial pseudo-tuberculosis has been often described in animals. First designated zooglear tuberculosis by Malassez and Vignal, this disorder has since been studied by numerous observers, and most carefully by Preisz. The cause of this affection consists in thick, short rods, frequently resembling cocci, and forming filaments. Spores do not develop, and Gram's method fails to stain. Upon gelatin-plates colonies form resembling those of the typhoid-bacillus, but not causing liquefaction. Upon gela-
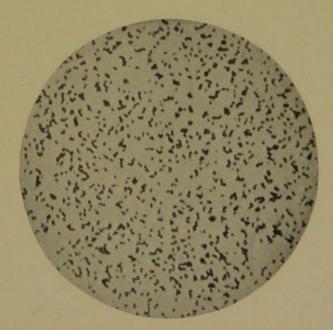
Fig. 60.—Bacillus pseudo-tuberculosis from agar-agar; × 1000 (Itzerott and Niemann).

tin stab-cultures a flat, nail-like growth takes place; upon agar a grayish, fetid coating forms, and upon potatoes a yellowish deposit. In bouillon there is at first flocculent turbidity, then the formation of a precipitate and a clearing up of the supernatant fluid. The bacillus pseudo-tuberculosis is pathogenic for all rodents; in slighter degree, also, for dogs and horses. The postmortem findings are strongly suggestive of true tuberculosis, particularly in the abdominal organs, which are especially attacked by pseudo-tuberculosis. The differential diagnosis is made with great ease. The readiness with which the bacilli stain and their rapidity of growth permit of a decision without difficulty.

Pseudo-tuberculosis due to more highly organized vegetable parasites likewise occurs preferably in animals. Various kinds of streptothrices and aspergilli act as the etiologic factors under these conditions, particularly aspergillus glaucus and fumigatus. Pigeons frequently succumb to a form of miliary pseudo-tuberculosis, in which the aspergillus fumigatus is found inclosed within giant-cells in the interior of the granulations. In individuals engaged in the fattening of pigeons pulmonary affections have been observed apparently dependent upon the same microbes ; at least the aspergillus fumigatus has been found in the sputum of such patients. It is assumed that the parasite adheres to the grain used in feeding the pigeons.

Eppinger has observed in, and cultivated from, a case of pseudo-tuberculosis a variety of streptothrix that he designates cladothrix asteroides. With pure cultures of this organism he succeeded in inducing the same disease in animals. (See Actinomycosis.)

✳

LEPROSY.

The **bacillus of leprosy** was discovered by Armauer Hansen. It has thus far not been successfully cultivated. In morphologic appearance the bacilli closely resemble tubercle-bacilli. Often, they appear somewhat shorter, and, like these, they are nonmotile. With regard to tingibility, the bacilli of leprosy occupy a position midway between tubercle-bacilli and other bacteria. As a rule, they prove susceptible only to the methods of staining that are applicable to tubercle-bacilli. They stain and decolorize somewhat more readily, however, than tubercle-bacilli, and they can be stained also by simple aqueous solutions of aniline dyes, especially violet and fuchsin, at room-temperature (Baumgarten). They can be stained, further, by Gram's method.

In the leprous new-formations (leprous nodules) the leprosy-bacilli lie mainly within the tissue-cells, in the so-called leprosy-cells. They have been found in the blood only during the febrile periods.

E. Levy has cultivated from a case of leprosy a bacterium that in glycerin-agar cultures bears some resemblance to the bacillus of mammalian tuberculosis, but that after

staining proves resistant to neither acid nor alcohol. Microscopically, it bears a distinct resemblance to the anaerobic actinomyces. It presents bulbous enlargements and ramifications.

Distribution of Leprosy.—Leprosy, which formerly was indigenous to all of Europe, is now restricted to Norway, Livonia, Turkey, the Crimea, and Southern Italy. Of late, isolated cases of the disease have again been encountered in Eastern Prussia. In countries outside of
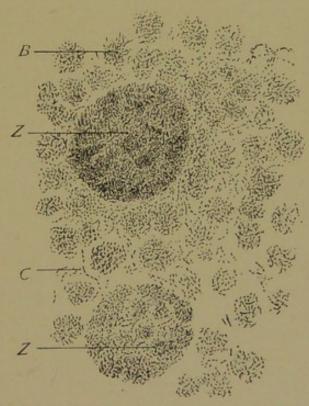
Fig. 61.—Bacilli shown in a section of the tongue in a case of tubercular leprosy; × 600. The bacilli are extracellular: *B*, Bacilli in groups; *Z, Z*, zooglear masses, large rounded masses of bacilli; *C*, bacilli in chains (Leloir).

Europe leprosy is quite common. The small number of cases that are observed in other places may be explained by importation from leprous localities. The disappearance of leprosy is undoubtedly to be attributed to the isolation —formerly practised with barbaric severity—of those affected in special leprosy-houses (leproseries). From this disappearance of the disease it may be concluded that it is contagious, but this has not been demonstrated with certainty, and is doubted by some physicians.

Experiments on Animals.—The experimental develop-

ment of leprosy in animals has thus far not succeeded. The only observations that can be considered as positive are those of Melcher and Ortmann, in which, after inoculation of the anterior chamber of the eye, general infection developed in rabbits; but these are interpreted by most observers as indicating that in the case or cases from which the infecting material was obtained there existed mixed infection with leprosy and tuberculosis, and that the inoculated animals died of tuberculosis. All of the attempts at inoculation of human beings have proved equally unsuccessful, or at least not above criticism. Danielsen, who inoculated himself and others with nodular masses, blood, etc., from leprous patients, obtained completely negative results. On the other hand, the experiments of Arning terminated positively; this observer succeeded in transmitting leprosy to a prisoner condemned to death. This case has, however, been seriously doubted, as the period of incubation was strikingly short, being only sixteen months, whereas the whole course of the disease covered only five years, and as, besides, the inoculated person was a member of a race highly susceptible to leprosy, and cases of leprosy had already occurred in his family.

Bacteriologic Diagnosis.—Cover-slip preparations are made from the contents and the tissue-juice of leprous nodules, and are stained in precisely the same way as tubercle-bacilli. Great care must be taken in the process of decolorization. The sections of leprous tissue are not permitted to remain for so long a time in the staining solution as sections of tissue containing tubercle-bacilli; half an hour is quite sufficient.

In most cases of leprosy, as R. Koch was the first to observe, the nasal mucus contains leprosy-bacilli. These are derived from areas of ulcerated or swollen mucous membrane on the cartilaginous portion of the nasal septum. The bacilli may, however, be present when such visible changes are absent.

Heredity of Leprosy.—According to Baumgarten, leprosy, like tuberculosis, is transmissible by inheritance. If this opinion is correct, a long period of latency must be assumed for the inherited leprosy-bacilli, as the disease never appears before the second or the third year of life.

Prophylaxis.—The best prophylaxis unquestionably consists in isolation of leprous patients in special hospitals.

INFLUENZA.

On the first occurrence of influenza in the winter of 1889–90 the numerous bacteriologic investigations undertaken failed to yield conclusive results. In the secretions of influenza-patients the ordinary exciting agents only of inflammation, especially streptococci and lanceolate diplococci, were encountered. Of the latter it was stated that in appearance and growth they presented certain points of distinction as compared with ordinary pneumococci. A specific bacterium was, however, not found. In subsequent epidemics (1891 and 1892) Pfeiffer, in the Hygienic Institute at Berlin, recognized a special bacillus as the exciting agent of influenza, and developed it in pure culture. The statements of Pfeiffer have since been completely confirmed.

Morphology of the Influenza-bacillus.—The influenza-bacillus is an extremely small organism (0.2 μ thick and 0.5 μ long), in thickness not quite equaling the slender bacilli of mouse-septicemia, and being only twice or thrice as long as wide. Its extremities are rounded. Rarely in the sputum and more commonly in recent pure culture, the bacilli form short pseudo-filaments. Long bands in cultures three or four days old are to be considered as beginning involution-manifestations. The influenza-bacilli possess no capsule, and are without movement of their own. Frequently, two especially short bacilli lie close to each other (division-forms). This may readily give rise to confusion with diplococci.

Influenza-bacilli appear not to possess *spores*. Spore-like formations have never been found in the secretions or in cultures, and, besides, the bacillus is but little resistant to the influence of temperature, drying, etc.

Staining of Influenza-bacilli.—The bacilli take the stain with considerable difficulty. Löffler's solution of methylene-blue may be employed, and, still better, a dilute, pale-red solution of carbolfuchsin in water. The preparation must be exposed to the action of the stain for from five to ten minutes. If the exposure is of shorter duration, or if other stains are employed, the central portion of the bacillus is often more feebly stained than the extremities. The bacilli are not stained by Gram's method.

Cultivation of the Influenza-bacillus.—The influenza-bacillus is strictly aerobic, and it develops only in the presence of hemoglobin or of leukocytes. The latter fact explains why cultivation of the influenza-bacilli remained for so long a time

unsuccessful.   Pfeiffer also was often able to cultivate the
bacilli from the sputum or pus from the lungs directly upon
agar, but this did not take place invariably, and at times it was
quite impossible to continue the growth of such cultures in any
way.   The explanation for this fact is that the bacilli developed
in the first culture when with the infecting material a trace of
blood was simultaneously transferred to the culture-medium.
Growth failed to take place, however, when the blood was
wanting, and thus also in all daughter-cultures.

The development of influenza-bacilli takes place regularly,
and the culture obtained may be continued indefinitely, if the
infecting material is inoculated upon a culture-medium contain-
ing blood, and best upon blood-agar tubes (p. 82).   For the
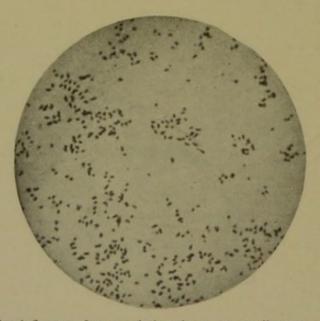
Fig. 62.—Bacillus influenzæ, from a gelatin-culture; × 1000 (Itzerott and Niemann).

development of pure cultures Pfeiffer recommends the following
method : The infecting material—bronchial sputum or fluid from
a bronchopneumonic infiltrated portion of the lung in a case
of influenza-pneumonia—is rubbed up with one or two cubic
centimeters of bouillon to a homogeneous emulsion.   By means
of a platinum loop some of this is inoculated upon blood-agar,
and also for control-purposes upon ordinary glycerin-agar, tubes,
care being taken to secure uniform distribution of the infecting
material upon the entire surface.   The dilution of the sputum
in the bouillon is intended, in the first place, to separate the
influenza-bacilli to such a degree that isolated colonies will form
upon the blood-agar tubes.   In the second place the hemoglo-
bin that may be present in the infecting material will be so
greatly diluted that the influenza-bacilli will be incapable of

developing in the control-tubes not previously treated with blood. The inoculated test-tubes are placed in the thermostat. After the lapse of twenty-four hours the influenza-colonies will become visible in the blood-agar tubes as densely packed, watery drops, whereas the control-tubes will be either sterile or present isolated colonies of streptococci, diplococci, or other bacteria, which were present in the infecting material in addition to the influenza-bacilli.

The water-like drops of influenza-colonies are usually so small that they are only visible with the aid of a lens. They exhibit little tendency to become confluent. Should they be especially numerous and close together, they coalesce to form larger drops with curved margins, but these permit a recognition of the aggregation of individual colonies. If the colonies are isolated and widely separated from one another, they may grow to the size of a pinhead, but in this case also they retain a vitreous transparency. The water of condensation of influenza-cultures generally remains clear. When mixed with blood derived from the oblique surface of the culture, delicate white flocculi form in it.

In *bouillon* mixed with blood and spread in a thin layer the influenza-bacillus thrives quite abundantly.

The *plate-procedure* is greatly to be recommended for the isolation of influenza-bacilli and for diagnostic purposes, if some blood is added to the liquefied agar before inoculation, or if agar is permitted to solidify in Petri dishes and blood is added, and the diluted sputum, etc., is smeared upon the surface in several streaks. The colonies present the same appearance as those in agar-tubes.

The *temperature-optimum* for the cultivation of influenza-bacilli is that of the body. The upper limit for growth is 42° C. (107.6° F.), the lower between 26° C. (78.8° F.) and 27° C. (80.6° F.). The bacilli do not grow at room-temperature.

Oxygen is always necessary for the development of the influenza-bacilli; they do not grow in an atmosphere of hydrogen or of carbon monoxid, even in the presence of blood.

Pfeiffer undertook to determine what constituent of the blood the influenza-bacilli require for their development. On making transfers from blood-serum or blood-fibrin to agar-tubes no growth took place. Red blood-corpuscles were invariably necessary, and particularly the hemoglobin contained in them, as was later shown, was the active substance. Hemoglobin-agar (p. 82) is equally adapted with blood-agar for the cultivation of influenza-bacilli. Pfeiffer endeavored to associate this indispensability of hemoglobin for the growth of influenza-bacilli at first with its relations to oxygen, with its faculty of acting as an oxygen-carrier. He succeeded, however, in obtaining growth upon an agar-layer in the presence of carbon-

monoxid hemoglobin. Exposure of blood-agar tubes to a temperature of 70° C. (158° F.), and even boiling of the hemoglobin, failed to prevent entirely the development of the influenza-bacilli. Pfeiffer was then led to believe that the iron-content of the hemoglobin was the important factor, but he was unable to cultivate the bacilli in culture-media containing iron other than that of the blood.

It may be mentioned further that all kinds of blood exhibit the same specific activity with relation to influenza-bacilli. Pfeiffer obtained growth upon the blood of rabbits, guinea-pigs, pigeons, and fish, and in more luxuriant degree and more speedily on pigeon's blood—which is rich in hemoglobin—than on human blood.

Resistance of Influenza-bacilli.—Influenza-bacilli are destroyed in a few minutes when exposed to a temperature of 60° C. (140° F.). They cease to develop at a temperature of 43° C. (109.4° F.), but they are only coagulated, not destroyed, for if tubes that have been exposed for forty-eight hours to a temperature of 43° C. (109.4° F.), and have remained sterile, are then exposed to a temperature of 37° C. (98.6° F.), colonies will yet develop abundantly. In unsterilized drinking-water the bacilli die quickly—in from twenty-four to thirty-six hours. Upon blood-agar and in bouillon they retain their vitality for from fourteen to eighteen days, and in moist sputum they appear to preserve their infectivity for at least fourteen days. The influenza-bacilli are quite sensitive to drying. When dried in blood or sputum at a temperature of 37° C. (98.6° F.), they succumb in an hour or two, and when dried at room-temperature, within not more than from thirty-six to forty hours.

Occurrence of Influenza-bacilli.—The influenza-bacilli occur regularly in the secretions of influenza-patients. In the secretion of the nasal cavities the specific bacilli have been found in enormous numbers; although generally associated with other microorganisms, yet, however, in preponderating number. The secretion in a case of ordinary coryza, on the other hand, is remarkably free of bacteria, being almost sterile. The sputum in cases of bronchitis and pneumonia complicating influenza is viscid, mucopurulent, globular, and not seldom also purulent and confluent, in color often yellowish green, not rarely pure white and only seldom rusty brown, and it contains the

influenza-bacilli in almost absolutely pure culture, and always in surprising number. The bacilli usually lie in the mucous ground-substance arranged in nests and groups; they are found also within the pus-corpuscles, at the beginning of the disease in small number, and during convalescence in preponderating number. In the latter condition they surround the nucleus and are not included within it. Sputa containing influenza-bacilli are often ejected for days and months. Especially in cases of tuberculosis have such chronic complications of influenza with bronchopneumonic localization not been rare. The bacilli have been found in the bronchopneumonic foci in cases of influenza-pneumonia, rarely in the pus in cases of influenza-empyema. Canon observed in the blood of influenza-patients delicate bacilli resembling those of influenza. According to Pfeiffer's investigations, these organisms, if influenza-bacilli at all, are exceptional, for, as a rule, the organisms are not present in the blood.

With regard to the *localization of the bacilli* in the gastric and nervous forms of influenza, unequivocal investigations are wanting. Likewise, the numerous complications and sequelæ of influenza have thus far been little studied from the bacteriologic standpoint, so that it has not yet been determined whether they represent results of the activity of the influenza-bacillus or its toxin, or are secondary infections. In a case of influenza-aortitis Pfeiffer found, in addition to the diplococci of Fränkel, numerous influenza-bacilli, and in the exudate from a case of influenza-meningitis diplococci exclusively.

The bacillus of influenza has never been found outside the human body, in the earth, or in water. It could scarcely persist for a long time under these conditions on account of its feeble powers of resistance.

Portals of Infection for, and Distribution of, the Influenza-bacillus.—The influenza-bacillus is probably taken up by the air-passages exclusively. Its distribution by means of dried and powdered sputum can play an etiologic rôle only in restricted degree, as the bacillus withstands drying so badly. The ordinary mode of conveyance is certainly by means of the moist nasal and bronchial secretions of influenza-patients. The widespread and often pandemic distribution of influenza may be explained by the fact that in the first place the susceptibility of human beings to

the disease is quite considerable; further, that the disease appears in many cases in the form of a mild coryza and of a harmless bronchial catarrh, and these at the beginning of the epidemic are not immediately recognized as influenza; that even after the epidemic character of the disease is established they do not confine patients to the house, so that opportunity is thus afforded, in sneezing and in coughing, to disseminate innumerable influenza-bacilli among those not yet infected. The sudden recrudescence of apparently extinct epidemics is rendered comprehensible by the fact just mentioned, that there are cases of chronic influenza that act for a long time as carriers of bacilli capable of inducing influenza.

Experiments on Animals.—Even in the most extensive epidemics of influenza domestic animals escape the disease. Success in the transmission of influenza-bacilli to animals was therefore improbable from the outset. Pfeiffer undertook experiments on mice, rats, guinea-pigs, rabbits, swine, cats, dogs, and monkeys. Only in the last-named animal was it possible to induce an infection resembling influenza, and then only by introducing the bacteria through the chest-wall directly into the lung, and also—which was more important—in a monkey, by introducing an influenza-culture into the uninjured nose. The disease manifested itself by some cough and fever of several days' duration. Multiplication of the inoculated bacteria was, however, not observed. By introducing large amounts death can be caused in rabbits quite rapidly, with antemortem depression of temperature (to 32.2° C.—90° F.); under these circumstances the symptoms are probably the result of intoxication. Symptoms of intoxication (fever, muscular paresis) also appeared in rabbits after intravenous injection of considerable amounts of bacteria. Further, cultures devitalized by chloroform proved quite toxic. The influenza-bacillus thus appears to generate an active toxin, a fact that sheds considerable light upon the nervous manifestations frequently observed in cases of influenza in human beings.

Immunity.—The monkeys in Pfeiffer's experiments reacted much less vigorously to a second injection of influenza-bacilli than to the first; and Pfeiffer believes this to be an indication of immunity. Human beings certainly can be attacked several times by influenza, even in the course of the same epidemic. This fact does not, of course, exclude the possibility that a certain degree of immunity follows an

attack of influenza, but this must, however, be considered as only temporary.

Pseudo-influenza-bacilli.—In a number of broncho-pneumonic foci in patients not suffering from influenza (but from diphtheria) Pfeiffer found bacilli that in form and staining properties resembled influenza-bacilli, and that, like these, developed exclusively upon blood-agar. Similar bacilli have since been isolated by various observers from cases of otitis media and of influenza. Pfeiffer believes these organisms to be related to influenza-bacilli, and he designates them pseudo-influenza-bacilli. They are to be distinguished from true influenza-bacilli by culture, in which, after the lapse of twenty-four hours, they appear considerably larger in all dimensions, and they exhibit a marked tendency to the formation of long pseudo-filaments. In similar cultures of the true bacillus the latter are wanting entirely or appear but exceptionally.

Bacteriologic Diagnosis of Influenza.—The bacteriologic diagnosis is made from examination of the sputum derived from the deepest possible portions of the air-passages, and thus preferably from examination of the bronchial sputum. Microscopic examination alone is usually insufficient, but, as a rule, cultivation of the bacillus is necessary, the method for which has already been described in detail (p. 283, Plate-procedure). Bacteriologic examination may acquire differential diagnostic significance. Thus, Borchardt relates a case in which the diagnosis oscillated for a long time between typhoid fever and influenza, until bacteriologic examination decided in favor of the latter.

ANTHRAX.

Pollender, in Germany, in 1849, and Rayer and Davaine, in France, in 1850, were the first, independently of each other, to detect bacilli in the blood of animals suffering from anthrax. The growth of the anthrax-bacillus in pure culture and the experimental development of the disease by means of the bacillus were successfully accomplished first by Koch in 1876.

Morphology of the Anthrax-bacilli.—Anthrax-bacilli appear as transparent, homogeneous, nonmotile rods. They are from 1 to 1.5 μ thick, and from 5 or 6 to 10 μ long, but they

are subject to great variations in size. In cultures the bacillus is prone to be considerably longer than in the animal organism. In the blood of human beings it is shorter than in that of rodents; in cattle it is shorter than in white mice and in guinea-pigs. The broad side of the bacillus is slightly rounded. The surfaces of two adjacent bacilli in direct contact are, however, plane. In the blood of animals suffering from anthrax the bacilli are at times collected into small filaments of two or four, or at most five, members. Only attenuated bacilli, just capable of causing death in experimental animals, form long filaments in the organs of the animals. Such filaments are usual in the body of the frog. In cultures, on the other hand,
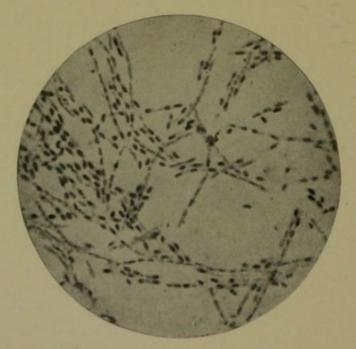
Fig. 63.—Bacillus anthracis, stained to show the spores; × 1000 (Fränkel and Pfeiffer).

the anthrax-bacillus exhibits an especial tendency to the formation of long, intertwining chains. Frequently, bacilli obtained from the lesions of the disease present a bright border that some observers have looked upon as a capsule. This supposed capsule may be demonstrated by the staining method of Johne in the following manner: The preparation made from the blood or visceral fluid and dried in the air is passed three times through the flame; stained for from fifteen to thirty seconds in a slightly warmed, two per cent. aqueous solution of gentian-violet; washed in water; exposed for ten minutes to the action of a one per cent. solution of acetic acid; and examined in water. It is important to make the examination in water, as the mucoid capsule is not visible when the specimen is

mounted in Canada balsam. The bacillus can be stained with all aniline dyes and also by the method of Gram.

Cultivation of Anthrax-bacilli.—The anthrax-bacillus is extremely indifferent with regard to its nutrient material. It will develop in the absence of oxygen, but it does not then generate a peptonizing ferment. The temperature-minimum is 12° C. (53.6° F.); the temperature-optimum, 35° C. (95° F.); the temperature-maximum, 45° C. (113° F.).

On *gelatin-plates*, with a magnification of from 80 to 100, the superficial colonies appear as round discs of yellowish color, constituted of a tangle of threads which form a dense, impenetrable convolution at the center. Especially the border presents
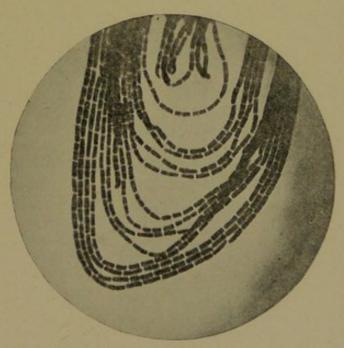
Fig. 64.—Bacillus anthracis: colony three days old upon a gelatin-plate; impress-preparation; × 1000 (Fränkel and Pfeiffer).

quite distinctly this filamentous structure, and from it frequently passes off a delicate network of convoluted and curled processes, which gives the colonies their characteristic appearance. The gelatin in the neighborhood is softened, and begins slowly to undergo liquefaction.

In *gelatin stab-cultures* the line of inoculation forms a whitish band, from the circumference of which numerous ramifying processes extend into the gelatin. Liquefaction of the culture-medium progresses slowly, the nonmotile bacilli, by reason of their weight, sinking to the bottom of the liquefied area.

In *bouillon* small fragments appear and fall to the bottom; they represent a union of the anthrax-bacilli for the formation of convolutions of filaments.

19

On *agar-agar* a dense, creamy, coherent deposit forms; and upon *potatoes* a dry, whitish-gray layer. *Blood-serum* is liquefied. *Milk* is coagulated and peptonized.

Sporulation.—If anthrax-cultures are exposed to temperatures above 18° C. (64.4° F.), the bacilli soon exhibit *spore-formation*. Slight development of spores takes place, besides, down to the temperature-minimum. The anthrax-spore invariably lies exactly in the middle of the mother-cell, being much shorter, but as wide, and possessing an oval shape. After a time the germinating bacillus disintegrates, and the spore is set free. If the spore is introduced into a sterile hanging drop of bouillon, gelatin, or agar, its germination can be accurately followed under the microscope (possibly with the employment of a warm stage). The spore first loses its glistening appearance, and increases in volume. Its membrane then ruptures at one extremity and permits the escape of the newly formed bacillus. The young bacillus enlarges in the direction of the long axis of the spore, and soon throws off the still adherent spore-membrane. Spore-formation takes place only in the presence of free oxygen—thus never in the animal body and never in the uninjured cadaver.

The higher the temperature the more rapidly do the spores develop—at 37° C. (98.6° F.), as early as twenty hours; at 21° C. (69.8° F.), not before seventy-two hours; while above 42° C. (107.6° F.) the anthrax-bacilli do not generate spores at all. It is possible artificially to deprive the anthrax-bacilli of their capability of generating spores. For this purpose it is only necessary to add certain antiseptic substances to the nutrient medium (Chamberland and Roux): for instance, carbolic acid in the proportion of from 1 : 600 to 1 : 1000, potassium bichromate in the proportion of 1 : 2000. The bacilli cultivated upon such nutrient media remain sporeless permanently in all subsequent generations, and in this way special asporogenous anthrax-bacilli are developed.

Resistance of Anthrax-bacilli and of Anthrax-spores. —Anthrax-spores, like all other spores, are exceedingly resistant structures; while the fully developed bacilli succumb after exposure for a quarter of an hour to temperatures in the neighborhood of 60° C. (140° F.), the spores die in compressed steam at a temperature of 107° C. (224.6° F.) only after the lapse of five minutes; and in live steam at a temperature of 100° C. (212° F.) only after from twelve to fifteen minutes. Five per cent. carbolic acid destroys the mature forms of anthrax-bacilli in ten seconds, but the spores not before from thirty-seven to forty days. In

1 : 1000 solutions of mercuric chlorid the spores are not destroyed earlier than after the lapse of twenty hours (Geppert). In sterilized distilled water or tap-water anthrax-bacilli survive only for three days, but the spores for from one hundred and fifty-four days to a year. The lower, further, the temperature, the better do the anthrax-organisms withstand the injurious influence of the water. Anthrax-bacilli in distilled and other water have been demonstrated to undergo sporulation at a temperature of 20° C. (68° F.). The bacilli are rapidly destroyed by the influence of putrefaction, while the spores have been found alive in putrefactive mixtures after a month.

The statements made apply only to robust spores, the descendants of highly virulent bacilli. Anthrax-spores of indifferent source do not invariably react alike; thus, von Esmarch was able to render some anthrax-bacilli innocuous by means of 5 per cent. carbolic acid within two days, and by means of live steam at a temperature of 100° C. (212° F.) within three minutes.

The Occurrence of Anthrax in Animals.—The animal most susceptible to anthrax is the sheep, and it is often attacked by the disease spontaneously. There is, however, one variety of sheep that is immune—namely, the Algerian sheep. This immunity is not dependent upon climatic conditions, for European sheep transported to Algiers were as readily attacked by anthrax as at home. White mice and guinea-pigs succumb likewise with regularity to experimental anthrax-infection; rabbits are somewhat more resistant; but none of these three species of animals is scarcely ever exposed to natural infection. Cows and calves not rarely succumb to spontaneous anthrax, although they prove rather refractory to experimental subcutaneous infection. Horses, antelope, deer, goats, at times acquire anthrax; swine more rarely. With regard to the much-discussed immunity of white rats to anthrax, it appears that young animals die of anthrax after experimental infection, whereas old animals usually exhibit only a local lesion, from which they recover. Carnivorous animals (dogs, cats, etc.) rarely are attacked spontaneously by anthrax. Experimentally, the disease can be induced in mature animals only by intravenous injection of the bacilli. New-born or quite young dogs, on the other hand, prove extremely susceptible. Birds, reptiles, batrachians,

possess a considerable degree of immunity to anthrax. If, however, on the one hand, the high temperature of birds is reduced artificially ; or if, upon the other hand, the low temperature of reptiles is elevated artificially, these animals react precisely like other animals—that is, they succumb to inoculation with anthrax. Pigeons may also be rendered susceptible to anthrax-infection by starvation.

Occurrence of Anthrax in Human Beings.—Human beings are infected, as a rule, through contact with animals suffering from anthrax, and with the cadavers of those dead of the disease. Not only the fresh cadaver is infectious, but also, owing to the presence of spores, which develop rapidly in summer with unobstructed access of oxygen, every individual portion of the body—wool, hair, horns, etc.—even after the lapse of a long time. For this reason tanners are exposed to danger in manipulating the skins of animals dead of anthrax. Even after the hide has been tanned, the spores are not always destroyed with certainty, and shoemakers, furriers, and harness-makers have been known to be infected by means of such leather. Workers in horsehair, turners of horn, brush-makers, dealers in hides, etc., are likewise exposed to the danger of infection with anthrax in so far as the raw material with which they work is derived from animals suffering from anthrax. The following instructive example, in this connection, has been reported by Einike : An ox dies of anthrax. Two individuals who partake of its meat die of the same disease. The hide of the animal, after it has been macerated for a time in a small lake, is used by a harness-maker for making halters. This man is attacked by anthrax. The two horses that wear the halters likewise succumb to this disease. Of a herd of sheep that bathe in the small lake mentioned twenty are attacked by anthrax.

The transmission of anthrax from animals to human beings may further be effected by certain varieties of flies that possess a stiff and pointed sting (stomoxys, glossina, ixodes). In the bodies of such insects as have fed upon anthrax-cadavers anthrax-bacilli have been repeatedly demonstrated.

Human beings are not especially susceptible to anthrax. For this reason only a local lesion generally forms at first— the so-called *malignant pustule* (anthrax-carbuncle)—which frequently terminates in recovery. In other cases the car-

buncle is succeeded by septicemic *general infection*, leading to death. Anthrax may be further transmitted from one human being to another. In the nature of things such an occurrence is rare, although it has been reliably observed. Jacobi has reported four cases in which the disease was conveyed by means of a hypodermic syringe that had previously been employed in a case of anthrax.

The infectivity of malignant pustule was formerly denied, and upon the basis of experiments in which the serous contents of the pustules were inoculated in healthy individuals, without the development of any reaction whatever. Such experiments are, however, not conclusive, as the serous contents of the vesicle, which surround the central eschar, contain only a small number of bacilli.

Natural Portals of Entry for the Anthrax-bacillus.—
(*a*) *Skin.*—Infection through the skin is possible only in the presence of a breach in continuity, however slight. In human beings this mode of infection is the most common, and particularly in individuals that come in contact with anthrax-cadavers or constituent parts thereof. The course of infection has already been outlined. At the point of infection a carbuncle develops, and this leads either to recovery or to general infection.

(*b*) *Digestive Tract.*—Spontaneous anthrax of herbivorous animals almost always involves the intestine (pasture-anthrax). If the herds graze on some meadow-lands in certain districts (champs maudits), anthrax is sure to occur among them. In Germany such regions exist in some parts of Saxony and in the Bavarian Alps, in France especially in the district Beauce, in Austria in the Hungarian lowlands, and in Russia in the neighborhood of Novgorod. It is believed that the earth in these meadow-lands contains spores, which are swallowed by the animals in grazing. The question naturally arises, How are the anthrax-spores brought to the surface of these fields? Pasteur, who was the first to occupy himself with this question, found anthrax-spores in the earth of graves in which years before anthrax-cadavers had been buried. It was his opinion that these spores are carried to the surface from the depth by earth-worms. The worms swallow the contaminated earth, later crawl upward to the surface, and there deposit the spores with their excrement. Such a possibility has been demonstrated experimentally, and that

it may actually be realized would appear from the observations of Bollinger, who succeeded in finding anthrax-spores in the bodies of worms collected from the anthrax-fields of the Bavarian Alps. The view of Pasteur was vigorously attacked by Koch. At the depth at which anthrax-cadavers are usually buried (from ½ to 1 meter) the temperature, even in the hottest summer-months, is only between 14° C. (57.2° F.) and 18° C. (64.4° F.), a temperature that is extremely unfavorable for sporulation. According to Soyka, the addition of porous particles of earth to artificial cultures furthers spore-production quite materially. Then, it is further to be borne in mind that the temperature of the buried cadavers is certainly raised somewhat in consequence of the putrefactive processes taking place. Kitasato buried gelatin-cultures and agar-cultures of anthrax in the earth and showed that at a depth of one meter sporulation proceeded in the months of June, July, and August. The conditions provided experimentally and artificially are, however, not entirely comparable with those observed practically. Animals dead of anthrax are, or were formerly, first dissected and skinned, the secretions, the blood, etc., being spilled indiscriminately; burial also is not effected immediately; in brief, abundant time and opportunity are afforded the bacilli, at least in summer, to form spores. The cadaver, which is now buried, contains not only mature forms, but also permanent forms; quite apart from the fact that numerous anthrax-bacilli are already present upon the surface of the earth in consequence of the various manipulations at the place where the autopsy was held. It is true that if the animal is buried at a depth of two or three meters immediately after death, without further manipulation, the formation of spores would certainly be entirely prevented; for, then, one of the most important conditions for spore-formation—namely, unobstructed access of oxygen—would be completely wanting.

In addition to earth-worms anthrax-spores may be disseminated by means of snails, flies, etc. Further, the diseased animal furnishes during life sufficient material for subsequent infection through the feces and the urine, which contain the bacilli in large number. In these, and also in vegetable culture-media, the bacilli, which are so indifferent with relation to the nature of the nutrient material, multiply abundantly, and in summer also form spores. The excre-

ment of animals fed with anthrax-matter contains spores regularly. Also, the feces of healthy animals (sheep) that have grazed upon anthrax-fields contain spores under certain conditions. The introduction of anthrax-bacilli into the gastro-intestinal tract does not invariably induce anthrax. The spores in some cases pass through the digestive tract without causing injury, although they remain as infective for other animals as they were formerly. Also, this mode of infection presupposes the existence of an injury or a lesion of the mucous membrane. With hay obtained from anthrax-fields the spores gain entrance into stables and give rise to so-called stable-epidemics. As a result of floods in the infected districts, the germs are carried far and wide, and thus give rise to cases of anthrax at places where previously the disease was unknown.

In human beings gastro-intestinal anthrax is by no means so common as malignant pustule; it was described by earlier observers as *intestinal mycosis*. Probably it occurs more commonly than current reports would indicate. The cases pursue the clinical course of dysentery or of typhoid fever, and if bacteriologic examination is omitted at the autopsy, they may readily escape diagnosis.

(c) *Lungs.*—Pulmonary anthrax has been repeatedly observed in England (Bradford) in individuals who pull sheep's wool or work with goat's or camel's hair and the like (*wool-sorter's disease*), and in Germany in those who assort rags (*ragpicker's disease*).

Experimental Development of Anthrax.—All of the modes of infection that have been mentioned as possible in accordance with clinical experience may be imitated in experiments on animals. Susceptible animals die of anthrax-septicemia after cutaneous and subcutaneous inoculation. The spleen in such animals is quite considerably enlarged at autopsy, its consistency is soft and friable, its color dark. If spore-containing material is fed to sheep, four among ten animals, on an average, die after the introduction of small amounts; if large amounts are used, almost all die. The spores, thus, escape uninjured the influence of the acid gastric juice. Anthrax occurs but seldom in rabbits, guinea-pigs, and white mice as a result of feeding-experiments.

Buchner has made careful observations with regard to the occurrence of pulmonary anthrax. When the spores are inhaled experimentally, the animals die of general infection.

The permanent forms penetrate the intact mucous membrane of the alveoli, and gain entrance into the lymphatics and blood-vessels, in which they germinate. After inhalation of anthrax-bacilli, however, such migration does not take place. The bacilli remain inactive, and cause only local irritation—circumscribed inflammation of the lungs. In this connection it may be noted that in quite rare cases of anthrax the disease has also in human beings pursued the clinical course of septicemia—that is, without local infection and without malignant pustule. The portal of infection remained undiscovered in these cases, although it is possible that this part was taken by the lungs.

Distribution of Anthrax-bacilli in the Infected Body. —In cases of general infection with anthrax the bacilli are found in the blood, and in only a small number of instances in the larger vessels, the greater amount being present in the capillary area. The capillaries of all the organs, especially the spleen and the liver, are filled by the bacilli, sometimes actually packed with them, so that their lumen appears to be occluded. Most of the bacilli lie with their long axes parallel to the walls of the vessel—that is, they are directed in the course of the blood-stream. In the glomeruli of the kidneys and in the intestinal villi the mass of bacteria frequently induces capillary hemorrhages. The microorganisms in this way gain entrance into the uriniferous tubules, although generally they do not progress beyond the convoluted tubes (Koch). Anthrax in animals may, therefore, be considered as the type or paradigm of a true infection, the factor of intoxication remaining entirely in the background and the most prominent feature being the enormous multiplication of the exciting agents. On examining the viscera of animals dead of anthrax the impression is gained that death has resulted from a flooding of the capillary area with the bacilli. The anthrax-bacilli are, however, by no means found in the blood immediately after infection, but several hours always elapse before they appear. The animal, however, will previously have been sick. This indicates that the formation of toxin on the part of the bacteria is not entirely wanting—a circumstance whose certainty is established by the course of the disease in less susceptible animals (and in human beings), in which the lesion remains localized.

In cases of malignant pustule the bacilli are present in

considerable number only before the wall of leukocytes that separates the eschar from the subjacent tissues. They surround the hair-follicles and the sweat-glands, but their distribution bears no relation to the blood-vessels.

The infiltrates in cases of intestinal anthrax are, both histologically and bacteriologically, to be placed upon the same plane as malignant pustule. The mesenteric glands are swollen and filled with the parasites.

In cases of pulmonary anthrax if a local lesion has developed, the bacilli are found in the perivascular lymph-spaces. If a lesion is wanting at the portal of entry, the swollen bronchial glands nevertheless constantly contain the bacilli.

Death occurs in every variety of anthrax, usually as a result of general infection. In addition to the blood the parasites pass over into the milk, the bile, the saliva, and the feces. They are found less commonly in the urine.

Toxins of Anthrax-bacilli. — Practically nothing is known with regard to the metabolic products of the anthrax-bacillus. Hankin obtained a toxic albumose from pure cultures. With regard to the relation between this albumin-ous substance and the actual anthrax-toxin, the same state-ment may be made as that with regard to the toxalbumins of Brieger and Fränkel (pp. 29, 30).

Mixed Infection.—In cases of anthrax mixed infection is not without significance. The limiting suppuration, which brings about the exfoliation of the malignant pus-tule or of the intestinal filtrate, may be excessive and give rise to phlegmons and to septicemia or pyemia ; many a pa-tient who has survived the anthrax-infection proper has died later of these secondary suppurative processes. From the blood and from the internal viscera staphylococci and streptococci may then be cultivated.

Heredity.—In the case of anthrax it has been demon-strated experimentally that the bacillus passes from the mother to the fetus only when changes have taken place in the placenta—minimal hemorrhages will suffice. Intrauter-ine infection with anthrax has also been observed in human beings (Marchand). As bearing upon the question whether the fetus in utero may infect its mother, the experiments of Lingard are interesting. This observer infected rabbit-fetuses in utero with anthrax. As a rule, the mothers were not infected, but they later proved immune to the disease,

the immunity lasting for more than eight months. In this way would be demonstrated the law for anthrax that Colles propounded for syphilis (p. 318).

Bacteriologic Diagnosis.—From the deeper parts of a suspected pustule some tissue-fluid is removed and plates are made therefrom. If suspicious colonies develop, pure cultures are made and animals infected therewith. If intestinal anthrax is suspected, the vomited matters and the feces should be examined bacteriologically. In cases of pulmonary anthrax the abundant frothy sputum at times contains the bacilli. Examination of the blood will yield information as to whether general infection with anthrax is present or not. Such examination is, therefore, always of the greatest importance in the formulation of the prognosis. It must, however, always be borne in mind that the bacilli are present especially in the capillary area.

For sanitary purposes it is frequently necessary to decide whether an animal has died of anthrax or not. If death has taken place but a short time previously, microscopic examination of fluid from the spleen and of the blood will suffice. The presence of the bacilli with capsules, demonstrable by the method of Johne (p. 288), renders the diagnosis positive. If, however, one or more days have elapsed since the death of the animal, then there develop in the body of animals dead of anthrax cadaver-bacilli that can be differentiated from the specific exciting agents of anthrax only with exceeding difficulty. Under such conditions gelatin-plates must be made, and mice and guinea-pigs inoculated with fluid from the spleen.

In inoculating the animals the possibility of the presence of the bacillus of malignant edema must be borne in mind, as this microorganism likewise is not rarely found in the bodies of dead animals. On subcutaneous inoculation the latter predominates, and the animals die of malignant edema notwithstanding the presence of anthrax-bacilli. This source of error can be avoided by inoculating the animals cutaneously with the suspected material. The anthrax-bacillus alone will then develop, and the diagnosis can not go astray. If putrefaction has progressed too far in the body to be examined, then the anthrax-bacilli may be completely suppressed by the competition of the other varieties of bacteria, so that the diagnosis is no longer possible.

Immunity and Vaccination.—Recovery from malignant pustule does not confer immunity, at least none of any duration. It has been repeatedly observed that the same individual has been attacked two or three times with malignant pustule, and even at intervals of a few months. The second attack often pursues even a more virulent course than the first. Nevertheless, it is possible experimentally to immunize animals to anthrax. The anthrax-bacillus is the microbe with which Pasteur demonstrated for the first time that the action of heat diminishes the virulence materially. Pasteur cultivated anthrax-bacilli at a temperature of 42° C. (107.6° F.). They still developed at this temperature, but gradually suffered in virulence from day to day. If after the lapse of several days the bacilli thus attenuated are transferred to a new nutrient medium and are exposed to a more favorable temperature (35° C.— 95° F.), they then grow luxuriantly again, but their virulence remains attenuated; the bacilli do not recover. Upon the basis of these fundamental facts Pasteur prepared two varieties of attenuated anthrax-bacilli, one of which had been exposed for from fifteen to twenty days to a temperature of 42° C. (107.6° F.), and which was capable of destroying only guinea-pigs not more than twenty-six hours old (Vaccine I). The other had been exposed from ten to twelve days to a temperature of 42° C. (107.6° F.), and it was capable of destroying guinea-pigs, white mice, and rarely rabbits (Vaccine II).

According to the method of Pasteur, animals (rabbits, sheep, cows) are inoculated first with Vaccine I. They become sick and exhibit fever of greater or less intensity. After the disease-symptoms have disappeared, the animals are inoculated with Vaccine II, and the same symptoms are repeated. After the animals have recovered from this second disturbance they are thoroughly protected against inoculation with highly toxic anthrax-material.

The attenuated anthrax-cultures generate alkaline substances upon the nutritive media; whereas the virulent cultures generate a considerable amount of acid. The vaccines are distinguished from the highly virulent cultures, further, by the fact that they give rise to febrile movement. This fact is usually interpreted as indicating that the fever represents a reaction of the organism to the invading parasites. As a matter of fact, it has for a long time been

a prognostic rule clinically, peculiarly in the case of anthrax, that complete apyrexia is a sign of ill omen.

Pasteur's method of vaccination confers complete and secure protection against subcutaneous anthrax. As to its protective power with regard to field-anthrax, opinions have differed. Koch, Löffler, and Gaffky express themselves with some reserve in this connection. They do not believe that the protective inoculation confers absolute immunity to intestinal anthrax. Statistics, however, appear unquestionably to favor the view of Pasteur. Since the practice of vaccinating the herds has been instituted in France, the mortality from anthrax among grazing cattle has been materially reduced.

The blood-serum of animals immunized to anthrax does not, so far as is known, transmit immunizing properties to other animals.

Prophylaxis.—The prophylaxis against anthrax consists in rendering innocuous the bodies of animals and human beings dead from the disease. This end is attained in the simplest manner by incinerating the bodies as a whole. The danger of infection, however, is maintained by the introduction and manipulation of suspicious material (hair, wool, rags, leather) from foreign countries in which the sanitary precautions observed by most intelligent nations are not enforced. In England, the home of wool-sorter's disease, regulations have been put in force requiring that wool, which is derived principally from Asia, should be boiled before it is assorted. Since, the disease has materially diminished. In the same spirit the disinfection of skins, hair, wool, etc., of suspicious origin should be everywhere insisted upon before being manipulated.

GLANDERS.

The exciting agent of glanders was discovered in 1882 by Löffler and Schütz.

Morphology of the Glanders-bacilli.—The glanders-bacilli are small, slender, nonmotile rods, at times curved, with rounded extremities, from 2 to 3 μ long, and from 0.2 to 0.4 μ thick. As a rule, they lie singly. The question whether glanders-bacilli form *spores* is still an open one, though answered in the affirmative by Baumgarten and Rosenthal, who succeeded in staining spores, and in the negative by most observers, because

the germination of the so-called spores has not yet been observed.

The glanders-bacillus takes all stains without difficulty, but it is decolorized with equal facility. The best specimens are obtained in cover-slip preparations by treatment with hot Löffler's solution or hot carbolfuchsin, and decolorization with distilled water. The organisms do not stain by Gram's method. In stained preparations the bacilli exhibit almost regularly unstained deficiencies. The bacilli frequently appear in the form of short structures resembling cocci. They are facultative anaerobic. The temperature-minimum is 25° C. (77° F.) ; the temperature-optimum, 37° C. (98.6° F.) or 38° C. (100.4° F.) ; the temperature-maximum, 42° C. (107.6° F.).

Fig. 65.—Bacillus mallei, from a culture upon glycerin agar-agar ; × 1000 (Fränkel and Pfeiffer).

Cultural Properties.—On *glycerin-agar plates* glistening, granular colonies, with a yellowish tinge and a smooth border, appear.

Streak-cultures upon Glycerin-agar.—Along the line of inoculation a moist, whitish coating forms. Upon blood-serum isolated, translucent, yellowish drops appear, which do not liquefy the culture-medium. Upon *gelatin* slight growth takes place at 25° C. (77° F.), with slight liquefaction. *Bouillon* is rendered densely turbid. The growth of glanders-bacilli upon *potato* is characteristic. The inoculated surface exhibits after two days a thin, honey-yellow deposit, which after a week becomes quite dark, brownish-red, and surrounded by a slightly blue, iridescent zone. The best culture-medium is glycerin-agar.

Resistance of Glanders-bacilli.—For the continued cultivation of glanders-bacilli it is important to know that the organisms rapidly undergo *natural attenuation* as early as the fourth or fifth generation. If, therefore, it is desired to obtain virulent bacilli, it is necessary to interpolate inoculations of animals every two or three culture-generations.

In the dry state the glanders-bacilli, according to Löffler, retain their vitality for three months. Other observers, however, have found them dead within ten days when dried in a thin layer. Toward heat the glanders-bacilli prove quite resistant. They are destroyed in two minutes at a temperature of 100° C. (212° F.); in five minutes at a temperature of 80° C. (176° F.); in fifteen minutes by 1 : 1000 mercuric chlorid; and in an hour by five per cent. carbolic acid.

Susceptibility of Animals to Glanders.—Among domestic animals there are susceptible in diminishing degree asses, mules, horses, goats, cats, sheep, dogs, swine. Cattle are immune. Among experimental animals field-mice, wood-mice, and guinea-pigs exhibit the most pronounced, and rabbits a much slighter, predisposition. White mice and domestic mice prove entirely insusceptible to glanders. If, however, white mice are previously poisoned with phloridzin, they succumb to infection with glanders (Leo). Birds, with the exception of pigeons, are refractory to glanders. In all animals glanders is at first a local disease, but later it becomes generalized and attacks all of the viscera.

Occurrence and Distribution of the Bacilli in the Products of the Disease.—From a pathologic-anatomic standpoint glanders belongs to the group of diseases that, like tuberculosis, give rise to the formation of nodules that exhibit a marked tendency to disintegration and softening. The small, often only submiliary, grayish newformations consist of epithelioid cells and mainly of leukocytes. The glanders-bacilli are present especially at the center of the granulations. They are demonstrable with difficulty by staining. The best mode of procedure consists in placing the sections for from six to eight hours in carbol-methylene-blue (methylene-blue 1, absolute alcohol 10 cu. cm., carbolic acid, 5 per cent., 100 cu. cm.) or carbolfuchsin, next decolorizing in dilute acetic acid, and then in distilled water, drying upon a slide, and, after clearing in

xylol, mounting the preparation in xylol Canada balsam. The sections may also be treated according to Weigert's method (p. 106), Löffler's alkaline methylene-blue solution being selected for staining. In this way positive results are obtained only with the relatively recent nodules. If necrosis has taken place, the bacilli are only rarely found in the products of disintegration.

Experiments on Animals.—Small numbers of bacilli inoculated subcutaneously into susceptible animals (generally guinea-pigs or field-mice), or larger numbers rubbed into the uninjured skin, cause death with considerable certainty. The mice die quickly—within three or four days— and the spleen, the liver, and the lungs are filled with an enormous number of nodules scarcely visible to the naked eye. Guinea-pigs are better adapted for the study of the course of infection. In them a local infection first develops, an infiltration, that soon is converted into an ulcer with indurated margins. Then follow swelling and suppuration of the adjacent lymphatics and lymph-glands, and finally, general infection with the characteristic new-formations. The process advances by way of the lymph-paths. Apart from cases pursuing a very acute course, the blood almost never contains the bacteria. The infection of the lymphatic apparatus extends with exceeding rapidity. As early as an hour after inoculation of a superficial skin-wound cauterization of the latter will no longer suffice to prevent the development of the disease. The urine, the seminal fluid, the sweat, the saliva, and the aqueous humor of infected animals may contain the parasites. The spleen and the bile are said to be free from them.

Portals of Entry For and Course of Glanders.—In human beings, who, in the vast majority of cases, are infected by contact with horses suffering from glanders, the skin, without doubt, constitutes the principal portal of infection. The individuals in question, usually hostlers, coachmen, farmers, cavalrymen, and the like, may be infected through the intermediation of the most superficial and most insignificant wounds of the skin. Laboratory-infection with glanders, in manipulating infective material and glanders-bacilli, has been observed repeatedly. Infection sometimes takes place also through the mucous membranes. Cases are on record in which hostlers have acquired the disease by drinking from the same pail as their sick horses, and the

like. Whether or not infection may take place by way of the respiratory apparatus has not yet been decided with certainty. Nevertheless, it is remarkable that in horses glanders, in its initial stage, is seated in the nasal cavity (glanders-ulcer). Bollinger believes that, especially in those attacked by glanders who present general symptoms in advance of the local manifestations, the virus has gained entrance into the body through the respiratory passages. The use of meat from animals suffering from glanders may, likewise, give rise to the disease. At least a number of feeding-experiments in animals are in favor of this view. Such experiments have yielded positive results in cats, dogs, lions, and bears. On the other hand, other experiments with the feeding of glanders-material have yielded negative results. The muscles themselves do not harbor the parasites, but rather the lymphatic vessels and glands lying within them or near them.

The clinical picture of glanders in human beings is a rather variable one, in accordance with the site of infection. Usually there occurs locally at the portal of infection a swelling, and this is soon followed by tumefaction and suppuration of the neighboring lymphatics. Then multiple abscesses form in the skin, the muscles, and the internal viscera, and often suppuration takes place in joints. The clinical picture resembles that of pyemia. Upon the mucous membranes, and particularly in the nose, characteristic glanders-nodules appear, which soon disintegrate and give rise to ulcers. Death results from the general infection, which takes place in human beings by way of the lymphatics. An acute and a chronic variety of glanders have been observed. In the former suppuration is the more likely to occur; in the latter—so-called farcy—tissue-proliferation is the more conspicuous.

Heredity.—The transmission of glanders-bacilli from the mother to the fetus has been observed repeatedly. The following observation by Löffler is interesting. A female guinea-pig that had recovered from inoculation with glanders gave birth to offspring five months later. Apparently healthy at birth, the young animal died of visceral glanders at the age of a week.

Bacteriologic Diagnosis of Glanders.—The bacteriologic diagnosis has to contend with the difficulty that in the course of suppurative destruction in the foci of disease the

bacilli also are destroyed. In suspicious cases, however, agar-plates should always be made with the pus from the ulcers in question. In addition male guinea-pigs should receive intraperitoneal injections of the suspected secretion. If the disease be glanders, swelling of the testicles takes place in the course of two or three days. This is absolutely characteristic, and is subsequently followed by suppuration of the organs (Straus). The injection must always be made in the median line, as otherwise there is danger of injuring the seminal vesicles, and as by direct injection into these structures other microorganisms also may give rise to swelling and suppuration of the testicles.

Mallein.—In exactly the same manner as tuberculin is prepared, von Preusse and Kalning obtained from cultures of glanders-bacilli a lymph—mallein—representing a proteid substance of the glanders-bacilli. Mallein is employed in veterinary medicine for diagnostic purposes. Horses suffering from glanders react to injection of the lymph with fever.

Prophylaxis.—The most efficient prophylaxis against glanders consists in the destruction of animals suffering from the disease, and the incineration of their carcases. The remaining horses in a stable in which the disease has appeared must be subjected to rigid quarantine, and the attendants must have their attention called to the danger of infection and be enjoined to careful disinfection.

MALIGNANT EDEMA.

The exciting agent of malignant edema, which was described in 1881 by Koch, is identical with the vibrion septique of Pasteur.

The **bacillus of malignant edema** (vibrio septicus) is a slender bacillus about as long as the anthrax-bacillus, but somewhat narrower (from 0.8 to 1 μ), with rounded extremities. It possesses spontaneous motility, which is due to from three to twelve lateral flagella. The vibrio septicus has a tendency to form long filaments, both in culture and also—in contradistinction from the anthrax-bacillus—in still greater degree in the living body. The chains not rarely present pretty, arch-like curves. The temperature-optimum is 37° C. (98.6° F.), although the bacillus will grow at room-temperature. Above

20

20° C. (68° F.) it is capable of sporulation. The spore, situated centrally, is at times wider than the bacillus, which then assumes a spindle-shaped, distended appearance. The vibrio septicus is a rigidly anaerobic organism. It stains without difficulty with all of the aniline dyes, but not by Gram's method.

Cultural Properties.—On *gelatin-plates* the colonies present the appearance of glistening, hollow globules, filled with fluid. At their center, with a magnification of from 80 to 100, a dense network of intimately intertwined threads may be seen, with radiating processes at the periphery. On more careful examination it will be observed that the colony is in motion.
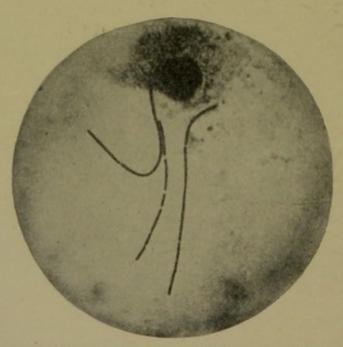
Fig. 66.—Bacillus of malignant edema, from the body-juice of a guinea-pig inoculated with garden-earth; × 1000 (Fränkel and Pfeiffer).

On *agar-plates* small, irregular, whitish, translucent colonies appear, with a dense center, from which innumerable delicate ramifications proceed.

In *high gelatin stab-culture* liquefaction and turbidity of the underlying gelatin take place, with abundant formation of gas, especially on addition of reducing substances.

In *high agar stab-cultures* the line of inoculation exhibits serrated branched margins. Abundant gas-production takes place.

Bouillon is rendered turbid, and later a precipitate forms. The reaction is not changed, but carbon dioxid and hydrogen develop.

All cultures generate a disagreeable, fetid odor.

Experiments on Animals.—Horses, swine, sheep, goats, dogs, pigeons, ducks, hens, rabbits, mice, and guinea-pigs are susceptible to malignant edema. In guinea-pigs sub-acute infection pursues quite a characteristic course. The animals cower, their hair stands erect, and signs of great fear set in. On the slightest touch the animals cry out. Death takes place within twelve hours. On autopsy there is found at the site of inoculation quite considerable bloody edema, with a small number of gas-bubbles, involving the subcutaneous connective tissue and the superficial muscles. In this area the bacilli with spores are found in large numbers. On the other hand, the blood and the internal viscera are always free from bacteria if the autopsy is performed immediately after death. As long as the animal continues to live, the anaerobic edema-bacilli are not capable of multiplying in the oxygen-containing blood. Only after death has taken place do they advance, and soon the viscera and the blood are filled with them. The spleen of the animal is large and diffluent. The liver and the lungs are pale, and the latter are of a peculiar grayish-red color. In mice the bacilli gain entrance into the blood and the internal viscera during life.

In the main, malignant edema represents an *intoxication*. As a matter of fact, precisely the same clinical picture can be developed experimentally in animals if the mature bouillon-culture or the serum from the edema freed of bacteria by passage through a porcelain filter is injected intra-peritoneally into guinea-pigs in rather considerable amount.

Occurrence of the Vibrio Septicus.—The bacilli of malignant edema, or rather their resistant permanent forms, are widely distributed in nature. They are the attendants of putrefactive processes, especially those that take place in the absence of oxygen. They are found present in manure, in dust, and in the earth of gardens and of fields. If a small amount of earth be introduced into a pocket beneath the skin in a guinea-pig or a rabbit, the animal will die of malignant edema. The picture developed under these conditions is, however, not a pure one. In addition to the septic vibrios a large number of other microbes will be found present in the edema, and as a result of this mixed infection the exudate is not merely bloody and serous, but putrid and fetid.

Malignant Edema in Human Beings.—Malignant edema occurs at present but seldom in human pathology. In preantiseptic times it was more common and more alarming. The so-called septicémie gangreneuse and the gangrène gazeuse of the French were in part manifestations of the activity of the vibrio septicus, and identical with our malignant edema. In order that the bacillus of malignant edema may exhibit its activity, the wounds through which infection takes place must be deep, because the strictly anaerobic parasite has no opportunity for development upon the surface. The bacillus may, further, be associated in mixed infection with other microorganisms, which consume the oxygen available, and thus artificially produce an atmosphere free from this gas. It may be mentioned that in two patients suffering from typhoid fever malignant edema has been observed after subcutaneous injection of tincture of musk.

The **portal of infection** is always constituted by a breach in continuity of the external integument.

Bacteriologic Diagnosis.—Plates are made from the putrid exudate in an atmosphere of hydrogen, and at the same time guinea-pigs are inoculated subcutaneously.

Immunity.—Malignant edema is one of those bacterial diseases in which artificial immunization through metabolic products was first effected. Chamberland and Roux immunized guinea-pigs by means of intraperitoneal injections of bouillon-cultures sterilized by exposure for ten minutes in the autoclave at a temperature of between 105° C. (221° F.) and 110° C. (230° F.). Immunity to malignant edema is also established without difficulty in animals by all other methods.

PROTEUS-INFECTIONS.

Morphology of the Proteus.—The proteus-bacteria, discovered by Hauser in 1885, are small, motile rods of exceeding activity and variable size.

The proteus-bacteria are usually arranged in pairs, and not rarely also in longer filaments. In addition to these fundamental forms coccus-like bodies and long, winding threads (spirulins) also are encountered. The proteus is characterized by the possession of an unusually large number of flagella surrounding the body of the cell. It is stained readily with carbolfuchsin, and less well with watery solutions of aniline dyes. It does

not stain by Gram's method. The proteus thrives equally well at room-temperature and at the temperature of the body. The temperature-optimum is between 20° C. (68° F.) and 25° C. (77° F.). The organism does not give rise to *spores*, and is destroyed by exposure for five minutes to a temperature of 55° C. (131° F.).

Cultural Properties. — On *gelatin-plates* small, round, yellowish colonies form at first, with a dense center and an irregular margin, from which bristle-like processes pass off. Other colonies are bounded by a zone of filaments surrounding the central opaque mass, in part circularly, in part in the most
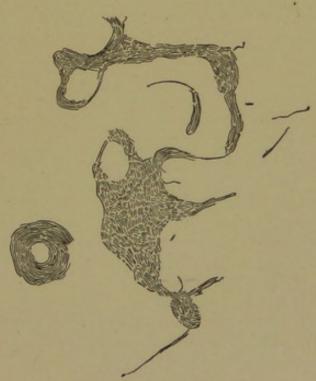
Fig. 67.—Swarming islands of proteus-bacilli on the surface of gelatin; × 650 (Hauser).

varied loops and convolutions. Extensive and rapid liquefaction of the gelatin takes place. Into the adjacent nutrient medium extend processes, both straight and tortuous, which frequently are cut off from the mother-area and move about as free islands in the partially liquefied gelatin. These conditions may be especially well observed upon five or six per cent. gelatin. There thus result peculiar figures and designs, to which the proteus owes its name of "*bacillus figurans.*"

Gelatine stab-culture is liquefied with exceeding rapidity.

In *agar streak-culture* a grayish, moist coating forms.

On *potato* a dirty grayish deposit appears.

Bouillon is rendered homogeneously turbid.

All nutrient media emit a disagreeable odor.

Formerly, Hauser distinguished three distinct varieties of proteus, the proteus vulgaris, the proteus mirabilis, and the proteus Zenkeri ; but later he abandoned this differentiation.

Experiments on Animals.—If rabbits or guinea-pigs receive intraperitoneal or intravenous injections of considerable amounts (three cubic centimeters) of proteus-culture, the animals die of acute enteritis and peritonitis. Intravenous injection of from five to ten cubic centimeters of a bouillon-culture is followed by much more typical phenomena in the dog. The animal suffers from bloody vomiting and bloody diarrhea attended with severe tenesmus. The temperature is elevated, and the scleræ are distinctly icteric. On autopsy the entire digestive tract, from the cardia to the anus, is the seat of an intense hemorrhagic inflammation. The blood and the internal viscera of the dog contain none or but a few bacteria. Exactly the same result is obtained with filtered cultures and with the bodies of the bacilli carefully destroyed. In mice, which, likewise, succumb to proteus-inoculation, the bacilli may be cultivated from the viscera. The organisms become the more virulent the oftener they are passed through the bodies of mice.

Occurrence of the Proteus.—Proteus-bacteria are present in all putrefactive processes, and also in the gastrointestinal canal. In human beings the proteus, in mixed infection with the ordinary exciting agents of inflammation, constitutes the cause of the *putrid, fetid phlegmons* at times observed in the sequence of cadaveric infection. Besides, the proteus gives rise to so-called *putrid intoxication*, by subsequently penetrating into a primary focus of suppuration or a traumatic lesion, multiplying there, and generating metabolic products that are absorbed. According to H. Jäger certain forms of *febrile icterus*, known as Weil's disease, are caused by the proteus. Jäger succeeded in cultivating a fluorescent proteus from the urine, and after death also from the viscera of individuals suffering from Weil's disease. Infection had taken place in these cases from bathing in streams the water of which was contaminated by proteus. On the banks of a tributary brook an epidemic had occurred among fowl, the exciting agent of which was likewise found to be the proteus fluorescens. Further, the proteus was found by E. Levy to be the cause of *hemorrhagic*

gastro-enteritis from which seventeen persons suffered after the use of spoiled meat.

Bacteriologic Diagnosis.—Plates should be made with pus from the putrid phlegmons and possibly also with the urine obtained with sterile precautions from cases of Weil's disease.

GONORRHEA.

Morphology of Gonococci.—The exciting agents of gonorrhea, the *gonococci*, discovered by Neisser, in 1879, are cocci, which are separated into two distinct hemispheres by means of a dividing fissure. The individual members
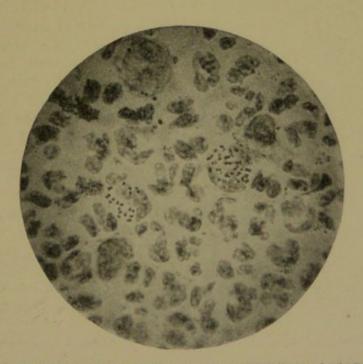
Fig. 68.—Gonococci in urethral pus; × 1000 (Fränkel and Pfeiffer).

suggest the appearance of a kidney or a biscuit. The cocci can be stained with all aniline dyes, but not by Gram's method. They are from 0.8 to 1.6 μ long, and from 0.6 to 0.8 μ thick.

Occurrence of Gonococci.—Gonococci are constantly present in the secretion from all gonorrheal inflammations (gonorrheal urethritis, cervical catarrh, blennorrhea, etc.). Their position within the pus-cells, in the immediate neighborhood of the nucleus, is characteristic. In cases that are no longer rare gonococci have been found also in the visceral

manifestations of gonorrhea (peritonitis, salpingitis, oophoritis, endocarditis, rheumatism, myelitis). The reported demonstration of gonococci in the normal urethra—thus their saprophytism—can not be considered as established.

Culture of gonococci (Wertheim) is possible only at a temperature of 37° C. (98.6° F.).

Plate-procedure.—Gonorrheal pus is introduced into a tube containing liquid human blood-serum at a temperature of 40° C. (104° F.), and from this two dilutions are prepared in two new blood-serum tubes, likewise at a temperature of 40° C. (104° F.). Into each of the three tubes a like amount of liquefied two per cent. peptone-agar, cooled at 40° C. (104° F.), is then introduced, thorough admixture practised, and three plates are made, which are at once placed in the thermostat. As early as twenty-four hours later isolated gonorrheal cultures will have developed. The *superficial colonies* present a dark, punctate center, from which a delicate, finely granular deposit extends toward the periphery; the *deeper colonies*, of whitish-gray color, possess a nodular appearance, and, in the course of two or three days, assume the shape of blackberries. If inoculations are made from the colonies, it will be found that they consist of a mucoid, viscid mass.

Streak-culture upon Solidified Blood-serum Agar Slants (one part of liquid human blood-serum at a temperature of 40° C. (104° F.), mixed with three parts of liquefied agar-agar also at a temperature of 40° C. (104° F.), and solidified in an oblique position).—Luxuriant growth takes place at first in the form of individual gray colonies that later coalesce into a moist, glistening, viscid-mucous deposit, from the periphery of which a thin, veil-like coating extends. The water of condensation is covered by a membrane.

A good liquid culture-medium is constituted by human blood-serum, with the addition of twice as much peptone-bouillon. Upon this a superficial membrane forms, while the culture-medium itself remains almost entirely clear.

In the preparation of culture-media animal blood-serum may be employed in place of human blood-serum, although the gonococci do not thrive so well upon the former as upon the latter. If, also, gonorrheal pus is smeared upon the surface of several agar-tubes covered with a thin layer of human blood (blood-agar, p. 82), a pure culture of gonococci may be obtained. A mixture of blood-serum with urine has also been employed with success in the cultivation of gonococci; further, a mixture of 2 parts of peptone-agar with 1 part of human acid urine, or of 1 part of ascites-fluid with 1 part of agar nutrient medium of the following constitution: Five per cent. peptone, 2 per cent.

glycerin, 3½ per cent. agar, 0.5 per cent. sodium chlorid. Wassermann has recommended the following culture-medium for gonococci: To 15 cu. cm. of swine blood-serum, from 30 to 40 cu. cm. of water, from 2 to 3 cu. cm. of glycerin, and 0.8 gram of nutrose are added, and all are thoroughly mixed and boiled for fifteen minutes. It is best to repeat the boiling on the following day. The fluid thus obtained is heated to a temperature of between 50° C. (122° F.) and 60° C. (140° F.), and mixed at the same temperature with 2 per cent. peptone-agar in equal amount.

Microscopically, the gonococci thus cultivated present exactly the same appearances as those present in gonorrheal pus. The organisms survive in the cultures for from four to six weeks. They are exceedingly sensitive to drying, disinfectants, and temperatures above 42° C. (107.6° F.).

The specific pathogenic significance of gonococci is demonstrated by the fact that pure cultures introduced into the normal human urethra (of paralytics) give rise to true gonorrhea. Animals do not acquire gonorrhea.

Bacteriologic Diagnosis of Gonorrhea.—Microscopic examination of the urethral secretion is of the greatest importance, and in doubtful cases almost indispensable. With the suspected discharge (gonorrheal threads) dry cover-slip preparations are made, passed three times through the flame, and simply stained with an aqueous solution of methylene-blue. The cocci and the nuclei of the pus-cells are stained blue, the former more deeply than the latter. The characteristic shape of the cocci (kidney-shaped or biscuit-shaped), their position in the leukocytes, and their failure to stain by Gram's method justify a positive diagnosis of gonorrhea.

Double staining may be successfully effected if the preparations are first stained with an alcoholic solution of eosin (the eosin being absorbed with bibulous paper), and then subsequently treated with methylene-blue. Cocci and cell-nuclei will then appear blue, the cell-bodies red.

Cultivation of gonococci for *diagnostic purposes* is not necessary.

Although positive evidence of the presence of gonococci in microscopic preparations renders the diagnosis of gonorrhea certain, negative evidence from examination of the urethral secretion must be accepted with caution. It is known that when gonorrhea has existed for a consider-

able time gonococci are not always demonstrable in the rather mucoid secretion then present, but that, nevertheless, the gonorrhea may persist and still be infective. The gonococci are situated in the depth of the mucous membrane, and the superficial secretion may be entirely free from them. It is, therefore, advisable in doubtful cases to induce irritation of the urethral mucous membrane (through the use of beer, etc.), and thereby to stimulate the secretion. Only when, after repeated examination and after previous irritation, the secretion is found free from gonococci—it then generally still contains other cocci in abundance— may the gonorrhea be considered as terminated, and only a catarrhal urethritis remains.

Prophylaxis.—Gonorrheal infection occurs almost exclusively through sexual intercourse with those suffering from the disease. It is a noteworthy fact that intercourse with an individual suffering from gonorrhea does not necessarily give rise to infection. It is, however, not necessary to invoke a special predisposition to the disease for those who are infected, and, on the other hand, a special immunity for those who escape. The gonococcus must encounter a lesion of the mucous membrane in order to gain lodgment and to give rise to gonorrhea. When such a lesion is wanting, infection may not take place, especially if the gonococci taken up are soon removed mechanically by subsequent irrigation (perhaps through the urine in micturition). Recovery from gonorrhea predisposes to repeated attack. Rigid sanitary supervision of prostitutes alone is capable of securing certain prophylaxis against gonorrhea. As a prophylactic measure against blennorrhea of the new-born, instillations of astringents into the conjunctivæ are in general employ.

SYPHILIS.

The exciting agent of syphilis is not yet known. Of the numerous bacteria that have been found and to which etiologic significance has been attached, the bacilli described by Lustgarten seem worthy of mention.

Lustgarten in 1884 found in syphilitic lesions and discharges special bacilli that he demonstrated by means of the following method of staining: Sections of tissue hardened in alcohol, or cover-slip preparations made from discharges and passed but once

through the flame, are kept for from twelve to twenty-four hours at room-temperature and then in a solution of aniline-water gentian-violet for two hours more at a temperature of 40° C. (104° F.). They are next decolorized in absolute alcohol, and are then exposed for ten seconds to the action of $1\frac{1}{2}$ per cent. aqueous solution of potassium permanganate, from which they are transferred to an aqueous solution of chemically pure sulphurous acid. They are then washed in water and again immersed in the potassium-permanganate solution, but now only for three or four seconds, and from this they are transferred to the solution of sulphurous acid; and this is repeated until the preparation appears entirely decolorized, which usually is brought about after the manipulations have been repeated three or four times. The

Fig. 69.—Bacillus of syphilis (Lustgarten), from a condyloma; × 1000 (Itzerott and Niemann).

preparations are then dehydrated in alcohol, cleared in oil of cloves, and mounted in xylol Canada balsam.

Lustgarten believed that by this method of decolorization all bacteria but the syphilis-bacilli, the tubercle-bacilli, and the leprosy-bacilli yield up their stain. The last two, however, are distinguished by their resistance to hydrochloric acid and nitric acid, both of which rapidly decolorize the syphilis-bacilli. Lustgarten found the bacilli stained in this way in all syphilitic infiltrates, and in smaller number at the center and in larger number at the periphery, as well as in the adjacent apparently healthy tissues. They rarely lie free, but singly or in groups of from two to nine within large lymphoid cells. On one occasion Lustgarten encountered them in the lumen of a large lymphatic.

On examination of syphilitic papules he found the bacilli between the prickle-cells of the rete Malpighii. The bacilli are from 3.5 to 4.5 μ long and about 1 μ thick, straight or bent, in part irregularly curved, and their surface presents a wavy contour. They are found constantly in the syphilitic lesions in varying number, but, on the whole, not abundantly.

A number of observers have confirmed Lustgarten's statements, while others have failed to find his bacilli. The significance of Lustgarten's observations was severely shaken when Matterstock and Alvarez and Tavel found bacilli in smegma that could be stained by the method of Lustgarten, and that also closely resembled the bacilli of syphilis morphologically. Doutrelepont maintained, subsequently, that after staining for forty-eight hours in aqueous methyl-violet solution, and decolorization with solution of ferric chlorid and alcohol, the smegma-bacilli yielded up their color, whereas the Lustgarten bacilli retained theirs. Nevertheless, the opinion is quite generally held that the so-called bacilli of syphilis are identical with the smegma-bacilli, of which not a few take the stain characteristic for tubercle-bacilli. This circumstance must, further, be borne in mind in examinations for genito-urinary tuberculosis. Confusion between tubercle-bacilli and smegma-bacilli may be avoided by a knowledge of the fact that the latter exhibit less resistance to the action of hydrochloric acid and nitric acid than the former. The smegma-bacilli exhibit still less resistance to the action of alcohol.

The bacillus of Lustgarten can not, therefore, be considered as the exciting agent of syphilis, although it may possibly bear some relation to this disease. Whether bacteria are the cause of syphilis at all is wholly doubtful. A bacterial etiology for syphilis is generally assumed because of the varied resemblance of its clinical manifestations to those of other bacterial infectious diseases—as, for instance, tuberculosis and leprosy. Perhaps, however, the exciting agent of syphilis is of an entirely different nature. It is only certain that it is an organized body, a contagium vivum, but nothing further is at present known with regard to it. Investigation in this field is attended with great difficulties because syphilis is not transmissible to animals, and experimentation fails. According to some observers, monkeys are said to be capable of acquiring syphilis; while others deny this assertion. At least, it appears that not all varieties of monkeys are susceptible to the disease.

Syphilitic infection takes place through the conveyance of the syphilitic virus, which is contained in the degenerated products of the syphilitic sclerosis, as well as in all secondary lesions, and in the course of florid syphilis also in the blood of syphilitic patients. This transmissibility has been demonstrated repeatedly by experiments on human beings, and first through the famous experiments of the palatinate Anonymus. Inoculations with sweat, saliva, urine, milk, and seminal fluid of syphilitics have been unsuccessful.

The *conveyance* of the infecting material takes place either *directly*, usually through sexual intercourse, less commonly through kissing, through the nursing of a syphilitic child, through contact with syphilitic lesions by the fingers (physicians, midwives), and the like ; or *indirectly* through instruments, articles, etc., contaminated by the syphilitic virus (house-epidemics through eating-utensils and drinking-utensils, infection through cigar-tubes, gloves, etc.). A most important rôle is played by *hereditary transmission*, which will be fully discussed later.

The **portal of infection** may be constituted by any portion of the skin or mucous membrane where a slight breach of continuity, any lesion of the epithelial covering, exists, so that the syphilitic virus can penetrate deeply. The virus remains localized at the site of infection, where it manifests itself as the so-called *primary lesion*, or hard chancre. In accordance with the varying frequency of the different modes of infection already named, this lesion is most common on the genitalia, but it not rarely occurs also in other situations : on the lips, on the tonsils, on the nipples, on the fingers, etc. After a period of incubation of not less than from three to six weeks, and frequently much longer, the virus is disseminated throughout the entire body, and the disease becomes generalized. The exciting agents themselves are contained in the blood and in the secondary lesions. A poison alone, absorbed perhaps from the site of the initial lesion, could not give rise to these secondary lesions, as the disease is transmissible indefinitely through them. The exciting agents further must possess an extraordinary length of life. They must survive throughout the whole duration of the secondary stage, extending over many years, for the disease is transmissible throughout this entire period. Only in the tertiary stage is this transmis-

sibility wanting, and the manifestations of this stage can not be dependent upon the virus in a living form, or at least capable of multiplication, and infectious. In the case of hereditary syphilis the virus is taken up by the blood, and the primary lesion, which ordinarily indicates the portal of infection, is wanting, and the disease sets in at once with the secondary manifestations.

Immunity.—All ages and all races are equally susceptible to syphilitic infection. Natural immunity to the disease in human beings does not exist. Syphilis is characterized by a marked tendency to relapses, which often appear after long periods of latency. On the other hand, one attack of the disease confers protection against subsequent attack— that is, it gives rise to immunity. Renewed infection (reinfection) occurs—even after disappearance of all previous morbid manifestations—only exceptionally.

According to a law laid down by Colles, the mother who gives birth to a child syphilitic through the father, without herself being attacked, is rendered immune by the fetus. By analogy with other immunizing procedures, it is easy to understand that the disease-germs do not pass over from the child to the mother, so that the mother is not infected, because the placental barrier is impassable to the microorganisms, but that, on the contrary, the toxins dissolved in the blood pass through this barrier from the child into the maternal circulation, and thus confer immunity upon the mother. The mothers of syphilitic children are actually immune. They may nurse their offspring at the breast without being infected, whereas healthy nurses nursing the same children would be infected with syphilis. Such mothers are thus certainly immune. The only point for discussion is whether they have been immunized by the children. According to the view of a number of syphilographers, they are immune because they have been, or are, themselves syphilitic. Their condition of health is only apparent. We shall return to this point later, in the discussion of heredity. According to the view just expounded, immunity to syphilis would always be acquired through previous infection.

The **specific therapy of syphilis** has never been essentially cleared up. Whether potassium iodid and mercurials operate by destruction of the infecting microorganisms—as seems the more probable—or by immunizing infected indi-

viduals, can not be determined with certainty, so long as the exciting agent of syphilis is not known. The efforts to check florid syphilis by means of the serum of individuals who have recovered from syphilis, or of animals into whom syphilitic products have been injected, have each and all failed; and for the present, at least, they are without any experimental justification.

Heredity of Syphilis.—Children of syphilitic parents are frequently syphilitic at birth, or present sooner or later evidences of *hereditary syphilis*.

The mode of transmitting the disease may, under such conditions, be as follows:

1. Through congenital transmission from the father—that is, transmission of the disease by means of the spermatozoids (*paternal infection*). According to the opinion of most observers, this mode of transmission is actually operative, the spermatozoid of the syphilitic father carrying the disease-germ into the earliest indication of the fetus. This view, however, has not been proved. Experimental attempts to induce syphilis in healthy individuals by inoculation of the seminal fluid of syphilitic men have been made, but without inducing syphilis in the persons experimented on. The assumption of a direct paternal infection is thus based solely upon the clinical experience that syphilitic men may procreate syphilitic children without infection of the mother. The majority of clinicians accept this as a fact. According to some, the disease of the fetus is always to be ascribed to the father, and should the mother suffer at all, she is believed to have first acquired the infecting material from the child infected by the father (retroinfection, choc en retour). It has already been mentioned that the validity of this proposition has been attacked from various sides. Distinguished syphilographers are of the opinion that no child is syphilitic in the absence of syphilis in the mother (A. Wolff); that healthy women who give birth to syphilitic children, and are thus immune, are only apparently healthy, but in reality are infected; that often, even though primary or secondary manifestations are not observed, they later suffer from tertiary manifestations. If this be true—that is, if in a given case the mother of a syphilitic child is herself syphilitic—then the illustration can not be employed in support of paternal infection; the disease of the child may then arise also through the mother.

From all that is known the question as to the direct inheritance of syphilis by the child from the father, although this is theoretically quite conceivable, and is accepted by the large majority of clinicians as actually occurring, must yet be considered an open one.

2. Congenital transmission from the mother—that is, transmission of the disease with the ovum—is accepted upon all sides as a possible and frequent mode of conveyance. As a matter of fact, the offspring of syphilitic women, if the disease has not advanced to the tertiary stage, are almost without exception syphilitic, independently of whether the father is syphilitic or healthy.

3. Intrauterine Infection.—If the mother, healthy at the time of conception, is infected with syphilis during the period of gravidity, the child also becomes syphilitic. Only when the infection of the mother takes place in the last two months of pregnancy are healthy children at times born. The fetus, under these conditions, must acquire the disease-germ from the mother through the placental circulation. Whether a lesion of the placenta is necessary for this to take place, as is assumed for all other forms of intrauterine infection, has not yet been decided. According to some observers, the offspring of a healthy woman may be infected in the uterus through sexual intercourse with a syphilitic man, and the mother, in turn, be infected by the fetus ; but this is as yet undemonstrated. The reverse procedure— infection of the woman, who then infects the fetus—is the probable one.

4. Extrauterine infection during parturition or in the first days of life, if the mother be suffering from recent syphilis and the child has remained uninfected until the moment of birth, is altogether conceivable, and probably also occurs. Whether, however, it plays an important part, it is difficult to decide, as probably the majority of such cases, in which a child develops syphilitic general manifestations about six weeks after birth, are designated as hereditary-syphilitic. Extrauterine infection of the new-born can be demonstrated by the presence of a primary lesion, which is wanting in placental (intrauterine) infection.

HYDROPHOBIA (LYSSA; RABIES).

The exciting agent of hydrophobia is not yet known. Nevertheless, the specific treatment of this disease has been successful, owing to the genius of Pasteur.

The **susceptibility for hydrophobia** exists among all warm-blooded animals. Human beings are infected through the bites, in the first place, of rabid dogs, then of cats, wolves, foxes, jackals, and other animals, and, in rare cases, of human beings suffering from hydrophobia. The *saliva* must thus contain the virus of the disease, and as a matter of fact this had already been demonstrated experimentally at the beginning of the last century by inoculation of dogs from human beings. The parotid is the gland most concerned, while the remaining salivary glands, though virulent, are not so constantly so as the parotid. The saliva of dogs contains the virus of rabies as early as two days before the appearance of the first symptoms of the disease. The lacrimal glands, the adrenal glands, the pancreas, and the mammary glands of rabid animals are further infectious; the milk, at times is so; the blood, never. Besides, the central nervous system is virulent—the brain and the spinal cord and, in a conspicuous and constant manner, the medulla oblongata.

Experiments on Animals.—The saliva is not used for inoculation-experiments because, in addition to the virus of rabies, it always contains a number of pyogenic micro-organisms that act as a disturbing factor. The medulla oblongata of animals or individuals that have died of rabies is used exclusively for inoculation-purposes. A watery emulsion is made from a small portion of the medulla oblongata, and a few drops thereof are injected beneath the dura mater or into the anterior chamber of the eye in dogs, rabbits, etc. After a period of incubation of from twelve to fifteen days the animals develop, with almost absolute certainty, the symptoms of rabies. Subcutaneous injection is not quite so trustworthy. In order to obtain positive results the injection must be made deeply, and preferably into the exposed and divided muscle-bundle. Direct injection of the virus into a peripheral nerve is likewise attended with success. Healthy mucous membranes (as of the nose and the conjunctiva) also absorb the virus. The possibility of intrauterine

21

transmission of rabies has been established experimentally in a small number of instances.

Infection takes place by way of the nervous system. If the spinal cord of a dog is divided transversely, and the virus of rabies is injected into a nerve of the hind-paw, only the cord below the point of division proves virulent after the death of the animal. The reverse conditions prevail after inoculation of a fore-paw. Having reached the central nervous system from the periphery (site of inoculation or of bite) through the intermediation of the nerves, the virus descends into the peripheral nerves of the opposite side. For this reason, if the disease has developed slowly, the nerves of the uninjured side are also found poisonous in experiments on animals.

The as yet unknown excitant of rabies appears to exert its influence through its metabolic products. At least, according to Italian observers, the filtrate through porcelain of an emulsion of the spinal cord from animals suffering from rabies induces paralytic manifestations in dogs. Rabies may, therefore, as suggested by Romberg, be designated a *toxoneurosis*.

The dissemination of the virus of rabies in the course of the nerve-paths explains why in human pathology the prognosis of the disease varies so widely in accordance with the number, the seat, and the depth of the bite-wounds. Everything depends upon whether the virus gains entrance into a nerve or not. Deep wounds are, therefore, much more dangerous than superficial ones ; injuries in regions with an abundant nerve-supply (as, for instance, the finger-pulp) more so than in other parts of the body. The greatest danger is involved in wounds of the head and the face. From these the virus of rabies quickly reaches the medulla oblongata, the main seat of the disease. The morbidity and the mortality of rabies (the developed disease is incurable) are, according to the most reliable statistics, about 16 per cent. of those bitten.

Incubation.—The duration of the period of incubation depends upon the same factors that have been mentioned as significant in prognosis. The period is the shorter the nearer to the head the portal of infection is situated. The usual duration of the incubation-period is from twenty to sixty days. The trustworthy minimum observed has been fourteen days ; the maximum, eighteen months.

Resistance of the Virus of Rabies (Medulla Oblongata of Dogs Dead of Rabies).—The virus is destroyed by exposure for an hour to a temperature of 50° C. (122° F.); further, to 5 per cent. carbolic acid for fifty minutes; to mercuric chlorid, 1 : 1000; to acetic acid; to potassium permanganate. The spinal cord of (Paris) rabbits dead of rabies, kept in dry air and protected from putrefaction, loses its toxicity only after fourteen or fifteen days. The smaller the animal, the thinner the spinal cord, the more rapidly does the loss in virulence take place.

Immunization and Vaccination.—Pasteur showed that the virus of canine rabies slowly diminishes in intensity when inoculated from dogs upon monkeys in a progressive series. This gradual loss of virulence is distinctly appreciable in the increase in the period of incubation. If the infecting material is reconveyed from monkeys to rabbits, an increase in virulence takes place, which constantly augments on further inoculation into rabbits. The period of incubation becomes shorter; and, finally, after the hundredth passage through the body of the rabbit, the period is not longer than seven days. It was impossible to produce a more active virus. The virus retained its virulence now unchanged, and Pasteur, therefore, designated it *virus fixe*. Pasteur in this way prepared a series of rabies-viruses that, beginning with the spinal cord of monkeys and progressing to the spinal cord of rabbits that succumbed to the virus fixe, possessed a steadily increasing virulence. On inoculating successively dogs subcutaneously with these spinal cords, of from the lowest to the highest degree of virulence, the animals treated failed to develop rabies, but became immune even to subdural infection with the virus fixe and to the bites of other dogs suffering from ordinary rabies.

A year later, in 1885, Pasteur and his collaborators, Chamberland and Roux, developed a still more practical method of immunization. Proceeding from the fact already mentioned that the medulla of animals suffering from rabies is completely deprived of its virulence in from fourteen to fifteen days by desiccation, they dried the spinal cords of rabbits that had succumbed to the virus fixe for from one to fourteen days with all aseptic precautions in high sterilized glass cylinders. The spinal cord fourteen days old had lost all its virulence; that thirteen days old and that

twelve days old, in part; and the others, successively less and less. Through successive inoculations with these gradations of spinal cords Pasteur established complete immunity in dogs. Upon the basis of these facts Pasteur proceeded to the vaccination of human beings therapeutically against rabies. In view of the long period of incubation of rabies in human beings the attempt was justified and hopeful; for, if it were possible to establish immunity through vaccination immediately after the bite of the rabid animal and before the period of incubation had elapsed, then it could be hoped that the disease would not break out. The results have completely confirmed Pasteur's anticipations. At first, the injections were so made that on the first day the spinal cord of rabbits kept for fourteen days, on the second day that of rabbits kept for thirteen days, and so on for ten days until the cord of rabbits kept for five days was reached, were successively injected subcutaneously into the subject to be treated (methode simple). Pasteur, however, soon recognized that this procedure was not sufficient for the severe cases with deep and numerous wounds.

Vaccination is at present practised in the Pasteur Institute in the following manner (methode intensive): A piece of spinal cord about three millimeters long is rubbed up in sterile bouillon and injected beneath the skin in the hypochondrium, and on the first day in the morning medulla fourteen and ten days old—an injection being made on either side—in the evening medulla twelve and eleven days old; on the second day in the morning medulla ten and nine days old, in the evening medulla eight and seven days old; on the third day two injections of medulla six days old, and from now on an injection every twenty-four hours of the more toxic spinal cords up to that three days old. With the spinal cord three days old a new series is begun, commencing with the medulla five days old. Upon this succeeds a third, and possibly a fourth, series of like character.

Vaccine may be prepared also by dilution with sterile water (Bardach) instead of by desiccation. This fact indicates that also in the dried spinal cord the virus is not actually attenuated, but only diminished in amount. As a matter of fact, Pasteur, after injecting desiccated medulla into a guinea-pig and the animal dying after thirty days, observed the medulla of this animal destroy a second guinea-pig

in exactly seven days; the virus thus remained a virus fixe.

The immunity to rabies appears to persist for a long time —in dogs for two years.

Results of Pasteur's Procedure.—The great utility of the method of vaccination against rabies is no longer seriously doubted by any one. From 1886 to January 1, 1894, 14,430 persons in all were treated in the Pasteur Institute, of whom 72 died. In the year 1891, 394 persons were treated, the diagnosis of rabies in the biting animals being established with all possible certainty; and not a single patient developed the disease. In 1892, 128 persons were treated, with 1 death. In 1893, 132 bitten persons were treated, of whom none died. In the year 1894, 1387 persons were vaccinated, of whom 7 died, and in the year 1895, 1520 were vaccinated, with 2 deaths.* These statistics require no comment: they speak for themselves.

SMALLPOX (VARIOLA).

Smallpox, like syphilis and rabies, is one of the diseases whose exciting agents are yet unknown.

The **virus of smallpox** resides in the contents of the variolous pustules, in the desiccating scales of the skin, in the sputum, and in the nasal secretion of those suffering from the disease. It is transported with the linen and the clothing of the sick. Also the air in the neighborhood of the sick must, from clinical experience, be considered a source of infection. Under suitable conditions the contagium may retain its vitality for an exceedingly long time, apparently for years.

The **portal of infection** for the contagium of smallpox has not yet been definitely determined. According to the common opinion, the disease is generally acquired through direct or indirect contact with the sick, and the skin would thus seem to constitute the portal of infection. In other cases the disease may be attributed to simple inhalation in the neighborhood of smallpox-hospitals, etc. Finally, articles of food (such as milk) and insects are thought to convey the infection, which, under these conditions, would

* The figures for 1896 were 1388, with 4 deaths; for 1897, 1521 cases, with 6 deaths.—A. A. E.

take place through the mouth or the lungs. Naturally, however, under any circumstances other modes of infection can not be excluded.

Predisposition and Immunity.—The susceptibility to smallpox exists at all ages. Even new-born children have been observed to present the disease. In the great epidemics of smallpox that traversed Europe before the institution of compulsory vaccination the morbidity varied in different localities. The local predisposition was the greater the more impoverished the community. It thus appears that not merely the telluric conditions themselves, but rather the vital conditions and the mode of life of the people living upon a given soil, constituted the cause for the varying distribution of the disease. The marked increase in smallpox that regularly takes place in winter has been considered evidence of a temporal predisposition. This may, however, be readily explained by the changed mode of life in winter (with greater confinement in closed rooms, the wearing of heavier and a greater amount of clothing, the greater difficulty of cleanliness, etc.), without the necessity for invoking a direct influence of the weather upon the germ of the disease.

Recovery from an attack of smallpox confers *immunity* that lasts on the average about ten years. A second attack of the disease before the expiration of ten years is most exceptional, but has been frequently observed after a longer period. Third attacks of smallpox have been reported nine times in the literature, and Cantani reports one case in which seven attacks occurred.

Upon the ancient experience that immunity is acquired by recovery from smallpox is based the procedure of *variolation*—that is, the inoculation of healthy persons with true smallpox for purposes of immunization, which was formerly practised and entailed not a few sacrifices.

Vaccinia and Vaccination.—Edward Jenner, a physician of Berkeley, in England, between 1749 and 1823, convinced himself, after study and investigation pursued for years, that the pock-disease of cows (vaccinia), conveyed to human beings, protected them from infection with variola. On May 14, 1796, Jenner vaccinated a youth with cowpox-lymph obtained from the hand of a maid who had infected herself in milking a cow suffering from cowpox of the udder. The vaccinated subject was protected against sub-

sequent variolation. After a large series of further successful vaccinations Jenner, in 1798, published his famous communication, in which he announced the established fact of the protective influence of cowpox-lymph against smallpox. Since then, vaccination against smallpox has been gradually adopted in all civilized countries ; in Germany it has been made obligatory through a law passed in 1874. In those countries in which the general practice of vaccination against smallpox is regulated by law, variola, which formerly was responsible for a large proportion of the mortality, has almost completely disappeared. Devastating epidemics now occur only in uncivilized countries. Vaccination against smallpox is practised in Germany in the first and twelfth years ; it consists in the cutaneous introduction of the contents of the pocks of young calves in a fresh state or rubbed up with glycerin (animal vaccination). The lymph obtained from the vesicles of inoculated persons has the same effect (humanized lymph). Of late, however, animal lymph is almost universally preferred, as the danger of simultaneous transmission of other disease-products (syphilis, etc.) can not with certainty be excluded in the use of humanized lymph. Animal lymph is obtained in Germany by systematic vaccination of calves in institutes under State supervision.

In accordance with existing knowledge, it can be definitely accepted that cowpox is identical with variola in human beings, and that vaccinia is only a form of variola mitigated by passage through the body of the cow. Fischer, of Karlsruhe, succeeded in grafting the virus of variola on the body of the calf by collecting and mixing together the liquid and the contents obtained by cureting from variolous pustules in human beings in their various phases, from development to suppuration. The mixture was then rubbed into the largest possible surfaces (crucial incisions and scarification). Fischer was able in this way to develop in the calf directly with the virus of variola typical pustules whose contents, subsequently upon reconveyance to human beings, from the third generation, proved to be vaccine. Vaccination against smallpox thus readily adapts itself to current views regarding immunity, inasmuch as it represents a protection against disease induced through preventive inoculation of an attenuated though similar virus.

As to **the nature of the virus of variola,** numerous

investigations have been made with variola and with calf-lymph. In spite of all the efforts that have been made the exciting agent of smallpox has not yet been discovered. It is possible that it does not at all belong to the class of bacteria, and in any event its requirements of the culture-media are different than are those of the bacteria whose gowth has thus far been successful. On ordinary bacteri-ologic examination staphylococci, pseudo-diphtheria-bacilli, and rarely streptococci are found in animal lymph. It has been maintained by Landmann that the evidences of irrita-tion that are sometimes apparent in marked degree around the pustules are due to the activity of these pyogenic cocci. On the other hand, the Commission for the Investigation of the Vaccine Question (1896) emphasized that the staphy-lococci found were of moderate, and only exceptionally of considerable, pathogenicity for animals, and that virulent streptococci could no longer be found in the lymph after the lapse of eighteen days. The streptococci found in older specimens are to be looked upon as only harmless skin-epiphytes, such as can not rarely be isolated from bac-terial mixtures. Frosch, the reporter of the Commission mentioned, reached the conclusion that no etiologic relation exists between the bacteria of the lymph and the irritative and inflammatory manifestations of the inoculation-pustule, and that in the preparation of animal lymph any noteworthy reduction in the number of germs or their complete exclu-sion even with the observance of strict antisepsis is not attainable ; that, therefore, it is impossible to obtain an unirritating lymph-supply.

Varicella (chickenpox) has nothing to do with the ex-citing agent of variola and of vaccinia, and recovery from the disease does not protect against smallpox.

ACUTE EXANTHEMATA.

The exciting agents of the acute exanthemata are, like-wise, thus far unknown.

Measles is contagious in an extraordinary degree. The susceptibility of human beings to the disease is exceedingly great between the second and tenth years of life ; it is less in the first year and in adults. *Natural immunity* to measles practically appears not to occur. The *contagium* is con-

tained in the nasal mucus, the conjunctival secretion, and the sputum of those suffering from measles, and, as experimental inoculation appears to have shown, also in the blood. The contagium is taken up through contact with the sick, or—as is generally accepted clinically—through inhalation of the exciting agent. The persistence of the virus of measles is not so marked as that of small-pox or of scarlet fever. In a dry state the virus of measles is said to persist for about six weeks. Measles is rarely conveyed by means of articles of clothing, linen, etc., and not for long distances. The *infectivity* of measles exists as early as the last days of the period of incubation—which is between eight and ten days—and in the stage of eruption. After the exanthem has entirely appeared, the danger of infection rapidly subsides.

In measles also a large number of bacteria have been found, but, above all, again cocci, which have also been demonstrated in the blood. None of these observations is worthy of consideration, because they depend upon accidental or secondary infection or upon contamination.

The *immunity* established by recovery from an attack of measles is rather active and enduring. Only thirty-six cases are recorded in the literature in which two attacks occurred, and only one with three attacks.

Scarlet Fever.—The *virus* of scarlet fever is much more resistant than that of measles. It adheres for months to the clothing worn and the rooms occupied by the sick. It appears, further, to possess considerable resistance to temperature-influences. Infection with scarlet fever, in accordance with clinical experience, occurs principally through direct contact with the sick or with articles belonging to them, but also through breathing of the air in rooms saturated with the virus of the disease. The *period of incubation* is of varying duration (from two to twenty-four days). Even toward the end of this period, according to Gerhardt, scarlet fever is infective, and it remains so until the cessation of desquamation and for weeks longer. Wherein the infective material resides, whether in the secretions, in the blood, or in the cutaneous scales, is not known. Some observers claim to have transmitted scarlet fever by inoculation, while others have failed to induce the disease experimentally by means of the blood, the scales, etc. There is much in the clinical picture of scarlet fever, especially the nephritis, in

favor of the view that the disease is a toxic infection, and that the exciting agent is not itself disseminated into the viscera. The relations between scarlet fever and diphtheria are well known. Scarlet fever is sometimes complicated by true diphtheria with diphtheria-bacilli in the membranes. More frequently, however, scarlatinal angina is due to streptococci. Altogether scarlet fever exhibits a tendency to be complicated by streptococci, and the secondary suppurative processes that not rarely occur are attributable to these microorganisms.

The susceptibility of human beings to scarlet fever is greatest between the third and eighth years, though not so great as that of measles. After the tenth year the disease is less common, and still less so in the first year of life.

Like measles, scarlet fever is more common in winter than in summer. Certain local influences in the distribution of scarlet fever can not be ignored. Although the disease is, on the whole, everywhere endemic in uniform distribution, noteworthy variations occur. Thus, some cities have remained free from scarlet fever for thirty, and even for fifty, years, although in constant communication with other infected cities.

The immunity after recovery from scarlet fever is active and enduring. Only twenty-nine cases in all of second attacks and four of third attacks are recorded in the literature.

The large number of bacteria found in cases of scarlet fever, both micrococci and bacilli, require no particular mention. Streptococci have been found most frequently, and these, as in the case of diphtheria, appear to constitute a frequent, perhaps a regular, complication of the virus of scarlet fever.

WHOOPING-COUGH (PERTUSSIS).

In its infectivity whooping-cough stands close to measles. Our knowledge of the virus of the former disease is yet quite scanty. The contagium is contained in the breath and, especially, in the expectoration of children suffering from whooping-cough. With the sputum it finds its way upon the linen, and it may also be conveyed through healthy persons. Probably infection takes place always through the respiratory tract. Children between

one and five years of age are most susceptible to whooping-cough ; the disease is less common after the tenth year of life, although it occurs also in adults, and not altogether rarely in the first year of life. Whooping-cough has been observed repeatedly in new-born children whose mothers have suffered from the disease toward the end of gravidity. The disease is especially common in spring and autumn. As a rule, it attacks human beings but once. A large number of bacteria have been cultivated from the sputum of patients suffering from whooping-cough, and animal parasites also have been found therein ; the demonstration of any etiologic significance for any of these is yet lacking.

ARTICULAR RHEUMATISM.

Although the exciting agent of articular rheumatism is entirely unknown, there is, however, no doubt that this disease is infectious. The febrile course, with the general manifestations, the frequent complicating inflammations of serous membranes and the endocardium, and the secondary nephritis, point forcibly to the infectious nature of poly-arthritis. The disease is well known to be dependent in a high degree upon meteorologic influences, but the necessity for certain influences increasing the predisposition also in the etiology of other infectious diseases has already been demonstrated. Acute articular rheumatism is in no wise contagious. Rheumatic polyarthritis is one of those infections after whose termination immunity lasts for only a short time, if it occur at all, to be replaced soon by heightened predisposition. Frequently the same individual suffers from repeated attacks of this disease. It is doubtful if all cases that are grouped clinically under the designation of acute articular rheumatism are associated etiologically. The fact that one-quarter of the cases are refractory to the influence of the known specifics (salicylic acid, antipyrin) appears indicative of etiologic multiplicity. In any event it is to be borne in mind that the clinical picture of poly-arthritis may also be produced by the exciting agents of other diseases (gonorrhea, scarlet fever).

RELAPSING FEVER.

The cause of relapsing fever was, in 1873, found by Obermeier, a former assistant of Rudolph Virchow, and who died prematurely, to be a special spiral bacterium, which he designated the *spirocheta of relapsing fever*. Obermeier's discovery was the more far-reaching because with it an organism of the class of bacteria was for the first time recognized as the cause of a human infectious disease.

The **spirilla of relapsing fever** (*spirilla Obermeieri*) are delicate, wavy threads, with numerous turns (from ten to twenty), varying in length from 16 to 40 μ, with distinctly pointed extremities. In form and size they closely resemble
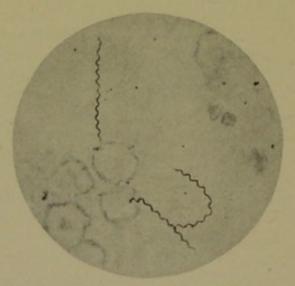
Fig. 70.—Bacillus of relapsing fever, from human blood; × 1000 (Günther).

cholera-spirilla, though they are only from one-half to one-quarter as thick as these, and they never appear in the form of a comma, or in S-shaped segments of a screw, but always only in the form of a complete screw. The spiral thread can not be differentiated into individual members. The spirilla possess flagella and are actively motile. They glide in rapid, twisting movements from one side to the other across the field of vision. With regard to their propagation, nothing certain is known; probably this takes place by division. The formation of *spores* has not been observed.

Artificial cultivation of the spirilla of relapsing fever upon nutrient media has thus far not been successful. The spirilla must be looked upon as strict parasites that flourish only within

the animal body. In leeches that have sucked themselves full of the blood of patients suffering from relapsing fever, and have been preserved upon ice, the spirilla remained alive for ten days. Little is known with regard to their temperature-relations. In blood from patients suffering from relapsing fever kept in glass tubes at a temperature of from 16° C. (60.8° F.) to 22° C. (71.6° F.) the spirilla survive for as long as fourteen days; at a temperature of 37° C. (98.6° F.), for about twenty hours; at a temperature of from 39.5° C. (103.1° F.) to 41.7° C. (107.1° F.), only from four to twelve hours; and at a temperature of 42.5° C. (108.5° F.), scarcely three hours (Heydenreich). Conclusions with regard to the growing bacteria in the living blood can not be drawn from these observations, as the blood removed from the body is no longer a favorable nutrient medium for the spirilla. Nothing definite is known also with regard to the requirements of the spirilla for oxygen.

The relapsing spirilla can be readily stained by means of watery solutions of aniline dyes; they do not take acid stains. For the demonstration of relapsing spirilla in blood-preparations the method of Günther is useful; this consists in introducing the dried cover-slip preparation, fixed by heating, before staining with gentian-violet or
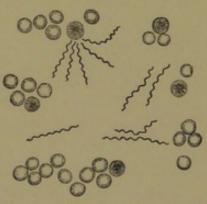
Fig. 71.—Spirochæta Obermeieri in the blood (v. Jaksch).

fuchsin, for ten seconds in five per cent. acetic acid, in order to extract the hemoglobin from the blood-corpuscles. The relapsing spirilla are not so resistant as all other bacteria to the action of dilute potassium hydroxid or concentrated acetic acid. In their reactions they resemble the protoplasmic rather than the nuclear substances. The spirilla do not stain by Gram's method.

Occurrence of Spirilla of Relapsing Fever.—The spirilla are present, and in varying number, only in the blood of those suffering from relapsing fever, in which they appear a short time before the onset of the fever, and they undergo considerable multiplication during the continuance of the fever, to disappear, a short time before the critical defervescence, until the onset of the next febrile paroxysm. During the afebrile period the spirilla have been found in the blood in but one case by Naunyn. They are not ordinarily found in the secretions and excretions of patients suffering from relapsing fever, and with equal rarity outside the

human body. They have been found once in the urine in a case of relapsing nephritis. The relapsing spirilla are, therefore, most rigid blood-parasites.

Transmission of Relapsing Fever.—Relapsing fever has been repeatedly transmitted to healthy human beings, and with complete success to apes, by means of blood containing spirilla (Koch, Carter, and others). The spirilla can thus with certainty be considered as the exciting agents of relapsing fever. How they induce the disease, and whether the fever is the result of a poison generated by them, can not yet be decided positively. Equally little is known with regard to the manner in which the disease is transmitted naturally. The disease is contagious ; as, however, the spirilla soon lose their vitality outside of the body— in water, on articles of food, in the air—the ordinary modes of infection, by way of the respiratory or the digestive tract, are scarcely to be considered. The disease can be conveyed only with the blood, as the spirilla apparently do not leave this fluid. Klebs refers to the possibility of conveyance through blood-sucking cutaneous parasites, but this has not been established with certainty. Intrauterine transmission of the spirilla to the fetus has been observed.

The mode of recovery from relapsing fever, which, as is known, thus terminates in the majority of cases, is essentially not yet understood. According to Heydenreich, the bacilli die as a result of the high temperature of the patient in the febrile attack. His observations, however, as has been pointed out, do not furnish adequate evidence in support of this view. According to Metschnikoff, the spirilla collect in the spleen during the precritical elevation of temperature, and are there destroyed by phagocytosis. Baumgarten does not consider this a healing process, but believes that the spirilla are deposited in the spleen, and also in the liver and the bone-marrow, where they undergo destruction, in part through inclosure in leukocytes, and in part independently of this process ; but this takes place only because they have ceased proliferating, because their vital activity has been impaired, and they are nearly or already dead. The cause of this attenuation Baumgarten believes to reside in spontaneous exhaustion of the proliferating power of the infecting microbes, which are endowed with only a short duration of life in consequence of immanent vital laws. The successive febrile cycles are attributed by

Baumgarten to new series of generations, which arise from individual spirilla that have not been destroyed. Final recovery may depend upon the development of an insusceptibility of the blood.

PART IV.

I. MYCOSES (INFECTIONS WITH FILAMENTOUS AND BUDDING FUNGI).

An incomparably lesser rôle in the etiology of disease is played by the *filamentous fungi* (molds) and the *budding fungi* than by bacteria (fission-fungi). The diseases generated by the former are designated mycoses.

MORPHOLOGY AND BIOLOGY OF THE FILAMENTOUS AND THE BUDDING FUNGI.

The **filamentous fungi** are chlorophyl-free, thread-like cells that exhibit progressive apical growth, and that, singly or branched, usually divided into segments by internal septa, unite to form a deposit and at times a dense felt-work of closely interlaced threads (*hyphæ*). This so-called vegetative portion of the fungus is known as *thallus* (fungous deposit) or *mycelium*. Distinct from this is the fructifying portion, the *fruit-bearer*, which arises from the mycelium and bears the fruit (*spores* or *conidia*). The spores again grow into threads; the enlargement takes place steadily through progressive apical growth of the threads, which in turn give rise to new spores. The structure of the fruit-bearing apparatus is so peculiar in a number of filamentous fungi that this external feature has been made the basis of their classification. Of the numerous species of filamentous fungi, running up into thousands, only the following are of pathologic interest :

1. The Aspergilli (*Bulbous Molds*).—The fruit-hyphæ exhibit no division ; they swell into the form of a club at the extremity. This bulbous enlargement is densely occupied by short, flask-shaped structures, arranged radially, the in-

termediate fruit-bearers (*sterigmata*), upon which the consecutive spores are seated.

2. The Penicillii (*Brush-molds*).—The fruit-bearers, which generally arise vertically from the mycelium, are transformed, through manifold forked divisions in their upper third, into dense tufts of brush-shaped, ramifying, delicate processes (*basidia*), upon whose extremities the conidia are seated in long rows in the form of globules.

3. The Mucorini (*Globular Molds*).—The fruit-bearers, which are mostly unsegmented and undivided, arise vertically from the mycelium and present at their extremity a large spore-mother-cell (*sporangium*), which is separated from the fruit-hypha by a septum markedly convex upward
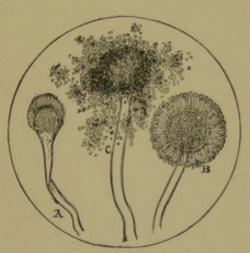
Fig. 72.—*A*, Aspergillus glaucus; *B*, aspergillus niger; *C*, ripe fructiferous head of aspergillus niger throwing off spores.
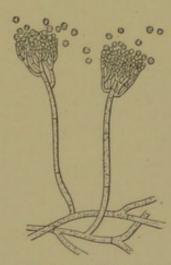
Fig. 73.—Fungi (penicillium glaucum).

(*columella*). The sporangium contains within its interior, separated by septa, the large cylindric-oval spores.

4. The streptothrices form, to a certain degree, a transition between the filamentous fungi and the bacteria. They consist of long, cylindric filaments, dividing by budding, and from which finally a true mycelium is formed. In many of these air-hyphæ develop, which simply give off special fruit-heads, spores (*segmentation*). These streptothrix-spores must not be placed upon the same plane as the permanent forms of the bacteria. In older cultures the streptothrix-threads disintegrate into degenerative products resembling bacteria, like rods, cocci, spirilla (*fragmentation*). If these structures are transplanted upon fresh nutrient material, a

22

true filamentous network will at once redevelop. The peculiarities of the streptothrices will be referred to in detail in the discussion of actinomycosis.

5. Oidia (*segmented molds*) are likewise simple in arrangement. They also possess no special fruit-heads, the spores being detached directly from the fruit-bearers that arise from out of the mycelium. Their most frequent representative is the oidium lactis, the white milk-mold, which vegetates upon sour milk. The oidia form a transition to the so-called budding fungi.

The **budding fungi or yeast-fungi** are chlorophyl-free cells of roundish or oval shape that multiply by *budding*—that is, a small, roundish, bud-like projection grows from the periphery of the mother-cell and, gradually increasing in size, assumes the shape of the mother-cell, from which it ultimately becomes detached. The newly formed cell undergoes the same process. If the various generations of cells remain attached to one another, long rows of yeast-cells result—the so-called *strings of buds*. Under special nutritive conditions—for instance, upon solid culture-media of
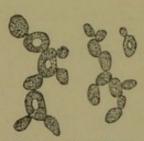
Fig. 74.—Yeast-fungi.

alkaline reaction or deficient in sugar—the budding fungi also form true mycelial threads. (See Thrush, p. 351.) The best known of the budding fungi are the yeasts (saccharomyces cerevisiæ) and the mold of wine-must (mycoderma vini).

Filamentous and budding fungi flourish at room-temperature. For their nutrition they require constantly preformed organic substances, water, and, further, oxygen as a rule ; a number of the filamentous fungi may develop also in the absence of oxygen. Acid culture-media are preferred, although the filamentous and the budding fungi grow also upon alkaline media. These fungi are present everywhere in nature, and are always numerous in the air and upon articles of food. They are capable of inducing fermentation (especially, though not exclusively, the budding fungi) and decomposition. Putrefaction, which is usually of bacterial origin, inhibits the development of filamentous and budding fungi in general.

Microscopic examination for filamentous and budding fungi is made on the whole in the same way as that for

bacteria. The filamentous fungi, with the exception of the streptothrices, which behave exactly as do bacteria with regard to stains, do not stain well in general, although they can be demonstrated with the aid of Löffler's methylene-blue. Generally, it is preferable to examine these fungi unstained. The filamentous fungi do not take up water, so that they are generally mounted in glycerin. It is advisable to undertake the demonstration of teased preparations from fungous vegetations in 50 per cent. alcohol containing a few drops of ammonia, in order to avoid the disturbing influence of air-bubbles; or the teased preparations are made in Unna's solution: gelatin 1, alcohol 25, solution of ammonia 25, glycerin 25, water 35. The following recommendation of Unna is useful: The cover-slip preparations are placed for a minute in 5 per cent. potassium hydroxid; then, after rinsing in water for five minutes, in 5 per cent. acetic acid; and finally they are exposed to the action of a strong aniline stain (for instance, gentian-violet), possibly under the influence of heat. Yeast is best stained with a dilute aqueous solution of vesuvin, as the other aniline dyes readily give rise to overstaining.

The *cultivation* of molds and budding fungi is carried out in the same way as that of bacteria. Isolation is effected by means of the plate-procedure, preferably with acid gelatin or agar (the gelatin or the agar is dissolved in acid fruit-decoctions, beer-wort, or potato-water (p. 81)), instead of alkaline bouillon. For further cultivation bread-pap is well adapted (p. 86).

PATHOGENIC ACTIVITY OF FILAMENTOUS AND BUDDING FUNGI FOR ANIMALS.

Although the majority of filamentous and budding fungi vegetate only upon dead organic material, a small number of varieties may flourish in the animal body and thus give rise to disease. A special position is occupied by the streptothrices, the most important representative of which, the streptothrix actinomyces (p. 354), will be fully considered. The best known among the remaining pathogenic filamentous fungi are the *aspergillus fumigatus* and *flavescens*, and the *mucor corymbifer* and *rhizopodiformis*. If an emulsion of these fungi in bouillon is injected into the

ear-vein of a rabbit, the animal will die after the lapse of
two or three days in consequence of a *general mold-myco-
sis.* In all of the organs, most abundantly in the kidneys
and in the liver, small, whitish-gray nodules are present,
which, when viewed microscopically, are found to consist
of a dense network of mycelial threads. These fungous
vegetations never, however, contain fruit-bearers or co-
nidia. Careful investigation has shown that germination of
the injected spores has taken place, but that fruit-formation
never takes place in the animal organism.

By reason of its deficiency in free oxygen and its alkaline
reaction the organism does not constitute a suitable nutrient
medium for most molds. The overwhelming majority of
these die when introduced into the body, whether through
the breath or in any other way. The few varieties that
survive at all prove pathogenic only by germinating, and
acting as foreign bodies inducing disturbances mechanically
through irritation and vascular occlusion, etc. They do
not undergo fructification or actual multiplication. Noth-
ing likewise is known with regard to any chemic activity on
the part of molds or budding fungi in the body. In a
certain sense the mold-mycoses are, accordingly, not true
infectious diseases, as they are not attended with multipli-
cation of the exciting agent, and with intoxication. Suc-
cess in inoculations with pathogenic molds depends, there-
fore, also upon the number of spores injected. The number
of disease-foci corresponds exactly with the number of
spores introduced, and the animals die as a result of the
extent of the foci of disease alone. The pathogenic molds
are, besides, inherently pathogenic, and likewise the non-
pathogenic molds are always nonpathogenic. Augmenta-
tion or attenuation of pathogenicity can not be effected.
The pathogenic varieties are not equally pathogenic for all
animals. Thus, the mucor corymbifer, which destroys rab-
bits, is harmless in dogs.

Pathogenic molds are quite widely distributed in nature.
It is only necessary, for instance, to expose unsterilized
bread-pap in a closed beaker for one or two days to a tem-
perature of from 30° C. (86° F.) to 40° C. (104° F.), in
order to see a dark-green fungous coating form, consisting
of aspergillus fumigatus. The culture is generally pure, as
at a temperature of between 30° C. (86° F.) and 40° C.
(104° F.) the aspergillus overgrows all other molds. If,

in addition, other molds be present, a pure culture can be readily obtained by injecting the fungous mass into an animal. The body of the animal at once differentiates pathogenic and nonpathogenic molds, inasmuch as the latter are destroyed with certainty. In spite of this distribution of the pathogenic molds, mycoses are not common in the animal kingdom. Spontaneously they occur relatively seldom. Only in the lungs of birds are fatal aspergillus-mycoses and mucor-mycoses found at all frequently, so that a certain susceptibility must be assumed for this particular tissue, and which, otherwise, is generally wanting in the animal body.

Of *pathogenic yeasts* but a small number are thus far known. Experimentally in animals they induce local suppuration, tumor-like swellings, and at times septic manifestations.

DISEASES IN HUMAN BEINGS INDUCED BY FILAMENTOUS AND BUDDING FUNGI.

DERMATOMYCOSES (PARASITIC DISEASES OF THE SKIN).

Favus.—The cause of favus was recognized by Schönlein in 1839 as a filamentous fungus, which, in his honor, has been named *achorion Schönleinii*, and which was the first of all organized causative agents of disease discovered. The disease is characterized by a peculiar crust or scutulum. On microscopic examination this is found to consist of a layer of cornified epithelial cells, beneath which there is a mass of fungous elements, in the form of concentrically arranged mycelial threads, which give off conidia toward the center of the favus-body. The center consists of conidia alone; in addition bacilli and cocci are always found. If a fragment of this scutulum be examined in water or in glycerin, the mycelia will be recognized as numerously partitioned or segmented ramifying filaments of varying thickness. The conidia are of variable form and size, roundish, oval, angular, in part provided with a yellowish nucleus, in part without a nucleus, but with granular turbid contents. This fungus, which from its form belongs to the oidium-group, is the cause of favus. It is found in association with both favus of the hairy scalp and in portions of the body free from hair, and with favus of the nails. It has been grown in

pure culture, and with this favus has been induced in animals and in human beings. It may be mentioned that Quincke succeeded in cultivating three distinct favus-fungi. More recent investigations have, however, rendered it quite certain that in all varieties of favus but one fungus is found, which, however, is not identical with the exciting agents of the other parasitic skin-diseases. The favus-fungus grows upon all nutrient media, both at the temperature of the body and at room-temperature ; it grows best somewhat beneath the surface, while only a small number of air-hyphæ are formed. The cultures are at first whitish, but later they become yellow, and from the periphery radiating processes
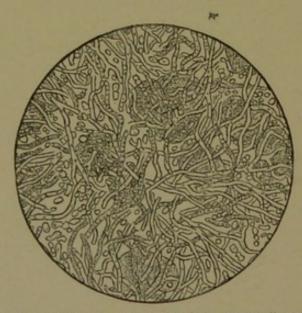
Fig. 75.—Achorion Schönleinii : × 450 ; showing simple mycelium in various stages of development, and free spores (after Duhring).

extend into the depth of the nutrient medium. Microscopically, a mycelium of branched hyphæ is visible. Individual hyphæ swell at their free extremity into the form of a roll, while others form lateral buds—the so-called yellow bodies of Kral—which burst, so that their contents escape as a free body. At these points the deep processes form. The filaments themselves finally disintegrate into oval, cellshaped structures.

Cultural Properties of the Achorion.—On *gelatin-plates* snow-white, star-shaped colonies, with an irregularly thickened center, form. The culture-medium is quickly liquefied.

In *gelatin stab-cultures* a thick, wavy, superficial deposit appears, which is stained yellow beneath the surface.

On *agar* a moderate, whitish, wavy coating forms, the lower surface of which exhibits a sulphur-yellow color.

Upon agar and gelatin the mycelium remains sterile.
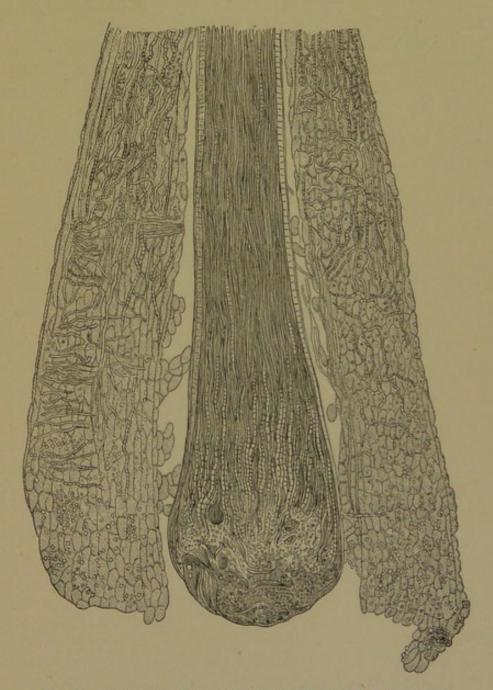
Fig. 76.—Achorion invading root-sheaths and bulb of the hair (Kaposi).

Upon blood-serum, at a temperature of 30° C. (86° F.), formation of conidia takes place.

Favus occurs in dogs, cats, and mice, as well as in human beings. It is generally transmitted to human be-

ings from others, less commonly through animals. On the whole, the disease is but slightly contagious. Whether an especial predisposition on the part of the skin is necessary for the vegetation of the favus-fungus is doubtful. Youth appears especially predisposed to favus.

Infection with favus probably occurs only when the fungus is deposited upon a macerated area of the epidermis or gains entrance into a hair-follicle. The morbid process takes place beneath the epidermis. It does not extend deeply, and the favus-fungus only exceptionally reaches the subcutaneous connective tissue. In rare instances favus has been observed upon the mucous membrane of
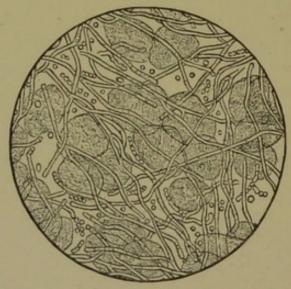
Fig. 77.—Trichophyton, × 450, as found in epidermic scrapings of ringworm, showing mycelium and spores (after Duhring).

the stomach. Under these circumstances it is probable that the fungus has been swallowed, as opportunity is scarcely ever afforded for embolic dissemination.

The *diagnosis* of favus is made upon both clinical and microscopic data. Culture is not necessary therefor. If it is desired to make a culture, the scutulum is rubbed up in a sterile mortar with sterile silicic acid, and plates are made.

Herpes Tonsurans.—The various forms of herpes tonsurans upon the hairy scalp and upon parts of the body free from hair are, like parasitic sycosis, eczema marginatum, onychomycosis trichophytina, and a number of other affections of the skin, due to the presence of the *trichophy-*

ton tonsurans (discovered in 1845 by Gruby and Malm-
sten), which, partly alone and partly in mixed infection
with bacterial excitants of inflammation, is the cause of the
disease.   The fungus appears especially in the form of
extended, slightly branched and straight mycelia, whose
width is less and whose segments are comparatively
much shorter than those of the favus-fungus.   The conidia
of the trichophyton resemble those of the achorion, al-
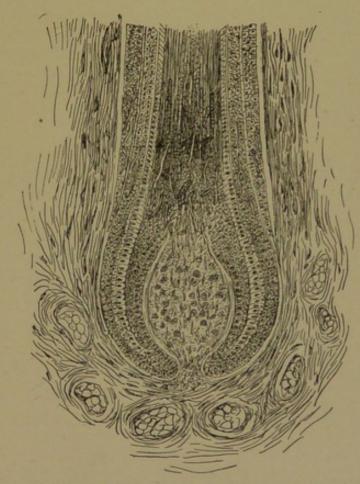
Fig. 78.—Root of hair in tinea barbæ, showing early invasion of trichophyton in root-
sheaths and upper part of bulb.

though they are somewhat smaller and, above all, less
numerous.   These fungous elements lie between the epi-
dermic scales, between and in the sheaths of the roots of
the hair.   In the less changed hairs the mycelium pre-
ponderates ; when the trichophytic infiltration is consider-
able, the spores are more abundant.

The trichophyton may be conveyed to human beings
from animals.   It occurs in dogs, cats, cattle, and horses.

More frequently, however, transmission takes place from one human being to another. Of all the dermatomycoses, herpes tonsurans is the most contagious. The predisposition of the skin for diseases due to the trichophyton is in general greater than that for favus. Infection is facilitated by all of the conditions favoring the vegetation of molds, such as a moist season of the year, living in damp cellars, and the like. The variability in the clinical picture of the diseases due to the trichophyton depends in the first place

Fig. 79.—Microsporon furfur, fungus of pityriasis versicolor; × 700 (Kaposi).

upon the varying reactivity of the skin and the varied localizations of the fungus, and in the second place also upon the simultaneous activity of various bacterial excitants of inflammation. The common cause of all varieties of trichophytosis has, however, been determined experimentally with certainty. In every instance the trichophyton was cultivated, and with a pure culture thereof all the varied forms of the disease were reproduced.

Cultural Properties of the Trichophyton.—Also in culture

the trichophyton tonsurans closely resembles the favus-fungus. The two are, however, not entirely alike. Lique-faction of gelatin takes place much more quickly with the trichophyton, and the deposits are much greater, than with the achorion. At a depth the trichophyton also develops a yellow color in growth.

For *microscopic demonstration* of the trichophyton the epidermic scales, removed with a sharp curet, or the epilated hairs, are treated with potassium hydroxid, preferably after previous treatment with chloroform and ether for the removal of fat.

Pityriasis Versicolor.—The cause of pityriasis is the *microsporon furfur* (first described by Eichstedt, in 1846),

Fig. 80.—Microsporon minutissimum ; × 1000 (after Riehl).

a fungus resembling the favus-fungus and the trichophyton, and, likewise, an oidium. The fungous masses, which are at once recognizable in the horny lamella, removed by means of the finger-nail or a sharp curet and mixed with six per cent. potassium hydroxid upon a glass slide, consist of unusually large and uniform conidia, which form regularly distributed masses, each of thirty or more, and of slightly ramified, short mycelia, which connect the conidial masses with one another.

The contagiousness of pityriasis is exceedingly slight. The microsporon obviously requires a quite special predisposition on the part of the skin. It is often found in tuber-

culous subjects. Experimental transmission of the disease has succeeded in several instances, but the culture of the fungus has not.

Erythrasma, a circumscribed erythema of the skin, is caused by the *microsporon minutissimum*, the small, round spores of which lie in the superficial horny cells, whereas the mycelium, consisting of small, delicate, convoluted and branched filaments, in part interlacing and with short segments, extends more deeply.

Psoriasis is no longer included by dermatologists among the parasitic diseases, its supposed excitant, the epidermophyton, having been recognized as a factitious product (Ries).

PHARYNGOMYCOSES.

In the pharynx mycoses are not at all rarely observed in certain situations. The fungous vegetations appear as small, porcelain-white plugs, mostly in the lacunæ of the tonsils. They consist, however, as a rule, not of true molds, but more commonly of filaments of the *leptothrix buccalis (mycosis pharyngis leptothricia)*, whose botanic position has not yet been clearly made out, but which is ordinarily included among pleomorphic bacteria. Miller differentiates a leptothrix buccalis innominata, maxima, and gigantea. The leptothrix is a constant inhabitant of the oral cavity; and its fine threads, from 0.5 to 0.8 μ thick, are frequently found on examination of the sputum. The straight, wavy, or spiral threads of the leptothrix are made up of rod-like or spiral segments. In the filaments a base and an apex may often be distinguished; at their free extremities spherical formations appear, which in part have been considered arthrospores. The appearance of this fungus is thus quite similar to that of the simple varieties of molds. According to some observers, these detached "spores" may, like true cocci, divide in pairs. In that event the fungus would belong to the bacteria, and would be included among the pleomorphic varieties. The leptothrix stains yellow with solution of iodin and potassium iodid.

Abundant opportunity is afforded for the entrance of the fungus into the lacunæ of the tonsils, as the inhaled air and articles of food always contain fungi. In the secretion of the oral cavity leptothrix and filamentous fungi are always present normally. The vegetation of the fungus in the

pharynx does not penetrate deeply and does not cause destruction. Frequently, it gives rise to no subjective symptoms whatever, and only accidentally comes under observation. In other instances it acts as a mechanical irritant, giving rise to the sensation of a foreign body, and also to signs of slight inflammation. The fungi adhere quite firmly; they can scarcely be removed by brushing and the like, but the plugs must be pulled out individually or removed with the galvanocautery.

KERATOMYCOSES, OTOMYCOSES.

Keratomycoses have been observed after injury of the intact cornea with soiled articles (pitchforks, etc.), their

Fig. 81.—Aspergillus fumigatus; × 500 (Fränkel and Pfeiffer).

cause always appearing to be the *aspergillus fumigatus.* The spores introduced through the agency of the traumatism develop into filaments whose unlimited growth is capable of causing destruction of the cornea. The fungous mass is surrounded by a wall of leukocytes. Fructification does not take place under these circumstances, nor have dissemination of the germs and the formation of metastases been observed.

Otomycoses, or myringomycoses, likewise occur, and are also mostly due to the *aspergillus.* The presence of

inflammatory processes in the auditory canal favors the lodgment of the fungi, which not only vegetate in the secretions accumulated in the external auditory canal, but also penetrate the living tympanic membrane.

PNEUMONOMYCOSES.

Pneumonomycoses have been observed repeatedly in human beings, and are due to both the *aspergillus* and the *mucor*. Mold-vegetations have not rarely been found in bronchopneumonic foci at autopsy, and also during life ; filamentous fungi have been found in the sputum, which is then sometimes characterized by a putrid odor. In general the occurrence is, however, rare.

In view of the frequency of occurrence of pathogenic molds in the air, and of the abundance of opportunity offered for the entrance of fungi into the lungs, it must be assumed that the human pulmonary tissue possesses an especially slight predisposition for molds. It has been mentioned, on the other hand, that the lungs of birds form a more suitable nutrient medium for the vegetation of filamentous fungi, and that birds not rarely succumb to spontaneous mycoses.

The pulmonary mycosis in human beings presupposes in general a primary disease, a hemorrhagic infiltration, a necrosis, etc., of the pulmonary tissue. The fungi proliferate, as a rule, only in such foci of disease. This mycosis also is unattended with a tendency to extension, and it is unassociated with evidences of general infection. When the primary disease undergoes recovery, the tissues are quite capable of disposing of the mycotic vegetations. Pneumonomycosis often terminates in recovery.

VISCERAL MYCOSES.

Mold-vegetations in internal organs (kidney, liver, etc.) have only exceptionally come under observation. The reason for this is readily made clear in experiments on animals. The opportunity for infection is wanting. The fungi can reach the organs in question only through the blood, and they are scarcely ever taken up into the blood-stream from existing mycoses, from the intestine, and wherever else in the body fungi are present.

THRUSH.

Thrush is a local disease, preferably attacking mucous membranes lined with pavement epithelium, and resulting from the lodgment and proliferation of the *thrush-fungus*. The lodgment of the fungus leads to the formation of milky-white deposits varying from the size of a millet-seed to that of a lentil. These gradually undergo enlargement toward the periphery, and in the absence of therapeutic intervention coalesce finally into large membranes.

Microscopic Examination of Thrush-deposits.—Microscopic examination of the white points that represent the first stage of the thrush-eruption discloses, in addition to squamous epithelium, molds and bacteria of various kinds, always in large number, the two phases of the thrush-fungus (*oidium albicans*): the mycelial threads and the conidia. The former are threads of double contour and of varying thickness and length, with transverse septa and indentations, from which often lateral branches of equal or less thickness pass off. The conidia, which grow from the mycelia, at their extremities or in the neighborhood of the septa, are more or less spherical, separate readily from the mycelia, and lie among them, at times isolated, at times in groups.

Occurrence of Thrush.—Thrush occurs most commonly in new-born children, principally about the second week of life. In older children and in adults thrush occurs only when protracted disease (typhoid fever, tuberculosis, etc.) has induced general enfeeblement of the organism. In the new-born, however, an eruption of thrush by no means presupposes a particular impairment of the general health. The mucous membrane of the infant is obviously quite especially predisposed to the vegetation of the thrush-fungus. In experimental transmission of thrush the disease will develop upon the healthy mucous membrane of well-nourished children.

The preferable seat of thrush is the mucous membrane of the mouth, and here the tongue, the inner surface of the lips and of the cheeks, are most commonly invaded. Next in frequency deposits of thrush appear upon the gums, in the pharynx, and also in the upper portion of the esophagus, on the anterior and upper surface of the epiglottis, and exceptionally upon the true vocal bands. In all of these

situations pavement epithelium is present, and it has been
assumed that the thrush-fungus is capable of proliferating
only upon mucous membrane covered with such epithelium.
The thrush-fungus is not rarely encountered in the vagina
of pregnant women, which likewise is lined with pavement
epithelium. It occurs, however, also upon mucous mem-
branes lined with other kinds of epithelium—as, for instance,
that of the stomach, the posterior surface of the epiglottis,
the deeper portions of the respiratory tract, although but
seldom in all of these situations. The thrush-fungus has
been demonstrated repeatedly in bronchopneumonic foci,
and in numerous instances upon the mammary glands and
mammillary areolæ of nursing women whose children suf-
fered from thrush, and in one instance in encephalitic sup-
purative foci in a child suffering from thrush.

Course of Thrush.—The thrush-fungus penetrates the
uninjured epithelial surface, and develops beneath the up-
permost layer of epithelium, within the deeper portions of
the mucosa. Only rarely does it invade the submucosa.
The abundant fungous vegetation raises the upper epithelial
layer, and gradually separates it from the connection with
the lower layers necessary for its nutrition, so that it under-
goes necrosis and exfoliation. The thrush-membrane is
then exposed to view. The thrush-proliferation usually gives
rise to mild local-irritative manifestations. The mucous
membrane generally appears somewhat altered at the site of
the thrush-deposits. It is dark red, slightly swollen, dry,
and distinctly sensitive to touch. The signs of an actual
inflammation are, however, not discoverable microscopi-
cally. It has been assumed that the thrush-fungus can
find lodgment only upon mucous membranes the seat of
catarrhal inflammation. This, however, does not appear
to be correct, but the slight evidences of irritation may
really be the result of the purely mechanical action of the
fungous vegetation. The saliva of patients suffering from
thrush invariably has an acid reaction. There is no sug-
gestion of chemic activity on the part of the thrush-fungus,
of any production of toxin. There is an absence of fever
and, in fact, of all constitutional manifestations. The
symptoms are of a purely local nature. The fungous
proliferation may become dangerous only when, by reason
of especially luxurious development, it obstructs the
esophagus or the larynx ; and, further, in small children,

through impairment of the nutrition, the tenderness of the mouth interfering with nursing. In general, however, the disease is a thoroughly benign one. The thrush-vegetations have no especial tendency to extend, and only exceptionally have extension of the thrush-fungus into the interior of blood-vessels and transmission through emboli been observed. The vegetations can be readily removed by rubbing the thrush-plaques with soft cloths dipped in a solution of borax (sodium biborate 2.0, glycerin 4.0, distilled water 34.0).

Sources of Infection for Thrush.—The thrush-fungus is present in the air of dwelling-rooms, in the feces of infants, upon rubber nipples, upon saccharine and amylaceous articles of food, etc., and it may gain entrance into the mouth with all of these. Frequently, as has already been mentioned, the thrush-fungus is found in vaginal mucus, and thrush in the infant has been attributed to conveyance of the fungus from the maternal genital canal during the act of parturition. This is, however, certainly not the ordinary mode of infection, as the period of incubation of thrush, as determined by numerous inoculation-experiments, is only four or five days, and the disease usually does not appear in infants before the second week.

Culture and Botanic Position of the Thrush-fungus.—In accordance with the appearance of the thrush-fungus in the thrush-membrane described, the fungus bears a close resemblance to the varieties of oidia that cause the dermatomycoses, and it has, therefore, been given the name *oidium albicans*. Grawitz, then, demonstrated subsequently by culture that the thrush-fungus is not a mold, but rather a budding fungus. As numerous later investigations have shown, the thrush-fungus grows upon culture-media of acid reaction and containing an abundance of sugar (prune-decoction agar) in filaments of actively budding yeast-cells. If these be transferred to ordinary meat-peptone agar—thus to an alkaline nutrient medium, deficient in sugar—distinct threads develop in addition to the budding cells. Retransferred to an acid medium, the threads again develop almost exclusively into yeast-conidia. The oidium albicans is thus a budding fungus that, under certain nutritive conditions, forms hyphæ. Other observers, however, maintain that the thrush-fungus is a mold. The organism does not liquefy gelatin. Upon gelatin-plates whitish colonies form,

23

and in stab-cultures whitish-yellow granules that send radiating processes into the nutritive medium. Upon agar a whitish-yellow, wrinkled deposit forms; upon potatoes a dense, white coating, and upon bread-pap a thin, white deposit. The temperature-optimum is 37° C. (98.6° F.).

It has been demonstrated experimentally that the thrush-fungus is the actual cause of thrush. It has been possible to develop thrush upon healthy mucous membranes both by means of the bands of buds, and with the filamentous cells. Rabbits die in from twenty-four to forty-eight hours after intravenous injection of emulsions of the thrush-fungus, a general mycosis resulting, and networks of thrush-vegetations being found in the kidneys, the liver, etc. (G. Klemperer). This pathogenic action of the thrush-fungus is, however, not constant.

The **diagnosis of thrush** is made by microscopic examination of the thrush-deposit; cultivation of the fungus is not necessary.

ACTINOMYCOSIS.

The **actinomycosis of animals** was recognized as a distinct disease in 1877 by Bollinger, and in its lesions peculiar vegetable structures (actinomyces, ray-fungus) were invariably found.

Actinomycosis is a disease peculiar to cattle, having been but seldom observed in other animals (swine, dogs). The usual seat of the disease in cattle is the lower jaw, less commonly the upper jaw, or also the adjacent soft parts, especially the tongue. The disease of the jaws leads to the formation of tumors of greater or less extent, which subsequently rupture outward through the skin, less commonly into the mouth, and thus come into view as ulcerated nodules. On section the tumor appears pale yellow; it is in general soft, but presents, in places, especially softened, yellowish areas of varying size, from which on scraping with the knife a substance resembling pus and numerous yellowish granules as large as grains of sand are obtained. Examined microscopically the tumor-mass represents granulation-tissue; the yellow coloration is dependent upon abundant fatty degeneration of the cellular elements. The essential feature and the peculiar characteristic of the actinomycotic nature of the new-formation consist in the yellow granules present, the so-called actinomyces-granules, to whose structure reference will be made later. These are fungous formations, and represent the cause

of the disease, as has been established with certainty by the successful transmission to calves of actinomycosis by means of such granules (Ponfick and others).

The disease in animals presents, in the first place, the characters of an inflammatory new-formation, which develops around the fungous proliferation as a foreign body; besides, there is considerable destructive tendency—the proliferating fungi in their growth displacing and destroying all opposing tissues.

Infection in animals probably takes place principally through the mouth in feeding. The ray-fungus is said to be present in feed, in wheat-grains, which play an important part in the process of infection. Infection through the superficial integument, through the lungs, etc., is possible also, but occurs less commonly. A case of actinomycosis of the udder has been observed in a pig, and another of miliary pulmonary actinomycosis in a cow.

Fig. 82.—Actinomyces (von Jaksch).

Actinomycosis in Human Beings.—Isolated cases of actinomycosis in human beings had already been observed by B. v. Langenbeck (1845) and by Lebert (1857), but the disease was first clearly recognized and accurately described as an independent affection by J. Israel in 1878. Actinomycosis in human beings differs from the same disease in cattle in its slighter tendency to tumor-formation, and its marked tendency to insidious, widespread extension, which may finally lead to involvement of all of the viscera. The chronic inflammation that takes place around the fungous deposit is identical with that which occurs in animals. The newly formed granulation-tissue undergoes fatty degeneration more quickly, however, with disintegration or suppuration, while the fungous proliferation progresses and gives rise to fistulous formation, undermining the skin, penetrat-

ing the muscles, and advancing without restraint. Thus, the fungus extends from the lower jaw, along the neck to the pleuræ and the lungs, and through the diaphragm into the abdominal cavity. Even invasion of the heart and the brain has been observed. In addition to the continuous advance of the disease, which usually is its dominating feature, the dissemination of the fungus may take place through emboli, in consequence of penetration of actino-myces into the lumen of the vessels.

The peculiar and the single characteristic feature of all actinomycotic lesions is also in human beings the presence of the *actinomyces-granules*. They vary from the size of a grain of sand to that of a mustard-seed, and are coarse, dense, at times calcareous, bodies of yellow color. Viewed with low powers of the microscope they appear as dark, finely granular globules, of roundish or irregularly nodular shape. If slight pressure is made upon the cover-glass beneath which the granules lie, they are broken up into a number of smaller portions. Among the latter certain radiate structures are especially characteristic, the so-called *actinomyces-druses* (spheres), from which the fungus has ac-quired its name of *ray-fungus*. These, however, can be observed only with higher magnifications, and best after staining the preparation. From a dense center there radiate in every direction uniform, glistening, variously ramified filaments, which enlarge toward the periphery and terminate in bulbous, club-shaped swellings. There thus result star-shaped figures, which have been compared with closed crystalline druses or filled asters. In addition to these, however, there are always present simpler structures radially branched, consisting of only a small number of filaments. These appear microscopically as gray, viscid, sometimes more consistent, granules. They are juvenile forms, which are found with especial frequency in softened foci. The filaments at times exhibit divisions, and are suggestive of strings of bacilli. Finally, there are present also collections of spherical bodies that have been con-sidered as masses of cocci.

Gram's method, as modified by Günther (p. 107), is well adapted for staining the fungus, as is also exposure for half an hour to the action of heated carbol-fuchsin solution.

The **portal of infection** for the ray-fungus is also in human beings generally the commencement of the diges-

tive tract, and most frequently, probably, carious teeth, or the wound left after extraction of a tooth. The frequent occurrence of the disease in the neighborhood of the jaw, and upon the neck, is suggestive of this site of invasion, although any other part of the body may constitute the portal of entry. Cases of actinomycotic perityphlitis have been reported, and Chiari has recorded one of primary actinomycotic disease of the intestinal mucous membrane in an insane person, and the like. It is certain that an injury of the skin or of the affected mucous membrane is necessary for infection to take place. The source of the infecting actinomyces-material is, however, only rarely demonstrable. The chewing of wheat-grains is often assigned as a cause. Some observers suggest the possibility of direct transmission from cattle, although this has not yet been demonstrated. In a considerable number of cases of actinomycosis a history of any industrial or accidental association with cattle is completely wanting.

The **course of actinomycosis** is exceedingly insidious. The extension of the chronic inflammatory process that attends the vegetation of the fungus can be restrained only by complete removal of the latter. The disease frequently leads to death, from the invasion of vital internal organs (kidneys, lungs, etc.) by the fungous vegetation. Frequently, secondary acute inflammation, amyloid degeneration, etc., are superadded. Secondary infection with the excitants of suppuration appears especially common. The extensive phlegmons, and especially the pyemia, which often complicate the disease, can be attributed to this source, although thorough investigations in this connection are wanting.

The question has not yet been studied whether the actinomyces is capable of generating toxic substances and whether it gives rise in the body to intoxication, or whether its action is purely mechanical—that is, whether it impairs the functions of organs by invading their structure. It is noteworthy in this connection that some severe cases of actinomycosis are entirely unattended with fever.

Pure culture of the actinomyces has been attained by M. Wolff and J. Israel (1890). These investigators cultivated the ray-fungus in the absence of air, both upon agar and in hens' eggs. Upon the surface of *agar* there form at a tem-

perature of 37° C. (98.6° F.) numerous small, isolated nodules resembling dewdrops, the first indications of which are visible with a lens as early as after two days, but which do not appear distinctly before from the third to the fifth day. These project above the surface as spheres, though at times they are not entirely round. They grow quite slowly, often only reaching the size of a pinhead after eight days, and they usually do not grow larger. At the same time they become opaque. As a rule, they do not become confluent, remaining isolated for weeks and months. If, after inoculation, a veil-like turbidity appears on the surface of the agar, the nodules described often become differentiated subsequently. At times, however, the nodules coalesce into a whitish coating. The actinomyces-granules used for inoculation undergo enlargement as a whole, if not carefully rubbed upon the inoculated surface, and form a whitish area in which, however, at times, also a differentiation into nodules is discernible. In addition to these fine nodules there are present in small number white nodules, rather larger than lentils, with a blunt, club-shaped, projecting center, and which become covered over toward the periphery, and frequently exhibit roundish depressions at quite regular intervals. These nodules in roset-form, whose periphery, further, is not always regular, but at times is only slightly excavated or cleft, are also characterized by their growth into the body of the culture-medium ; they send root-like processes into the agar. These large nodules are not common, and are usually encountered only in small number in culture. As, in addition to them, the smallest nodules first described are often present also, and as, further, nodules of intermediate size—transition-forms—with root-like processes appear, Wolff and Israel consider the characteristic large nodules only as the highest macroscopic form of development of the inoculated actinomyces-fungus.

In *stab-cultures*, in addition to enlargement of the introduced granules, there develops throughout the entire length of the puncture a delicate, gray, veil-like turbidity, and this likewise contains numerous small nodules. At times large, flat, white plaques form at the site of puncture.

The cultures described develop typically only in the absence of oxygen ; otherwise the growth is but scanty. A temperature of 37° C. (98.6° F.) is always necessary for the cultivation of the fungus, and no development takes place at temperatures between 16° C. (60.8° F.) and 20° C. (68° F.). For this reason growth does not take place upon gelatin.

In *bouillon* growth is quite scanty. After from three to five days white scales and dots appear ; the bouillon itself, like the water of condensation in agar-cultures, remains clear.

Culture in Eggs.—The actinomyces thrives well in pigeons'

and hens' eggs, and when raw, as well as when boiled for three or four minutes. The shell of the egg is carefully cleansed by means of mercuric chlorid and sterile water, an opening is bored into one end by means of a flamed needle, and a platinum wire mounted with actinomyces-material is introduced deeply into the egg, in which it is moved to and fro several times. The opening made into the inoculated egg is closed with sealing-wax, and the egg, with the perforated end upward, is placed in the thermostat. After the lapse of from nine to twenty-eight days the egg will be found odorless, without formation of gas, and not discolored. There will be seen small, opaque, whitish clumps and dots ranging in size up to that of a pinhead, in the raw egg, in both the white and the yolk, and in the boiled egg at the junction between the two ; or there may be present in the liquid white of the unboiled egg lines and streaks of a turbid mass resembling nasal mucus ; or, finally, the line of inoculation and the central surface of the coagulated albumin may become occupied by a smeary, granular mass.

On *microscopic examination* Wolff and Israel found the agar-cultures made up of short rods, mostly straight, but often, also, comma-shaped, or even more markedly curved. These vary in length and thickness, plump and thick rods lying side by side with short and thin and long and thick rods. Often the rods present a globular or an olive-shaped enlargement at one extremity. In addition to the rods, there are found a small number of filaments, rarely rectilinear, mostly wavy and curved, or also spiral. While these are uncommon upon agar, the occurrence of beautiful, long, filamentous networks is usual in egg-cultures. The filaments, also, whose convolution often exhibits a radial arrangement at the periphery, at times present a knob-like enlargement at the extremity. They, as well as the short rods, together with the terminal enlargements, can be stained both by Gram's method and with fuchsin. Filaments stained for an hour with heated carbolfuchsin exhibit at times segmentation into longer or shorter rods, ranging in size down to that of the shortest coccus-like bodies, arranged irregularly, and separated by unstained intervals of varying length. In addition to the filamentous network, the rods are also found in the eggs.

Finally, there are found in agar-cultures, as well as in egg-cultures, also micrococcus-like bodies, at times free, at times in dense masses. These are of varying size, in part spherical, in part oval, in part rather irregular and angular ; they stain deeply with gentian-violet and also by the method of Gram. They correspond completely with the granules, in which the differentiation of the stained filaments can often be made out, and the rods also when stained exhibit similar granulation. Wolff and Israel do not consider these coccus-like

bodies as spores, on account of their irregular shape and the readiness with which they take stains, but as disintegrated filaments and rods. They do not, however, represent dead detritus, for when inoculated upon fresh nutrient media, they again develop into rods and filaments.

Experimental Development of Actinomycosis with Pure Cultures.—The various cultures thus present the same structures as the actinomyces-masses in the foci of disease : groups of cocci, rods, and filaments ; the fully developed clubs alone are wanting. Culture shows, further, that the cocci, rods, and filaments represent various phases of development of one and the same organism. Each form is capable of further development in as many generations as may be desired, and in this process the characteristic features always appear. The actinomyces retains its vitality in culture for a long time—as long as nine months.

That the fungus described is the true ray-fungus, in spite of the absence of typical clubs, is demonstrated by the successful inoculation of animals with cultures. Wolff and Israel infected 18 rabbits, 3 guinea-pigs, and 1 sheep, in part with agar-cultures, in part with egg-cultures, by intraperitoneal injection, or by injection of a suspension of the fungus into the liver. All of the animals, after the lapse of from four to seven weeks, exhibited multiple tumors in the abdomen, which contained typical actinomyces-druses. With these tumors actinomycosis could be again inoculated. Further, from their contents new cultures could readily be obtained, presenting all of the peculiarities of the original culture, including absence of typical club-forms. The chain of evidence in favor of the specific etiologic significance of the actinomyces-fungus cultivated by Wolff and Israel is thus conclusively established.

The anaerobic actinomyces-fungus described in the foregoing was obtained by Wolff and Israel in two cases of actinomycosis in human beings. According to Kruse, the actinomyces of Wolff and Israel has thus far not been cultivated by others. On the other hand, it must be pointed out that one of the authors of this work has succeeded in obtaining the same cultures with precisely the same characteristics in five cases of actinomycosis in human beings.

A **second species of actinomyces (aerobic actinomyces)**, which is quite distinct from the actinomyces of Wolff

and Israel, has been cultivated by Boström, Affanassieff, and others. This second variety of actinomyces grows more energetically in the presence than in the absence of air—an important differential diagnostic point in relation to that already described. The temperature-optimum is 37° C. (98.6° F.), although the fungus will grow well also at ordinary room-temperature. Upon gelatin-plates, and still better upon agar-plates, there form at first fine, radiating colonies, which develop with comparative activity to opaque nodules, whose periphery presents a delicate filamentous network. In agar streak-culture a continuous deposit does not form, as a rule, but a series of more or less closely approximated nodules. The latter not rarely exhibit a brick-red color upon blood-serum, and become covered by a whitish, down-like coating consisting of air-hyphæ. From these there develop, through a process of segmentation, a series of roundish bodies arranged like chains, the so-called actinomyces-spores. These are destroyed only after exposure for five minutes to a temperature of 75° C. (167° F.), whereas the mature forms die at as low a temperature as 60° C. (140° F.). When kept in the thermostat for some time, a wrinkled, coherent membrane eventually develops upon blood-serum, while the nutrient medium is softened. Upon potato the aerobic actinomyces grows as a brick-red deposit, and likewise with air-hyphæ. In bouillon floating and in part granular membranes form that sink to the bottom, while the overlying fluid remains clear. Milk is gradually peptonized.

With regard to the microscopic appearances, the granules and the druses, which constitute the characteristic feature of the morbid process from which the aerobic actinomyces is obtained, resemble in every detail those already described, so that it is only necessary to refer to the account of these. Preparations made from pure cultures, however, display great differences. Long filaments with rectangular branches may be seen that have arisen through the process of budding. Terminal enlargements are encountered but rarely in old colonies that have developed in the depth of the culture-media. Transmission of the aerobic ray-fungus to animals has not yet been effected with certainty—a third point in contradistinction from the anaerobic species of Wolff and Israel.

With regard to the **botanic position of the actinomyces,** it was formerly included among the pleomorphic bac-

teria. Kruse (Flügge's *Microorganisms*) includes it among the streptothrices. These represent, to a certain degree, a connecting link between molds and bacteria. They consist of long, cylindric filaments, dividing by budding, and eventually developing a true mycelium. Individual species produce fruit-bearers, which, after the fashion of oidia (see p. 338), constrict off spores directly, without especial fruit-heads. These spores must, however, not be placed upon the same plane as the similarly designated permanent forms of the bacteria, as they succumb to exposure for five minutes to a temperature of 70° C. (158° F.), as has been mentioned in connection with the aerobic actinomyces. In old cultures the branching filaments break up into bodies resembling bacteria, bacilli, cocci, and even spirilla. If, however, these disintegrated products are transferred to new culture-media, true filamentous networks will again develop from them. According to our own investigations, we have likewise reached the conclusion that the entire group of actinomyces is to be included among the hyphomycetes.

The **bacteriologic diagnosis** of actinomycosis requires only microscopic demonstration of the actinomyces-druses in the pus. Culture is not necessary to establish the diagnosis.

Treatment.—According to recent statements, actinomycosis is specifically influenced by potassium iodid. It is said that recovery from the disease will take place without any surgical intervention upon administration of moderate doses of this drug—from two to three grams daily.

PATHOGENIC STREPTOTHRICES.

In connection with the actinomyces, brief mention will be made of the *streptothrix Eppinger* and the *streptothrix farcinica*. The former was found by Eppinger in an abscess of the brain. The fungus presented a branch mycelium. Air-hyphæ and spores are, however, found only upon potatoes. The fungus can be stained by Gram's method. It grows best at a temperature of 37° C. (98.6° F.) in the absence of air. Upon gelatin elevated, wart-like yellow colonies form, which do not liquefy the culture-medium. Upon glucose-agar a wrinkled, orange-colored deposit forms, and upon potatoes a thin, yellowish-red deposit. Bouillon remains clear, although flocculent islands develop. A variety of pseudo-tuberculosis develops in guinea-pigs and rabbits inoculated with the streptothrix Eppinger.

The *streptothrix farcinica* (also designated " bacille du farcin des boeufs Nocard ") likewise gives rise to the formation of a branched mycelium, with air-hyphæ and spores. Growth takes place between 30° C. (86° F.) and 40° C. (104° F.) in the presence of air. The fungus can be stained by Gram's method. Upon agar whitish, dry scales form that subsequently become yellow and finally confluent. Bouillon is not rendered turbid, but presents flocculi. A similar change takes place in milk, which is not otherwise altered. The farcin des boeufs is a pseudo-tuberculous affection of the skin and the internal viscera in cattle. Experimentally the streptothrix farcinica induces pseudo-tuberculosis in cows, sheep, and guinea-pigs.

PATHOGENIC YEASTS.

The recognition of the pathogenic yeasts has been made only within recent years, and is due especially to the labors of Busse, Sanfelice, Curtis, and Rabbinowitsch.

The following varieties of pathogenic yeasts have been found in human beings:

The *saccharomyces hominis* has been observed in an infectious disease that began with subperiosteal inflammation of the tibia, and finally terminated in the clinical picture of pyemia. The organism appears in the form of round or oval cells, with double contour and a capsule. Upon gelatin-plates it forms round, projecting colonies that do not cause liquefaction ; upon agar, a whitish deposit ; upon potatoes, a grayish-brown deposit ; in bouillon, marked turbidity, with the formation of a membrane ; and upon blood-serum, a dewdrop deposit. The saccharomyces hominis possesses the property of inducing fermentation of grape-sugar, with the generation of alcohol and carbon dioxid. It is pathogenic for guinea-pigs, inducing local suppuration, and for mice, which die exhibiting septic manifestations.

The *saccharomyces subcutaneus tumefaciens* has been cultivated from a myxomatous tumor of the thigh. It consists of oval or round cells that frequently possess a large, transparent capsule. In gelatin stab-cultures development takes place in the form of small colonies ; the culture-medium is not liquefied. Upon agar a thick, creamy deposit forms, and upon potatoes a whitish deposit, later becoming brown ; upon beerwort-agar a brownish coating, and upon beerwort, a dense sediment without development of membrane-formation. The fungus has slight fermentative activity for saccharose, generating ethylic alcohol and acetic acid. This yeast is pathogenic for white mice and rats, in which extensive local vegetation occurs. Microscopically, the tumor-like formations present no true structure, but are found to consist of enormous parasitic infiltrations.

II. INFECTIONS WITH THE LOWEST FORMS OF ANIMAL LIFE.

The *protozoa*, the lowest forms of animal life, have hitherto taken part in the etiology of but a small number of diseases in human beings. Perhaps the future will show that they occupy a larger field of activity. In a number of infectious diseases whose exciting agents are as yet unknown, animal parasites are believed to have been observed —as, for instance, in whooping-cough, in carcinoma, and others. The connection of disease with the lowest forms of animal life has, however, been established with certainty only for two affections—namely, dysentery and malaria.

DYSENTERY (AMEBIC ENTERITIS) AND TROPICAL ABSCESS OF THE LIVER.

The cause of dysentery was recognized by Lösch in 1871 as peculiar animal parasites belonging to the class of protozoa—*amebæ*—which were found in dysenteric stools. Koch, on the occasion of his cholera-expedition to Egypt, noted the same organisms in the base of the ulcers of the intestine in four fatal cases of dysentery. Kartulis, Ogata, and others, and in Germany recently Kruse and Pasquale, and, further, Quincke and Roos, have since studied the amebæ carefully, and confirmed their etiologic relation to dysentery.

Th **amebæ of dysentery** are unicellular organisms of varying size, with ameboid movement. The smallest cells are about 10 μ in diameter; the largest, 50 μ (*giant amebæ*); the majority, at rest, from 20 to 25 μ. The protoplasmic body of the amebæ can be differentiated on movement into an outer zone, the *ectoplasm*, which is homogeneous and less refractive, and the *entoplasm*, which in part is apparently almost structureless, containing a small number of disseminated granules and differentiated from the ectoplasm only by its greater refractive power, and in part highly granular, and completely filled with irregular, mostly quite fine granules (*granular plasma*), or, finally, exhibiting a greater or lesser number of large and small vacuoles. The two layers are distinctly differentiable from each other only when the ameba is in motion, while the differentiation is

lost in a state of rest. Frequently, foreign bodies, especially red blood-corpuscles, also bacteria, much less commonly leukocytes, may be seen within the entoplasm; at times the protoplasmic body is literally stuffed with blood-discs. The ameba invariably contains a nucleus, which, however, is not always clearly visible in the moving cells. The nucleus is usually eccentric, and with change in shape, often near the periphery. It has a diameter of between 6 and 8 μ, is round in shape, generally with a sharp contour, and a punctate nucleolus. At times the contents of the nucleus are slightly granular.

The *characteristic* of the ameba is its mode of movement, which is designated *ameboid*. The ectoplasm is extended at

Fig. 83.—Amœbæ coli in intestinal mucus (after Lösch).

any given point in the form of a blunt, roundish, homogeneous process, and the protoplasm flows after it. In this way an actual change in position can be brought about by a slow, at times backward, crawling movement. The process may further be retracted, to appear immediately at the same or at another point. The ameba is thus engaged in constant change in shape. At times the ectoplasmic processes move around the central mass in waves without locomotion taking place.

Little is known regarding the *nutrition* of the amebæ. In general the foreign bodies so frequently seen in the entoplasm, especially the red blood-corpuscles, are considered as nutrient material. These are taken up by a process of

intussusception. The ameba sends out processes—*pseudopods*—around the foreign body, and in a certain sense thus surrounds it. Nothing is yet known with certainty as to the need of oxygen on the part of the amebæ.

Propagation of the amebæ probably takes place by dichotomous division, although direct division of cell and nucleus has not yet been observed. *Spore-formation*, such as has been assumed by some, has thus far not been demonstrated. Of importance are certain *permanent forms*, which have acquired especial resistance as a result of encapsulation ; these are designated permanent cysts or encysted amebæ. They are generally small—from 10 to 12 μ long—have a much sharper, at times distinctly double, contour, and possess a translucent luster. The nucleus is only indistinctly recognizable. In form the bodies are round, and they are nonmotile.

Death of the amebæ is manifested principally by cessation of movement, the greater or lesser activity of which also is an expression of a corresponding degree of vitality. With the advent of loss of motility the ameba invariably assumes a round shape, the differentiation between ectoplasm and entoplasm disappears, and the nucleus becomes distinctly visible. The dead amebæ after a time undergo degeneration : either they become homogeneous, with a fat-like luster, or they disintegrate into granules, often after having become constricted into several round masses. In cover-slip preparations the amebæ, after cessation of movement—that is, after death—do not remain visible for more than two days ; in the stools they have already disappeared within twenty-four hours ; only the encysted forms persist for a longer time, being still distinctly recognizable after twenty days in cover-slip preparations, as well as in preserved stools, but no longer after four weeks (Quincke).

The *resistance* of the amebæ to thermal and chemic influences has been but little investigated. The most suitable temperature is that of the body. Outside of the organism the parasites rapidly lose their motility, which at room-temperature is lost after the lapse of a few hours—exceptionally eight hours, and in one instance twenty-four hours. Elevation of the temperature to that of the body (examination upon a warm stage) increases the motility and conduces to the survival of the amebæ for a longer time. It has, how-

ever, thus far been impossible, even under the most favorable conditions of preservation, to keep them alive for more than twenty-four hours. Efforts at cultivation of the amebæ upon any nutrient medium have, likewise, thus far been unsuccessful. Even when they were inoculated in pure culture no growth developed. (See Liver-abscess, p. 370.) Kartulis has reported successful cultivation of dysentery-amebæ in infusions of hay. His observations have, however, turned out to be incorrect, as the supposed dysentery-amebæ were hay-amebæ, which always develop abundantly in infusions of hay not sufficiently sterilized.

It may yet be mentioned that the amebæ are rather indifferent to the action of tannic acid (0.3 per cent.) and of boric acid (1 per cent.), while quinin, in a solution of 1 : 5000, speedily causes their death.

With regard to *staining* the amebæ, reference may be made to the sections dealing with the intestinal changes below and with examination of the stools for amebæ (p. 371).

Occurrence of Amebæ in the Stools of Dysenteric Patients.—The amebæ described are regularly present in the stools in cases of tropical dysentery, and especially in the mucous elements thereof. They vary in number. In the albuminoid masses of mucus, often streaked with blood, found in recent cases, they are present actually in hordes ; in older cases, sometimes only in small number. Medicinal treatment may reduce the number materially, and eventually cause their temporary disappearance. The amebæ can be demonstrated only when the fresh stool is examined. Accordingly, a negative result in a single examination of the stools can not be accepted as evidence of the absence of the amebæ. Only continuous and repeated examination of all the intestinal evacuations, especially at the beginning of the disease, will yield definite information as to the presence or the absence of the amebæ.

The Intestinal Alterations of Dysentery.—The clinical symptoms of dysentery are those of hemorrhagic catarrh of the large bowel. The pathologic-anatomic conditions in the intestine include the presence of the dysenteric ulcer, with elevated, wall-like, undermined borders. The ulcerative process responsible for these alterations originates primarily in the submucosa, which undergoes a peculiar necrotic transformation. If a section of the intestinal wall, after hardening in absolute alcohol, is stained with Löffler's

solution or by Gram's method, the amebæ will be found regularly in the ulcers. They lie almost exclusively in the submucosa, at times more deeply, upon the serosa. The smaller forms, about 1 ½ times or twice as large as white blood-corpuscles, are constantly found in the intestinal wall. They appear roundish, without a distinct nucleus; their bodies stain a deep blue, and vacuoles are often indicated. In addition to the amebæ, bacteria are always found in the floor of the ulcer.

In the intestinal wall also the amebæ, as in the stools, are only demonstrable when the tissues are subjected to examination in a fresh state. At autopsies made four and a half hours after death the amebæ were present in the intestinal ulcers in enormous number, while at autopsies made eighteen hours after death but few were present.

Pathogenic Activity of the Amebæ of Dysentery for Animals.—Dysenteric affections, with the evacuation of hemorrhagic stools containing amebæ, and with characteristic ulcerative changes in the large intestine, in which also amebæ are present, have been induced experimentally in large numbers of cats by injection of dysenteric stools through the anus. Lösch had induced dysentery in a dog in the same way, by the introduction of large numbers of amebæ, both by the mouth and by the anus. In other animals, however, infection has thus far not been induced. It is noteworthy that Quincke and Roos observed that of four cats that received by the mouth exclusively stools containing amebæ, with an abundance of the encysted varieties, two were seized with typical amebic enteritis. The encysted varieties thus appear to be able to escape the action of the hydrochloric acid of the stomach.

Occurrence of Bacteria in Dysentery.—In amebic stools, in addition to amebæ, bacteria are always present; and especially and in preponderant number, streptococci; not rarely, also the bacterium coli; and, further, the bacillus pyocyaneus, staphylococci, etc. In the intestinal wall also the amebæ are found constantly in association with bacteria, and here bacilli rather than streptococci are present. The bacteria lie within and around the amebæ. At times they penetrate independently into the tissues, and more deeply than the amebæ. The association of bacteria and amebæ occurs with such regularity that some connection must exist between the two. It is possible that the condi-

tion is one of mixed infection. This is, however, not probable, as all of the bacteria found were not virulent for the animal on infection by enema, although the pus from a liver-abscess that contains only amebæ and no bacteria will induce dysentery in cats. It is more probable that secondary infection with these bacteria, all of which are regular inhabitants of the intestine, has taken place. What modification of the clinical picture of the disease and what portions in the anatomic alterations are to be attributed to the secondary bacterial infection, and how much is due to the amebic infection alone can not for the present be decided.

Portals of Infection for and Transmission of the Amebæ.—Dysentery is, according to clinical experience, only transmissible in a limited degree from one person to another, and from one place to another. It has not yet been determined through what medium the amebæ gain entrance into the body. The results of the experiments of Quincke, already alluded to, render introduction by the mouth possible. Accordingly, infection from case to case could take place directly through the intermediation of the stools. In general, however, direct contagion is not considered probable by clinicians. It is rather assumed that, as a rule, the amebæ are taken up from surrounding nature. Drinking-water is believed often to play the rôle of the intermediary. As has been pointed out, the amebæ do not persist in their original form in the stools, but they quickly undergo degeneration. Whether they persist in some as yet unknown vegetative intermediate stage in nature or in another host, or whether after leaving the body they develop permanent forms, has not yet been cleared up. Kruse and Pasquale froze stools containing amebæ for a quarter of an hour in a cold mixture. After thawing, no amebæ could be detected, not even encysted forms. The stools, however, proved pathogenic for cats, hemorrhagic enteritis, with abundant proliferation of amebæ, taking place after introduction into the bowel. With the disappearance of the ordinary forms of amebæ observed the parasites evidently do not wholly perish.

It is an open question how the amebæ, when introduced into the intestine, penetrate the mucous membrane and gain access to the submucosa, and whether a lesion of the mucosa is necessary for this to take place, or whether, per-

24

haps, with the cooperation of the bacteria, they are capable of passing through the intact mucous membrane. According to clinical observation, exposure to cold and disorders of digestion favor the development of dysentery. It thus appears that an especial local predisposition for amebic infection is necessary in precisely the same way as for bacterial infection.

Abscess of the Liver.—Abscess of the liver, which occurs so frequently in tropical regions, has long been associated clinically with dysentery. It has mostly been considered as a sequel of the latter disease. As a matter of fact, the pus and the abscess-membrane in cases of tropical abscess of the liver almost invariably contain the same amebæ that cause dysentery. When the amebæ are absent from the pus, there is generally no relation to dysentery ; so that the presence of amebæ may be utilized as a point in the differential diagnosis between idiopathic and dysenteric abscess of the liver. Naturally, careful and repeated examination of the contents of the abscess is necessary, as not every specimen of the pus will contain the amebæ, which are present in the abscess in varying number.

Bacteriologic examination of the pus from hepatic abscesses containing amebæ has yielded a positive result in the majority of cases, streptococci, staphylococci, colon-bacilli, and other bacteria being found present also. Only a small proportion of the abscesses are sterile. If the pus free from bacteria contains living amebæ, practically a pure culture is present, which may then be employed experimentally.

It is an open question, further, what rôle in the development of abscesses of the liver is played by the bacteria, as all of them possess pyogenic activity. It is possible that the amebæ injure the liver-tissue, and that the bacteria then secondarily induce suppuration. It is a question, also, as to how the amebæ gain entrance into the liver. In this connection the portal circulation, the peritoneum, the lymphatic vessels, and the biliary passages, must be considered as possible media of infection. Dissemination of the amebæ through the blood-stream appears the more probable, as they have been found repeatedly in the intestinal wall, within the lumen of vessels. Penetration of the intestinal wall and infection from the peritoneum also appear

probable.   Amebæ have been encountered in peritonitic
exudates, and hepatic abscesses are frequently quite super-
ficial and preferably in the right lobe. Dissemination through
the lymph-channels appears improbable by reason of the
numerous lymph-glands interposed.

Finally, some observers consider hepatic abscesses as not
always the sequel of dysentery, but both diseases as due
to a common cause, and of which one may at times pre-
cede the other, and vice versâ.   If primary amebic ab-
scesses of the liver actually occur, and are, perhaps, fol-
lowed subsequently by dysentery, migration of the amebæ
through the biliary passages would naturally have to be
assumed under such conditions.

Amebæ in Normal Intestinal Contents.—In the stools
of healthy persons there occur, usually in small number,
and sometimes in rather considerable number, amebæ
that are indistinguishable microscopically from those that
give rise to dysentery. Thus, amœba coli (Kruse and
Pasquale) or amœba intestini vulgaris (Quincke and Roos)
is not pathogenic for animals (cats), whereas the amœba
dysenteriæ causes dysentery in animals. Whether this
difference in virulence is permanent, or whether the two
amebæ are identical, the one merely having lost its viru-
lence temporarily, or the other having acquired virulence
temporarily, can not be decided at present in the absence
of a method of culture.   Quincke observed as the causa-
tive agent in a case of rather mild dysentery of autoch-
thonous origin in Kiel an ameba closely resembling that
of dysentery, but equally nonpathogenic for cats as the
intestinal ameba.   He places this amœba coli mitis between
the amœba intestini vulgaris and the ameba of dysentery
(amœba coli (Lösch)). (See Fig. 83.) Such transitions
render it probable that the virulence of the amebæ is no
more constant than is that of the bacteria.

Examination of the Stools for Amebæ.—In examining
the stools for amebæ the most important requirement is
that the stools should be quite fresh and be examined as
soon as possible after evacuation.   It is best to receive the
stool in a vessel previously heated (in water at a tempera-
ture of 40° C.—104° F.). If the stool is thin, a drop is
simply placed beneath the cover-glass ; when blood-tinged
flocculi of mucus are present, these are examined. If the
stool is mushy, a dilution with a warm solution of sodium

chlorid (from 35° C.—95° F.—to 40° C.—104° F.) is made. If the stool is formed, only the superficial layer of mucus is examined. To fix and to preserve the amebæ, a portion of the material containing them is spread in as thin a layer as possible upon a cover-slip, which is at once introduced into absolute alcohol before it dries. The amebæ take stains less well than the bacteria; so that in cover-slip preparations they are distinguished by their pallor. Methylene-blue is best suited for staining. The nucleus stains more deeply than the protoplasm. More, however, is to be learned from examination of fresh material than from that of stained preparations, and if the observation cover a protracted period, a warm stage is a useful adjunct.

MALARIA.

The *exciting agent of malaria* was discovered in 1880 by Laveran in the blood of patients suffering from that disease.

The *parasites of malaria* are unicellular forms of life that at an early stage are endowed with ameboid movement. They belong to the class of protozoa, the lowest forms of animal life; but there is no unanimity of opinion with regard to their position in the zoologic scale. Metschnikoff included them in the class of sporozoa, and considered them as coccidia. Others placed them among gregarines, Kruse among the earliest, and he designated them as hemogregarines. Still others believe them to be amebæ, and include them among the rhizopods. Mingazinni suggests that the entire group of parasites that invade the red blood-corpuscles be designated hemosporidia. In consequence of this diversity of opinion a suitable name for the parasites of malaria is wanting. The designation malarial plasmodia, proposed by Marchiafava and Celli, is inappropriate, as plasmodium indicates a body resulting from the confluence of numerous amebæ, all of which preserve their nuclei.

Morphology and Biology of the Parasites of Malaria (Plate I).—The malarial parasites vary in size from one to ten microns in accordance with the age of the individual cell. The juvenile forms are in general flattened and disc-like, and their shape varies with their ameboid movement. The mature
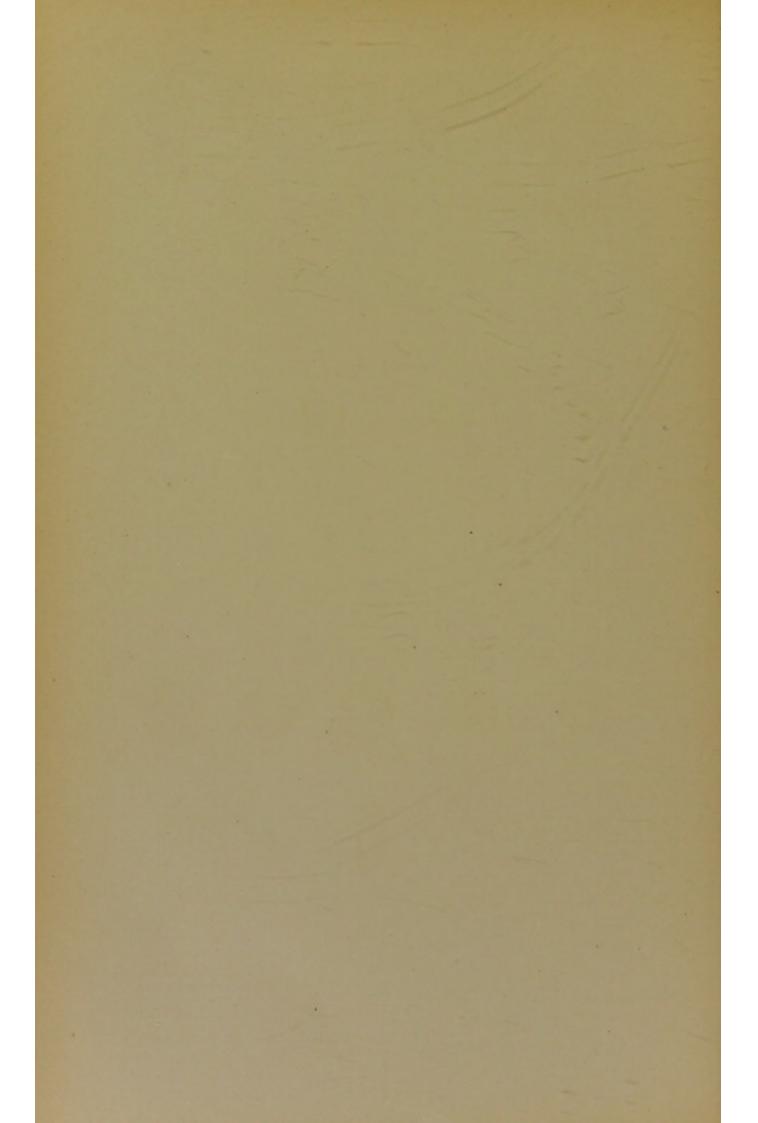
Various forms of malarial parasites (Thayer and Hewetson): Figs. 1 to 10, inclusive, tertian organisms; Figs. 11 to 17, inclusive, quartan organisms; Figs. 18 to 27, inclusive, estivo-autumnal organisms.

FIG. 1.—Young hyaline form; 2, hyaline form with beginning pigmentation; 3, pigmented form; 4, full-grown pigmented form; 5, 6, 7, 8, segmenting forms; 9, extracellular pigmented form; 10, flagellate form.

FIG. 11.—Young hyaline form; 12, 13, pigmented forms; 14, fully-developed pigmented form; 15, 16, segmenting forms; 17, flagellate form.

FIGS. 18, 19, 20.—Ring-like and cross-like hyaline forms; 21, 22, pigmented forms; 23, 24, segmenting forms; 25, 26, 27, crescents.

cells are spherical. A distinct cell-membrane, visible as a double contour, is not peculiar to most varieties. The protoplasmic body is relatively small in the juvenile forms, the nucleus preponderating. In the older cells these conditions are reversed. In the living parasites, especially at an early period, nucleus and plasma are usually not differentiable. In stained preparations (p. 393) the unstained nucleus is clearly distinguishable from the deeply stained cell-body. Differentiation into an outer layer of cytoplasm (ectoplasm) and a central portion (entoplasm) is, on the whole, not practicable. The plasma appears mostly homogeneous and hyaline, although mature parasites at times present dense, slightly refractive granulations. In the older cells the substance of the plasma generally contains from a brownish-red to a black pigment—the *malarial pigment*, or *melanin*, which is considered a digestive product of hemoglobin. This assumes the form in part of fine, dust-like particles, in part of coarser granules; or it appears as delicate needles up to 1 μ in length. The pigment often exhibits a peculiar dancing and wriggling movement, which only resembles, but is not identical with, the Brownian molecular movement, and is believed to be an active motor procedure. In addition to the ameboid movement and that of the pigment the malarial parasites are characterized by a third mode of motion—namely, that dependent upon flagella. From the fully developed round parasite arise flagella that move actively, in part become free, and then retain their motility. According to Kruse, these structures must be considered as evidences of degeneration. In addition to pigment the cell-body of the malarial parasites frequently contains vacuoles, mostly small and few. Often difficult of differentiation from these is the relatively large, vesicular, generally eccentric nucleus, which in the living cell is only recognizable by its distinct contour, and in stained preparations exhibits no nuclear membrane, but a distinct deep-black nucleolus at the periphery. The nucleolus is often surrounded by a feebly stained zone, while the nucleus itself does not take the stain. Kruse, upon the basis of his investigations, does not admit the significance of this large nucleus, but he considers the nucleolus as the nucleus.

Laveran's Crescents.—Morphologically distinct from these simple forms of malarial parasites (corps sphériques of Laveran) are the crescents of Laveran (corps en croissant) and the spherical and spindle-shaped bodies related to them (spheres of the crescentic series). The crescents have the shape of a half-moon, are from eight to ten microns long, at the middle from two to three microns thick, and are characterized by a considerable degree of refractive power. They always contain pigment, usually in abundance, but sometimes only a few granules. This

is usually collected at the center, frequently in the form of a figure of eight, although it may be distributed throughout the entire crescent ; in the latter event granules are frequently in tremulous movement. The concentrated pigment is invariably still. The crescentic bodies possess a membrane (Mannaberg), which, however, is not visible in all specimens. At times their poles are united on the concave aspect by a delicate curved line (remainder of the host-cell). The crescents are not endowed with ameboid movement ; they have, however, the faculty of slowly changing their form and becoming spindle-shaped, oval, and finally quite round bodies. This transformation, which can be observed under the microscope, takes place quite gradually, in the course often of several hours. In fresh blood-preparations containing numerous malarial parasites, there are always present a few spindles, ovals, and spheres, and Mannaberg is of the opinion that these changes in the shape of the crescents are dependent exclusively, or almost exclusively, upon the removal of the parasite from the human body, and that they do not occur at all, or but exceptionally, within the blood-vessels. By no means all crescents, further, undergo the changes in shape described, but the larger number retain their shape in blood-preparations preserved in a moist chamber. In the sphere resulting from the crescent the pigment is generally arranged in the shape of a wreath. After some time it begins to move, and, with tremulous movement, to intermingle, and soon the moving granules of melanin occupy the entire cell-body. Flagella also then form, and the entire body suddenly engages in jerking movements, while at the periphery projections and retractions take place. After some time processes resembling in form the finger of a glove appear, which are formed by the membrane of the body. This membrane ruptures, the processes retract, and long, slender filaments are thrust out from them, which actively lash themselves about. These flagella usually present a bulbous enlargement at their free extremity, and they often exhibit in their course knob-like swellings that appear to change place. From one to five filaments develop from a single sphere. Their active movement soon diminishes, and has entirely disappeared after from fifteen to thirty minutes. The resting filaments remain attached to the body ; although often they separate and then move actively about free in the blood. The membrane ruptured in the exit of the flagella becomes rolled together and occupies the periphery of the sphere in the form of a globule or a ringlet. Such globules distinguish the spheres resulting from the crescents from the other spherical elements of the malarial parasites already described. Further, the spherical bodies resulting from the crescents have, also after the loss of their membrane, when, therefore the double contour is no longer present, still a much

more sharply defined boundary-line than the other spherical forms. Some crescents undergo segmentation, usually dividing transversely through the middle of the body.

Relation of the Parasites to the Red Blood-corpuscles.—The parasites described appear in part free in the blood-plasma, and in part in association with the red blood-corpuscles. This association is considered by Laveran as one of simple adhesion of the parasites to the blood-cell, whereas Marchiafava and Celli, in opposition to this extraglobular mode of life, assume an actual penetration of the parasite into the blood-cell, an endoglobular mode of life. Both views are probably correct. The smaller forms appear impressed into the surface of the red blood-corpuscles, to which they are merely closely attached, whereas the older forms certainly occur within the bodies of the blood-corpuscles. This latter occurrence can be observed especially when the parasites leave the infected blood-corpuscle. In this process the residuum of the blood-corpuscle can be seen to rupture, while its contents, stained with hemoglobin, are distributed in fine drops in all directions. The endoglobular arrangement is the rule, especially for the crescents of Laveran, as Marchiafava and Celli have demonstrated. The infected blood-corpuscles are usually soon decolorized, while the parasites at the same time take up pigment. In the presence of some varieties of the parasite hypertrophy of the infected blood-corpuscle is observed to take place, while others cause diminution in size and shrinking of the blood-discs in which they occur.

Sporulation.—The propagation of malarial parasites takes place through spores that form in the mature cells. In the fully developed parasite a greater or smaller number of bodies develop, each of which exhibits complete cell-structure. This process of sporulation terminates the existence of the spore-forming bodies; the spores disperse, and of the mother-cell only a dead residual body remains, consisting mainly of the pigment of the mother-parasite. The systematic arrangement of the spores within the sphere around a mass of pigment often seated centrally gives rise to peculiar appearances that have been compared with those of daisies or sunflowers. Every spore contains a visible nucleolus, while the formation of the nucleus often takes place later.

Development of the Parasites.—According to Golgi, in the development of the parasites the unpigmented spore moves about free for a time in the blood-plasma, and meanwhile possibly grows somewhat; it then becomes attached to a red blood-corpuscle, in which it finds the conditions for its further development.

The young parasite, into which the spore within the blood-

corpuscle has, without especial external alteration, been transformed, grows and deprives the red blood-corpuscle of nutritive material. It converts the hemoglobin of the host into melanin, which it collects in the outer layer of its protoplasmic body. It grows until it reaches the height of its development, when it sometimes entirely fills the red blood-corpuscle, and it then gives rise to new spores, in the setting free of which the residuum of the blood-cell is ruptured.

With regard to the relation of the crescentic bodies to this process of development of the parasites, it is commonly assumed that the crescents result from the small ameboid parasites, of which Laveran considers them to be the encysted form, while Councilman believes them to be their spores. Mannaberg considers the crescents as copulation-bodies (syzygia), which result from the union of two ameboid malarial parasites. The two parasites do not completely coalesce (pseudo-conjugation), and they may subsequently separate (segmentation). Kruse believes the crescentic bodies to be no longer capable of infection, but he considers them the harmless residue of the infectious process.

Polymorphism or Multiplicity of the Malarial Parasites.—The appearances presented by the blood in the different types of malarial fever are most variable, and for a long time there has been a difference of opinion as to whether there are various forms of malarial parasites, with a special species of parasite for every type of fever, or whether the various forms of the parasite are but different phases of the same organism. Laveran and his adherents consider the parasites as polymorphous. They believe that the various types of fever are induced not by different species of parasites, but as a result of variations in the predisposition of the organism attacked. The Italian school, on the other hand, considers the exciting agents of the various types of fever as belonging to different species. Golgi makes three varieties : the parasites of quartan, those of tertian, and those of irregular, febrile type. According to his view, the quotidian type is dependent upon either two generations of parasites of the tertian or three generations of the quartan type, of which each generation is separated in its development from the other by a period of twenty-four hours. Golgi has described the cycle of development for these three varieties. Marchiafava and Celli recognize Golgi's tertian and quartan parasites. As the exciting agent of the irregular fever, however, they consider the small ameboid forms from which the crescents are derived.

At times these also give rise to typical quotidian fever. The different parasites appear to Marchiafava and Celli, however, as varieties of a single parasite. Grassi and Feletti have gone so far as to describe five species.

As cultivation of the malarial parasites is at present not possible, the question whether the forms of parasites are constant and variable, or whether each form gives rise to a definite type of fever, can only be determined by experimental conveyance of the parasites with the blood of malarial patients. Such inoculation-experiments have been repeatedly made in human beings. The results, as summarized by Mannaberg, are as follows: Of sixteen carefully performed experiments, there was in fourteen a complete concurrence between the forms of parasites from the source of inoculation and those from the inoculated person; whereas in two cases the inoculated person exhibited other forms than those from the source of inoculation. The two cases, however, that appear to support the view of polymorphism are, on more careful scrutiny, as Mannaberg shows, not entirely free from criticism, so that the results of the sixteen experiments render it highly probable that the individual varieties of parasites represent distinct species, which do not undergo a transformation into other varieties. From the experiments, the conclusion is to be drawn further that an unmistakable relation exists between the type of fever and the species of parasites.

Classification of the Malarial Parasites.—The fundamental types of intermittent fever are the *quotidian*, the *tertian*, and the *quartan*. In addition there occur frequently *irregular* types, which pursue their course in part continuously, in part with distinct remissions, or with attacks following one immediately upon another. Of the quotidian variety two forms have long been distinguished— namely, the *double tertian* and the *triple quartan*. The different types are dependent upon different forms of parasites. The following are among those best known:

1. The Quartan Parasite.—The juvenile form of the quartan parasite is an unpigmented body, with slow, ameboid movement, visible as a small, bright spot within the infected blood-corpuscle. It grows but little during the first twelve or twenty-four hours. Then pigment appears in the outer layer in the form of coarse granules and rods; this is nonmotile. With increasing pigmentation the para-

site loses its previous torpid motility, becoming spherical and filling the blood-corpuscle one-third or one-half. It then continues to grow slowly until it has attained the full size of the blood-corpuscle, so that no portion of this remains visible, and the parasite appears to be free. Sporulation now takes place, the pigment-granules concentrating at the center, while a radiate striation makes its appearance in the plasma, first at the periphery, subsequently also at the center, gradually becoming more distinct and dividing the parasite into from eight to twelve segments. These segments separate more distinctly from one another as oval bodies (daisy-form), and each contains a bright spot—the nucleolus. Spore-formation is now completed, and the spores are set free by rupture of the mother-cell. The entire process of development has occupied seventy-two hours. Segmentation takes place before and during the febrile attack. About three hours before the occurrence of the chill the first complete sporulation-bodies are visible in the blood. The red blood-corpuscles that have been infected with quartan parasites do not undergo change in size. The parasites at times progress to sporulation before they have attained the size of the blood-corpuscle. They then generally give rise to from four to six spores. The extrusion of flagella is observed seldom and only in the younger forms.

In the regular progress of development of the quartan parasites it is relatively easy to distinguish several generations from one another when these are present.

The quartan parasite induces *typical quartan* fever (100100100I); two or three generations separated by an interval of twenty-four hours give rise to *double quartan* (120120120) or *triple quartan* (123 123 123). The last may be considered a false quartan. If several generations of the quartan parasite are present whose development is separated not by intervals of twenty-four hours, but by shorter or longer intervals, *irregular types of fever* result.

2. *The tertian parasite* completes its cycle of development within forty-eight hours. The juvenile form resembles that of the quartan parasite: presenting a small plasmic body, with a nucleus free from pigment, in the living

state visible only as a bright spot within a red blood-corpuscle. It is actively motile, and extrudes numerous pseudopods. In the first twenty-four hours (first phase of Golgi) it develops gradually, and collects finely granular pigment within itself, which continues in active vibratile movement, with a preference for the outer layer of the plasmic body. With increasing deposition of pigment the activity of ameboid movement diminishes, without, however, ceasing entirely. After twenty-four hours the parasite has attained about half the size of the red blood-corpuscle, which itself has lost in color and has undergone increase in size— often to a considerable degree. " The blood-corpuscles infected with tertian parasites are frequently distended and chlorotic." After forty-eight hours, when the parasite has attained almost the size of the blood-corpuscle and is no longer in motion and the pigment also is at rest, *sporulation* takes place. Usually, the pigment moves again toward the center, the plasma divides into from fifteen to twenty round, highly refractive globules, which sometimes arrange themselves in two concentric rows (Golgi's sunflowers), but frequently lie together in the shape of berries, rosets, or grapes. The round globules are smaller than the spores of the quartan parasites. The nucleolus is only with difficulty to be distinguished in them. The spores then become free, and after a time infect new blood-corpuscles, in turn themselves to repeat the same process of development. By no means all parasites undergo sporulation, just as is the case with the quartan parasite. A large number of tertian parasites remain sterile. These barren elements generally become as large as the parasites that undergo propagation, or larger. In them, however, the pigment remains actively motile. Laveran considers them as hydropic and involved in degeneration. They may still be visible in the blood for hours after the attack, and even on the afebrile days. The act of sporulation corresponds also in tertian fever with the febrile paroxysm. Golgi has shown that as early as three hours before the chill the temperature begins to rise, and that then the first spores make their appearance in the blood. They are, however, most numerous at the time of the chill. In the mature tertian parasites the formation of flagella can often be observed. Kruse considers this process, likewise, as evidence of degeneration.

The tertian parasite causes *typical tertian* fever (1010101).
Two generations of the parasite may give rise to a *false
quotidian*, a so-called *double tertian* (1212121). Several
generations not separated from one another by intervals of
twenty-four hours give rise to *irregular fever.*

3. The Quotidian Parasite.—The commencement of the
cycle of development, which in its entirety occupies twenty-
four hours, is like that of the other parasites. The juvenile
form, unpigmented, consisting of plasmic body and nucleus,
infects the red blood-corpuscle. The parasite is actively
motile, and is often recognized by this property, while by
reason of its extremely delicate contour and its color, which
is only slightly less pale than that of the red blood-corpus-
cle, it is scarcely distinguishable from this. On removal of
the blood the parasite soon loses its motility, at the latest
after the lapse of an hour. A whitish ring is then seen to
form, with a reddish center. In consequence of a pro-
jection at one point of the periphery the ring frequently
resembles a seal-ring. Mannaberg believes that these
forms are only closely attached to the red blood-corpuscles,
and are not contained within them. The red spot in the
center is thought to depend upon dilution of the plasma and
the appearance of the underlying red blood-corpuscle as
seen through this. The annular form may return to the
ameboid. The ameboid parasite does not grow much—alto-
gether to only about one-third the size of the red blood-
cell. At the same time a fine pigment, which is often only
reddish and is but slightly motile, collects at the periphery.
After the lapse of twenty-four hours the pigment becomes
concentrated at the center or at the periphery in the form
of dark, resting clumps, and the parasite disintegrates within
the red blood-corpuscle into the smallest spores (from five
to ten). This process of *sporulation* takes place, according
to Marchiafava and Celli, only in the internal viscera of the
body, and scarcely at all in the peripheral blood. For this
reason the spores are encountered in abundance in the
blood obtained from the spleen, whereas they are found not
at all, or only isolated, in blood from the finger-tip. The
red blood-cells infected with the quotidian parasites con-
tract and become brass-colored (brassy bodies.) After the
quotidian parasite has been present in the blood for several
days the crescents already described invariably appear, with

their secondary form, the spindle-shaped (cigar-shaped) bodies and the spheres. With regard to the nature of the connection between these forms and the ameboid parasite there is as yet no unanimity of opinion. The various views with regard to the source and the destiny of the crescents have already been briefly outlined (p. 376).

The quotidian parasite gives rise to *typical quotidian fever*, and, when present in several generations, to *continued*, or *irregular, fever*. The fever induced by the quotidian parasite differs clinically from that due to the tertian and quartan parasites in its malignancy. It recurs obstinately, and often gives rise to profound anemia, and to other pernicious manifestations (diarrhea, cachexia, coma, etc.). The recurrences take place usually from seven to fourteen days after the first febrile paroxysm. The crescents are generally held responsible for the recurrences. These parasites are present in the blood in the afebrile interval, and are believed to be capable, through segmentation or true spore-formation, of giving rise to new ameboid forms. The new paroxysms would, thus, not be true recurrences, but, as Golgi believes, only the expression of a type with long intervals. This is denied by others, and the crescents are considered to be only degenerative forms that are incapable of contributing further to the formation of new individuals. It is true that with the presence exclusively of crescents and their spheres in the blood, fever is generally not present. On the other hand, the demonstration of these bodies in the blood indicates with certainty that fever existed a short while previously, and with great probability that new paroxysms will occur within a short time.

It is to be mentioned, finally, that there is an unpigmented quotidian parasite (Marchiafava and Celli), which is distinguished from the ordinary quotidian parasite only by the complete absence of pigment. This parasite, likewise, forms crescents, but these are unsupplied with pigment. The clinical course of the infection with these unpigmented parasites is in no wise different from that described for infection with the pigmented quotidian parasite.

4. *The malignant tertian parasite* has been separated by Marchiafava and Bignami as a special species. It stands close to the quotidian parasite, but is believed to differ from this in the fact that its cycle of development occupies

forty-eight hours, that it becomes somewhat larger (at the time of sporulation it is one-half or two-thirds the size of the blood-corpuscle), and that it remains unpigmented for twenty-four hours and then begins to acquire pigment, without, however, losing its motility. This parasite also forms annular bodies, and especially crescents. It generally develops from eight to fifteen spores. The blood-corpuscles infected by it become brass-colored, and usually shrink, but never increase in size. The parasite is distinguished by these last-named peculiarities from the ordinary tertian parasite (Golgi), which, besides, in all corresponding stages is larger, is provided with more pigment, and forms larger spores (from fourteen to twenty). The malignant tertian parasite gives rise to *severe tertian types* of fever, and also to *peculiar temperature-curves*, with quite short afebrile intervals often lasting only a few hours, and with regular pseudo-crises, and, finally, *continued*, or *irregular*, fever. All of the varieties of fever induced by it are characterized by the malignancy peculiar also to the quotidian parasite.

Mixed Infection.—In a number of cases of intermittent fever several of the parasites described are found together in the blood, and with especial frequency pigmented quotidian parasites, with unpigmented, and not rarely also quartan with tertian parasites. Golgi found in one patient three generations of the quartan parasite and two of the tertian parasite. All of the various components may find definite expression in the temperature-curve, or the fever may pursue an irregular course. It may be, further, that the temperature-course corresponds with only one variety of parasite, while the other appears to exert no influence.

Diagnosis of the Malarial Parasites (Method of Blood-examination.—A drop of blood is taken, as in ordinary examination, from the lobule of the ear or the tip of the finger, after previous cleansing by means of brush, soap, mercuric chlorid, alcohol, and ether. Slides and cover-glasses must be cleansed (with alcohol and ether) and dried with especial care. It is advisable not to manipulate them with the fingers at all, but to grasp them with the aid of suitable forceps (Ehrlich). A puncture is made with a lancet, the extruded drop of blood is received upon the cover-slip, and this is inverted simply upon the slide. It is especially important that the drop obtained be not too

large. The blood-cells must lie side by side somewhat scattered, as arrangement in rouleaux would conceal the parasites. The preparation is then examined with a good oil-immersion lens. If the examination is to be continued for some time, evaporation is prevented by surrounding the margin of the cover-slip with wax, or the uniformly distributed drop of blood is examined on an excavated slide, at the bottom of which is a drop of water. To stimulate ameboid movement, a warm stage should be employed. Examination in a warm room is possible, however, for an hour or more, without the aid of this device.

In the preparation of *dry specimens* also it is important to obtain quite a small drop of blood. The cover-slip is then quickly drawn over the surface of a second cover-slip, and both, protected from dust, are permitted to dry in the air. The dried preparations are placed for fixation for from five to thirty minutes in a mixture of equal parts of absolute alcohol and ether. They are now dried between layers of bibulous paper, and are stained—either in dilute aqueous solution of methylene-blue, and then, after rinsing with water, in two per cent. alcoholic (sixty per cent.) solution of eosin; or the specimen is exposed to the action of both stains at the same time. For this purpose Plehn recommends the following mixture:

Concentrated aqueous solution of methylene-
    blue, ...60
One-half per cent. solution of eosin (in sev-
    enty-five per cent. alcohol), ...20
Distilled water, ...40.

The preparation is kept in this solution for from five to ten minutes, and is then rinsed in water, dried, and examined in xylol Canada balsam. The hemoglobin-containing blood-cells are thereby stained red; the plasmic body of the parasites, more or less deeply blue. For rapid examination, simple treatment with aqueous methylene-blue suffices. For special examinations and for the determination of the finer structural relations, a large number of methods of fixation and staining have been proposed. For diagnostic purposes, examination of fresh specimens and of stained preparations by the method described is quite sufficient.

With regard to the *diagnostic utility* of the results of the examination, the following statements may be made:

The presence of even a single undoubted malarial parasite establishes the diagnosis with certainty. Should the result be negative, it must be borne in mind that after the action of quinin the parasites at times can not be found in the blood ; further, that they are sometimes wanting after quite recent infection during the first days of the disease. Negative results from a single observation are, therefore, not conclusive, even when a large number of specimens have been examined. Repeated examinations must be made, preferably from three to ten hours in advance of the paroxysm, when the parasites have reached the height of their development.

In the determination of the **variety of parasites,** and with regard to the **prognosis,** it is especially important to decide whether crescents and their spindles or spheres are present. The characteristics of these have been described. The recognition of the species, as well as the decision whether several generations of the parasite are present, naturally demands continued observations, to be repeated in the course of several hours. The differentiation of free bodies moving about in the blood is attended with considerable difficulty, and opportunity is here afforded for confusion from several sources. The parasitic nature of the bodies contained within the red blood-corpuscles is, however, more readily appreciated. The presence of pigment is here especially of significance. Only the unpigmented juvenile forms may occasion difficulty, as they may be readily mistaken in living preparations for vacuoles of the red blood-corpuscles. The structure, which can not be overlooked, especially in stained preparations (nucleus), is decisive in this connection ; the vacuoles of the blood-discs are structureless.

Explanation of the Symptoms of Malaria by the Presence of the Parasites.—The parasites of malaria are present exclusively in the blood. Even in the viscera they are found only within the capillaries. The melanemia of malarial patients, which has long been known, is explained by the conversion of the hemoglobin into melanin within the parasites. The melanin is set free in the process of sporulation, when it floats about in the plasma and is taken up by the leukocytes. The infection of the blood-corpuscles by the parasites furnishes a direct explanation for the anemia that arises in the course of malaria. The infected

blood-discs are destroyed in the process of development of the parasites. At the same time an injurious influence is exerted upon the uninfected blood-corpuscles by a parasitic poison dissolved in the blood-plasma. A chemic toxic action on the part of the parasites has, it is true, not yet been established. It is, however, rendered probable by the fact that the urine and the sweat of malarial patients, both of which secretions do not contain the parasite itself, are poisonous to rabbits, and are capable of causing death in these animals. The presence of a poison is also necessary for the explanation of the febrile attack. The paroxysm always sets in with sporulation—that is, with disintegration of the completely developed parasite. It is generally assumed that in this process, simultaneously with the spores, a poison is set free that is thrown into the blood and gives rise to the fever. Malaria would thus be a form of protozoan septicemia presenting analogies with ordinary bacterial septicemia. The toxic action would then without difficulty explain also the other symptoms—the diarrhea, the dyspnea, the ecchymoses, and especially the nervous symptoms. The bone-pains are usually attributed to the increased demands upon the blood-forming activity of the bone-marrow, and comparable with those observed in leukemia. Coma may be induced by the occlusion of the cerebral vessels with the parasites themselves, and this has been demonstrated microscopically in a number of cases.

Mode of Infection with Malaria.—Cultivation of the malarial parasites has thus far not been successful. Transmission of the disease to animals has likewise not been successful, although it has been attempted with numerous varieties. The only positive result that has been obtained in this connection consists in the survival for forty-eight hours of the parasites within the bodies of leeches that had been applied to malarial patients (Rosenbach). The malarial parasites are known only as blood-parasites of human beings. No conception has hitherto been possible as to where and in what form they exist outside of the human body. For this reason existing knowledge of the manner in which malarial infection takes place, and which is mainly based upon empiric observation, is not very extensive. Malaria may be transmitted from one human being to another by means of the blood. Gerhardt was the first to demonstrate this by subcutaneous injections of

25

malarial blood. Subsequently, infection was repeatedly transmitted with the blood of malarial patients by subcutaneous and intravenous injection. Malaria is, however, not a contagious disease. It does not pass from one human being to another under natural conditions. This could only be possible if the blood of a malarial patient gained entrance into the body of another individual, and probably few opportunities for this are afforded. The parasites are not present in the secretions and excretions of malarial patients; they appear to be present in the contents of the herpetic vesicles that malarial patients often exhibit. At least, malaria has been inoculated by means of the contents of such vesicles.

The malarial parasites must, however, be present somewhere in nature. They must live in some form in air, earth, or water, in certain swampy regions in which the disease is endemic, and at certain times becomes epidemic. The view is generally held that the parasites are inhaled; some believe that they are taken up with the drinking-water. Transmission through the bites of insects is theoretically possible, and is admitted by some observers, but it is not yet demonstrated.*

The *period of incubation* is in most cases from eight to fourteen days; cases are, however, known in which the disease appeared months after infection, and others in which the period of incubation was only one or two days, or even hours. These differences may be explained by the number of the infecting germs, and by the varying individual predisposition of those infected. That the germ is taken up in a form that must pass through certain variations within the body in order actually to become the malarial parasite is negatived by the cases with a short period of incubation.

The Action of Quinin and Spontaneous Recovery from Malaria.—Laveran has determined that addition of even a very dilute solution of quinin to a blood-preparation at once causes cessation of the ameboid movements of the parasites. Within the human body, also, the parasites undergo visible alterations after administration of quinin: they suffer in motility and undergo degeneration, and, in many, sporulation does not take place in the normal manner.

* Recent observations have demonstrated most conclusively that malaria is transmitted by some varieties of mosquitoes.—A. A. E.

According to Golgi, the spores are most susceptible to quinin, and the mature forms, before the beginning of the process of segmentation, are somewhat less so, and the endoglobular, juvenile forms still less so. The crescentic bodies are considered as entirely insensitive to the action of quinin. The parasites altered by the action of quinin are designated as " quinin-varieties." It is generally accepted that quinin is a specific fatal poison for the malarial parasites (Binz). Quinin certainly exerts a curative effect, and, according to some observers, given in small doses, it is also of prophylactic utility.

In explanation of *spontaneous recovery from malaria* Metschnikoff considers phagocytosis a most important factor. Probably this plays a not insignificant rôle, but other influences, besides, must certainly be taken into consideration. As has been mentioned, in every case of malaria a number of normally developed parasites regularly do not undergo sporulation. The remaining sterile elements are still visible in the blood for from twenty-four to forty-eight hours, when they undergo degeneration. Upon what this sterility depends is not known. It may, however, readily contribute to the process of recovery, should a considerable number of the parasites not undergo propagation. Further, the fever itself, as in the bacterial diseases, is believed to exert an injurious influence upon the parasites.

LEYDENIA GEMMIPARA SCHAUDINN.

In connection with the protozoa of dysentery and of malaria mention must be made of an observation by v. Leyden, who found an organism in the sterile ascites fluid obtained from two patients suffering from carcinoma. This organism is an ameba, which the discoverer investigated in conjunction with the zoologist Schaudinn. It is spherical or irregular in shape, and from 3 to 36 μ long. In hot weather it retains its motility for four or five hours without the employment of a warm stage. The ectoplasm sends out pseudopods, in whose formation the entoplasm also soon takes part. The latter possesses a honeycomb structure, and contains yellowish, refractive granules, perhaps hemoglobin derived from the inclosure of red blood-cor-

puscles. Within the entoplasm of the processes vacuoles, food-residua, and excrementitious granules resembling crystals are also found. Among the vacuoles one exhibits pulsation, undergoing contraction about every fifteen minutes. The nucleus is about one-fifth as large as the resting ameba. The pseudopods of adjacent bodies frequently unite, and there thus result plasmodia consisting of as many as forty segments. Multiplication takes place through division or budding. Leydenia belongs to the rhizopods. Von Leyden expresses himself with reserve in regard to its etiologic significance.

APPENDIX.

I. BACTERIOLOGIC EXAMINATION OF SOIL, AIR, AND WATER.

Although air and soil do not play the important rôle in the transmission of disease assigned to them by the pathology of the past (pp. 169, 191), they nevertheless frequently act as media for the transmission of disease-germs, as has been shown in the preceding chapters. Bacterioscopic examination of air and soil is frequently demanded of the hygienist when it is desired to determine the availability of ground for any public purpose. In individual instances such examination may become the duty of the physician, when suspicion is aroused as to a relation between existing disease and soil or air. Of much greater significance in the development of the infectious diseases is water, concerning whose essential participation in the causation of epidemics reference has been made (pp. 170, 190). Bacteriologic examination of water often devolves upon the physician.

SOIL.

Method of Investigation.—By means of a sterilized platinum spoon of known capacity (about $\frac{1}{50}$ cu. cm.) a specimen of the earth to be examined is taken up, and with this Esmarch gelatin roll-tubes are made, in which subsequently the number of colonies that have developed are counted and their nature is determined with the aid of a microscope. Ordinary plates may, likewise, be prepared. In order that a portion of the fragments of earth shall not remain behind in the test-tube when poured out, and the result of the estimation be, thereby, rendered quite uncertain, especial care must be taken to secure uniform distribu-

tion of the sample. To this end mortar and pestle are sterilized, and one gram of earth diluted with ten times the amount of sterile 0.6 per cent. solution of sodium chlorid is rubbed up. By means of a graduated platinum spiral a definite amount is removed, and with this gelatin-tubes (with three or four dilutions) are made in the usual manner.

In order to obtain deeper layers of earth a special boring instrument (Fig. 84), devised by C. Fränkel, is employed; this can be opened at the desired depth by a rotatory movement, and, when it is filled, is again closed and removed.

By these methods, however, information is gained only with regard to the aerobic bacteria present in the earth. As, however, anaerobic microorganisms occur in the soil, especially in manured garden-earth, in not inconsiderable number, it is advisable in making bacteriologic examinations of earth always to prepare also anaerobic plates. If it be desired to isolate a certain bacterium from the earth, animal experimentation is resorted to if the organism is pathogenic. If the bacterium sought possesses resistant permanent forms, the sample of earth may be distributed in water and heated for a considerable length of time to between 80° C. (176° F.) and 90° C. (194° F.), and then plates are made.

Fig. 84. — Fränkel's instrument for obtaining earth from various depths for bacteriologic study.

The Bacteria of the Soil.—The superficial layers of earth, even when uncultivated, contain large numbers of bacteria—about 100,000 to the cubic centimeter, and more. The further progress is made in depth the smaller the number of bacteria present in earth, and unless coarse gravel be present, germs will no longer be found at a depth of between ¾ and 1¼ meters, thus at the ground-water area. Porous soil filters the air, as well as liquids, in a perfectly reliable manner. This is naturally not the case in regions where fissures and breaks of any origin are present.

Bacilli especially are found in earth. Of innocuous mi-

crobes, the hay-bacillus, the potato-bacillus, and the root-bacillus (p. 404) are cultivated from earth with great frequency. Upon the bacteria of earth devolves the function of disintegrating dead organic substances, and converting them into materials from which new organic substances (plants) are formed. The carbon of organic substances is transformed into carbon dioxid ; the nitrogen, into ammonia ; and the hydrogen, into water. Certain bacteria of the earth oxidize the ammonia into nitrites (ferments nitreux, nitroso-bacteria) ; others, the nitrites into nitrates (ferments nitriques, nitro-bacteria) ; the entire procedure of the transformation of organic nitrogen into nitric acid is designated *nitrification*. This transformation-process, which is brought about by the bacterial flora of the earth, is absolutely indispensable to the vegetable world. If plants are placed in soil that contains all necessary nutritive elements, but has been sterilized artificially—that is, rendered bacteria-free—they will develop but incompletely, and soon begin to die (Duclaux). It must be pointed out that these nitrifying bacteria develop also in cultures that contain no trace of organic carbon-compounds, and that they, therefore, obtain their carbon-requirement directly from the carbon dioxid, without the aid of chlorophyl and light.

In the superficial layers of the earth, in addition to the bacilli, many *permanent spores* are present, which in part possess extraordinary resistance, and withstand for four or five hours the action of live steam. Favorable conditions for spore-formation must, therefore, be present in the superficial layers of the soil. Perhaps the circumstance is of significance that the individual particles of earth are surrounded by a capillary zone of fluid, and are thus protected from drying. It has been shown that anthrax-bacilli in cultures mixed with porous particles of earth undergo sporulation much more quickly and actively than otherwise. Anthrax-spores retain their infectivity for years in earth in which dissected animal carcases have been buried. Of pathogenic bacteria, cultivated and manured earth frequently contains, besides, the *bacillus of tetanus* and that of *malignant edema*.

The *malarial parasites*, likewise, must reside in the earth in certain localities ; at least, numerous clinical facts support such a view. Their demonstration therein has, however, as yet not been successful. Other infectious agents have

up to the present, been found in the earth only when but a short time has elapsed after the bacteria in question have been introduced at the given point with the disease-products (cholera or typhoid dejections, etc.). Typhoid-bacilli deposited at a depth of ½ meter retain their capability of development, under favorable conditions, for five and a half months, and perhaps still longer.

On the whole, it may be stated that pathogenic germs may multiply upon and within the surface of the earth if the atmospheric temperature be high ; but that, as a rule, they are suppressed by the competition of saprophytes. In the deeper layer the conditions for their propagation are much less favorable.

The burial of the bodies of individuals that have died from infectious diseases is scarcely capable of giving rise to infection. The accompanying bacteria are overrun by the saprophytes, and even if a small number—as, for instance, of tubercle-bacilli—may persist for months, perhaps for years, the opportunity is, on the whole, seldom afforded for their being carried from a depth to the surface of the earth or into the subsoil water.

AIR.

Method of Examination.—*1. Procedure of Hesse.*—A glass tube 70 cm. long and 3.5 cm. in diameter is sterilized and coated with gelatin, which, in the same way as in Esmarch's tubes, is distributed uniformly upon the inner surface by rotation in cool water. By means of an aspirator air is then drawn through this tube at the rate of a liter in from two to four minutes. At this slow rate of speed the germs of the air are deposited upon the gelatin, in which they subsequently develop into colonies. This method permits of examination of only relatively small quantities of air (from 10 to 20 liters).

2. Procedure of Petri.—A sand-filter, 3 cm. thick, supported upon two wire nets, is fastened in a short, glass tube, with a diameter of from 1.5 to 2 cm. ; the whole is sterilized, and air is permitted to stream through in a rapid current. The sand, which should have a grain of from ¼ to ½ mm. in size, filters with certainty all of the germs contained in the air. After from 50 to 100 liters of air have been drawn through the apparatus, the entire filter is intro-

duced into liquefied gelatin or agar, and plates are made. The amount of air is measured by means of a gas-meter.

3. If a precise quantitative result is not desired, gelatin-plates may simply be exposed to the air for a certain period of time, and the germs deposited may be permitted to develop into colonies.

Bacteria of the Air.—One cubic meter of air contains, on the average, from 500 to 1000 germs, including from 100 to 200 bacteria. Molds constitute by far the majority of the germs; next in frequency are yeast-fungi, and last,
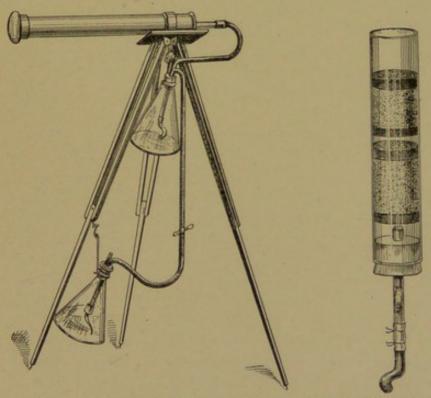
Fig. 85.—Hesse's apparatus for collecting bacteria from the air.

Fig. 86.—Petri's sand-filter for air-examination.

bacteria, which are usually represented only by micrococci and sarcinæ.

The number of germs present in the air is in marked degree dependent upon local and temporal variations. In inhabited localities, where dust is being constantly created and blown about, the air contains a larger number of micro-organisms than in deserts. On uninhabited mountains the air is almost entirely free from germs, as it is also at sea. At a short distance from land sea-air contains germs carried thither from the land by currents of air. After a heavy

rainfall and during the winter the number of germs in the air is considerably diminished. In the still air of rooms comparatively few germs are found, even in densely occupied quarters—as, for instance, in hospital-wards. As soon, however, as dust is set in motion, the number of microorganisms in the air increases enormously—to as many as 16,000 in the cubic meter. The majority of the bacteria, however, by reason of their weight, are soon again, after from half an hour to an hour, deposited upon the floor and the walls with the dust, and the air will then contain almost only the light mold-spores.

Sources of Aerial Germs (Air-infection).—The presence of spores of molds in the air in such large number is explained by the fact that generally the fruit-bearers of the fungous deposits project upward from the mycelium, and the spores can, therefore, be readily conveyed by currents of air.

Bacteria frequently find their way into the air with the small fibers separated from clothing, handkerchiefs, linen, etc.

With regard to the possibility of air-infection, recent investigations by Flügge and his pupils have yielded a wealth of facts that are well adapted to modify the views hitherto held. The earlier supposition that no germs enter the air from liquids or from moist surfaces is, according to Flügge, valid only in so far as the surface of the fluid remains unmoved and intact. A moderate wind (a velocity of four meters in a second) suffices, however, to set free *germ-containing small drops* from water-surfaces, moist articles, and the like. The conditions favoring the conversion of fluids into small drops in the air are often present, as in the open air, in a high degree in the neighborhood of the surging sea, of water-mills, etc., in lesser degree with every current of air that sets the leaves of trees in motion. In closed apartments, according to Flügge, small drops that enter the air are set free more frequently than is generally believed in sprinkling, in washing, in manipulating wet linen, and—upon which especial weight must be placed— in speaking, sneezing, and coughing. These droplets, whether they contain germs or not, are, as Flügge further demonstrated, carried away by the slightest currents of air for considerable distances, and not only in a horizontal, but also in a vertical direction. In order to demonstrate the

dissemination of such droplets in *speaking, coughing*, and *sneezing*, Flügge had the person under observation introduce a suspension of prodigiosus into the mouth. All the observations yielded positive results: The plates exposed at a distance of several meters became covered by characteristic colonies, and they remained sterile only after quiet speaking in a low tone.

With regard to the separation of *desiccated germs*, it may be said that this may likewise occur frequently. Flügge found that the movement of the finest dust already begins with a current of air having a velocity of one meter. In the open air the most varied mechanical influences (wagon-wheels, pedestrians) cause the separation of the smallest particles, which are then readily borne by the wind. In rooms particles of dust and fibers are set free by the vibration of the floor, by manipulation of utensils, furniture, clothing, etc. The smallest of these floating particles containing germs are, however, as Flügge has shown, moved in a horizontal direction by minimal currents of air of 0.2 mm., and in a vertical direction by currents of from 0.3 to 0.4 mm. per second. In the course of his experiments Flügge succeeded with the prodigiosus in demonstrating the distribution of dust-germs in all possible parts of a closed room. Germ-containing dust is not completely removed from rough surfaces even by the strongest currents of air. Airing of infected clothing, as frequently practised, does not, therefore, entirely attain the object desired.

Upon the basis of the results of these observations Flügge expresses the view that in the case of all infectious diseases air-infection may take place through the smallest droplets separated from fluid sources of infection. In cholera and typhoid fever this will occur but rarely—for instance, in the sprinkling of polluted water, in the washing of linen, and the like. The principal rôle in these affections is played by contact-infection, the dissemination through drinking-water, articles of food, etc. In the infectious dis-eases of the nose, the pharynx, and the respiratory organs, however, the possibility of transmission through small drops must be given greater consideration than it has received in the past. In diphtheria, influenza, whooping-cough, pneumonia, and pulmonary tuberculosis, droplets containing germs are sent into the air in coughing,

sneezing, and loud talking ; they are set in motion by min-
imal currents of air, and in a quiet room can be demon-
strated floating in the air for as long as five hours.

In cases of air-infection through *dry particles of dust,*
only the finest particles, capable of being carried by any
movement of air, demand consideration. Only such diseases
are transmitted naturally in this way whose exciting agents
are still capable of surviving in the dry state. For the
acute exanthemata, which have always been considered as
diseases due to volatile contagia, such a possibility must be
accepted.

Pulmonary tuberculosis has hitherto been generally
accepted as disseminated through inhalation of ejected
dried and pulverized sputum containing tubercle-bacilli ;
Flügge opposes this view. Attempts to infect animals
through inhalation of dried tuberculous sputum have never
quite succeeded ; and, further, the tubercle-bacilli do not
appear at all capable of being carried by the finest particles
of dust. Flügge expresses a warning against going to the
other extreme of attaching too great importance to the
danger of infection through drops of spray or even of con-
sidering it the only source of infection. Infection with
tuberculosis is dependent upon a large number of factors :
upon the environment of the patient, upon the presence
of tubercle-bacilli in the saliva, which forms fine droplets
much more readily than the viscid sputum proper, and
upon other conditions.

WATER.

Method of Examination.—The water to be examined
is collected in sterilized Erlenmeyer flasks, and is investi-
gated as speedily as possible. If the examination is deferred
for only a few hours, the indifferent saprophytic bacteria
that reside in water will have undergone multiplication, and
the estimation of the number of water-germs will not yield
reliable results. If the specimens of water to be examined
are sent from a distance, the water must be forwarded in
sterilized flasks, provided with glass stoppers, and packed
in ice. In obtaining water for examination care should be
taken that the material to be examined has not stagnated
in the conduit from the well or spring or other source of
supply. For this reason a certain amount of water is first

permitted to escape. Of the specimen obtained, 1, ½, and ¼ cu. cm. are removed by means of sterilized pipets, and introduced into liquefied gelatin, and plates are made. Greatly polluted water should be diluted with sterilized water from another source, and no more than $\frac{1}{10}$, $\frac{1}{100}$, and $\frac{1}{1000}$ cu. cm. should be employed. Before introducing the pipets the specimen is vigorously agitated, as the bacteria, by reason of their weight, quickly settle to the bottom. In the process of water-examination Koch's old method of making plates is still frequently followed, because these permit better than the Petri dishes the distribution of the gelatin in as uniform a layer as possible. When the plates have developed, the colonies are examined and counted, if present in large number by means of a special enumerator (a glass plate with etched squares). An estimate is made of the number of germs to the cubic centimeter of water, and the number as well as the identity of the varieties present is determined.

The Number of Bacteria Present in Water.—Spring-water, as well as subsoil-water, is free from germs at the point where it escapes from the earth. It has been mentioned that the earth at the level of the subsoil-water no longer contains bacteria. According to the most trustworthy investigations, pure tap-water and spring-water contain, on the average, from 2 to 50 bacteria per cubic centimeter ; pure pump-water, from 100 or 200 to 500 germs ; unfiltered water from streams kept unpolluted, from 6000 to 20,000 ; filtered river-water, from 50 to 200 ; polluted wells, as many as 100,000 germs ; and river tap-water, when the filtering apparatus is out of order, the same number. Drain-water and greatly contaminated streams contain from 2,000,000 to 40,000,000 germs to the cubic centimeter (Flügge). In the summer and after a heavy rainfall the number of bacteria in water is increased.

The Bacteria of Water.—The microorganisms living in water are mainly bacilli. A large number of them liquefy gelatin ; others generate offensive gases, and still others beautiful pigments. Of great interest are the so-called typhoid-like water-bacteria (p. 402) and the water-vibrios, which in a number of points resemble the comma-bacilli of Asiatic cholera (p. 404).

Among pathogenic bacteria typhoid-bacilli and cholera-vibrios have been repeatedly found in water. The method

for cultivating these bacteria from water has been considered in the discussion of typhoid fever (p. 178) and of cholera (p. 195). At this point it may be briefly repeated that by the addition of one per cent. peptone and ½ per cent. sodium chlorid, the water to be examined itself constitutes a nutrient medium, so that in this way large quantities of the fluid can be employed, whereas in earlier investigations a fraction of a drop had to suffice, and, as a result, the pathogenic germs were readily overlooked.

The saprophytic water-bacteria multiply in water to an unlimited degree. For the pathogenic bacteria, however, while the possibility of preservation often exists, but rarely is opportunity for proliferation in water afforded. To this end there are necessary a favorable external temperature (summer) and, further, solid particles of vegetable or animal origin, to which the bacteria adhere, which serve as nutrient medium, and which, at the same time, afford the bacteria protection from the competition of saprophytes. The exciting agents of typhoid fever and of cholera appear to survive in ordinary water for days, and even for weeks. Some observers even believe that cholera-vibrios may at first undergo multiplication in water. As a rule, however, the pathogenic germs are suppressed sooner or later in water in consequence of overgrowth by the saprophytes.

Self-purification of Water.—The microorganisms present in water are derived from the surface of the earth, the air, from waste water and the sewage of cities, emptying into the water-courses, from cesspools communicating with imperfectly constructed subsoil-wells, etc. The pollution of streams by cities is a most prominent factor; the Seine at Ivry, for instance, contains 32,500 germs to the cubic centimeter; below Paris, in Asnières, the number is 12,800,000. Fortunately, if renewed contamination does not take place, streams purify themselves (Pettenkofer). The microorganisms settle and are carried to the bottom, partly with the constituents suspended in the water and with the insoluble earthy combinations that form from calcium and magnesium bicarbonates after escape of the carbon dioxid. Light also in a high degree exerts an injurious influence upon the microorganisms present in water down to a depth of about two meters. The organic substances contained in water are gradually consumed by bacteria and algæ.

Sources and Mode of Water-supply.—In the practical application of bacteriologic examination of water for the construction of conduits, wells, etc., it is, in the first place, important to determine whether the water used contains pathogenic bacteria or not. If a given water contains many putrefactive bacteria (proteus) or the bacterium coli commune, it should be entirely excluded from use; for such conditions indicate with certainty that the source of supply is polluted. Formerly, great weight was attached to the number of germs present. Some difficulty was encountered in this connection in the establishment of a limit, beyond which water should no longer be considered pure. Some observers made this limit one hundred, others fifty, and still others five hundred. The absolute number of germs contained in a given water depends, however, upon such varied factors that an opinion based solely upon the number of colonies formed is not to be depended upon. It was then proposed to attach the greatest importance to the number of different varieties present. A large number of different species, it was thought, would, to a certain degree, be an indication of the greater probability of the occurrence of contamination of the water. This belief is not without justification, but too much significance must not be attached to the number of different varieties of bacteria in water. The fact can not be evaded that bacteriology has thus far not attained the importance in the hygienic determination of the usefulness of water that was originally attributed to it.

As a central source of water-supply for large communities *bacteria-free spring-water* or *subsoil-water* is most to be recommended, when this is obtainable in at all sufficient quantities. In the selection of a source of supply care should be taken to avoid undue proximity to communities or manured land. If spring-water or subsoil-water is not available, there is no alternative but to employ river-water or lake-water, which naturally must be obtained from a point above the city to be supplied. Such water is, as has been pointed out, exposed to numerous sources of contamination, and must, therefore, unconditionally be subjected to some process of *filtration* before being used. This may be effected by means of *sand-filtration*, which is carried out in large, cemented, covered reservoirs. The utility of sand-filters has been studied in recent years with especial care by

C. Fränkel and Piefke. At the bottom of a filtering basin large cobblestones should be placed to a height of 305 mm., upon these a layer of small cobblestones, 102 mm. high, then 76 mm. of coarse gravel, 127 mm. gravel of medium size, upon this 51 mm. of coarse sand, and, finally, 559 mm. of fine sand. Filtering properties reside solely in the layer of sand. Before the process of filtration is begun the reservoir must be permitted to remain filled with water for twenty-four hours. As a result, there will form a coating of sediment, and a slimy covering for the pores of the filter, which constitute the essential factors in the purification of the water. The rate of filtration should not exceed 100 mm. an hour. The form of filter described does not yield perfectly sterile water, but this will be found to contain between 50 and 200 germs in the cubic centimeter, which it must be noted are derived in largest part from the lower layers of the filter. The mechanism can be disturbed by tears in the cover of the filter, when the filtration-pressure, which will increase with increasing sliminess of the filter, becomes too great. The filter must, therefore, be cleansed from time to time. The working of the filter altogether demands unremitting attention. The water of every filter must be examined bacteriologically every day, and as soon as more than 100 bacteria are contained in the cubic centimeter, use of the water should not be permitted, and the filter should be renewed.

Of *house-filters* for the purification of water for domestic use numerous varieties have been recommended. Of all not one properly serves its purpose, as they are soon saturated with bacteria. For domestic purposes suspected water is rendered harmless in the simplest manner by boiling for five minutes (after the vapor of steam has arisen). Of wells the best as a source of water-supply are of the tubular variety, in which an iron tube leads to the subsoil-water area, and thus furnishes germ-free water.

Ice.—*Natural ice*, which is obtained in winter from streams and ponds, contains numerous bacteria—on an average 2000 to the cubic centimeter in ice-water, with a minimum of 50 and a maximum of 25,000 germs. Quite a number of bacteria, among them the cholera-vibrios, offer considerable resistance to freezing. Some even are capable of multiplication at this temperature (p. 22). *Artificial ice* prepared from distilled water contains from none to 10

germs in the cubic centimeter of ice-water. Distilled water itself, after standing for some time, contains many water-bacteria, but these are among those that do not bear freezing.

Artificial carbonated waters are often rich in bacteria, even after standing for months, and it has been shown experimentally that typhoid-bacilli, for instance, may survive for from days to weeks in such water. Care, therefore, should be taken to prepare artificial waters only from pure drinking-water or from distilled water.

THE BACTERIA PRINCIPALLY FOUND IN SOIL, AIR, AND WATER.

I. BACILLI.

1. NOT LIQUEFYING GELATIN.

(a) Chromogenic.

1. Bacillus Aurantiacus.—This is a short, plump rod, with slight spontaneous movement. Upon plates it appears in the form of an orange-colored, knob-like deposit. In gelatin stab-cultures it exhibits a glistening, orange-colored growth. Its appearance in bouillon is characteristic. The fluid itself remains clear, while upon the surface a membrane forms, presenting a small number of orange-colored spots, and at the bottom a somewhat lighter sediment collects.

2. Bacillus Constrictus.—This organism derives its name from the peculiar appearance it presents when stained by the method of Zimmermann. The rods exhibit a slight constriction between the individual segments, which are united into short chains, and they resemble biscuits in shape. In plates the colonies present the appearance of granular discs, with eroded margins. The color is between yellowish-gray and light sulphur-yellow.

3. Bacillus Fluorescens Nonliquefaciens.—This is a delicate, short, actively motile rod. Upon gelatin the colonies present a peculiar mother-of-pearl luster, which also exhibits fluorescence. Upon agar-agar the growth acquires a greenish tint. A subvariety has been described as *bacillus fluorescens nonliquefaciens immobilis*, which is distinguished by the absence of motility and of flagella.

4. Bacillus Fuscus.—This is a medium-sized rod, sometimes curved, which takes its name from the dark-brown pigment to

26

which it gives rise in all nutrient media. In gelatin stab-cultures a nail-shaped growth develops at first, the head subsequently extending.

5. Bacillus Rubefaciens.—This is a fine rod, consisting of two or more segments. Gelatin-cultures exhibit a pale rose-red color. Upon potatoes the substratum appears of a rose-red color, while the colony itself is between yellowish-gray and brownish-red.

6. Bacillus Subflavus.—Cultures of this organism give rise to a pale-red pigment; upon plates they exhibit a mother-of-pearl luster. The pigmentation is most distinct in agar-agar cultures. Several bacilli often lie attached to one another. The individual organism is from two to four times as long as it is thick.

7. Bacillus Brunneus.—The colonies of this small, nonmotile bacterium are characterized by diffusing a brownish pigment into the surrounding culture-medium.

(b) Nonchromogenic.

8. Typhoid-like Bacilli (Weichselbaum).—Under this designation is included a group of motile bacilli that resemble the bacillus of Eberth-Gaffky and the bacterium coli commune in both morphologic and cultural properties. On plates the colonies present easily the appearances of those of the typhoid-bacillus and the colon-bacillus (pp. 167, 120). Upon potatoes a deposit forms, at times brownish, at other times yellowish, at still other times scarcely visible. Coagulation is induced in milk. Some varieties cause fermentation of grape-sugar, while others do not. The nitroso-indol reaction is positive with some, and negative with others. These bacteria are free from all pathogenic activity in experiments on animals. That this group is constituted of a series of bacteria differing among themselves is demonstrated by the fact that any one variety is incapable of conferring immunity to any other. The attempt, also, to cause agglutination in the cultures of one variety with the blood-serum of animals that have been immunized to another variety has invariably failed.

2. LIQUEFYING GELATIN.

(a) Chromogenic.

9. Bacillus Arborescens.—This is a slender bacillus that frequently forms wavy filaments. It is incapable of spontaneous movement, and it is characterized by branch-like ramifications in gelatin plate-cultures, and by iridescence of its colonies. It gives rise to a yellowish or yellowish-red pigment, especially upon potatoes.

10. Bacillus Fluorescens Liquefaciens.—This is a motile

organism that closely resembles the bacillus pyocyaneus. It liquefies gelatin rapidly, with the formation of a greenish-yellow pigment, which is vividly fluorescent. Typical cultures form upon glycerin-agar, which acquires from an olive-green to a dark olive-brown color.

11. Bacillus Rubidus.—This is a medium-sized rod, actively motile, and arranged in threads of considerable length. It generates a brownish-red pigment, both in gelatin and in agar, and also in potato-cultures. Other ·than pigment-formation it presents scarcely anything else characteristic.

12. Bacillus Violaceus.—This is a small, slender, actively motile rod, that upon agar forms central spores. In its growth upon gelatin-plates a bluish-violet bacterial mass appears in the liquefied culture-medium. Upon agar-agar and potatoes the formation of pigment is intense, and the color is dark violet, almost black.

13. Bacillus Viscosus.—This organism closely resembles the bacillus fluorescens liquefaciens, from which, however, it is differentiated by the formation of a chocolate-colored coating.

14. Bacillus Ianthinus.—This is a motile bacillus of medium size. Its appearance in growth upon gelatin-plates is usually compared with the appearance of a drop of ink that has fallen upon them. It forms a violet pigment in all nutrient media.

15. Bacillus Helvolus.—This appears in the form of motile rods of varying length, which are often united into short threads. These generate from a yellowish to a sulphur-yellow pigment. Upon plates the colonies appear as circular, bright-yellow discs that lie in a funnel of liquefaction. Upon agar an abundant deposit of intensely yellow hue forms.

16. Bacillus Prodigiosus.—This is a small rod (formerly designated micrococcus prodigiosus or monas prodigiosa), often collected in small chains, of slight motility, occurring not rarely in the air, less commonly in water, rather frequently upon amylaceous nutrient media (bread, potatoes), upon meat, and in milk. It grows upon all nutrient media, with the development of a bright-red pigment; this is most intense on potatoes, which present a blood-red coating. Upon gelatin-plates rather deep, small, white dots appear, and also superficial, roundish, red colonies with an irregular border. Gelatin is energetically liquefied. Upon agar-agar a coating of moderately dark-red color appears, while the nutrient medium itself is not discolored. On cultivation at a temperature of 37° C. (98.6° F.) the prodigiosus loses its red color in the course of several generations. In cultures, especially upon potato, in addition to the red pigment, trimethylamin forms (odor of pickled herring). Coagulation is induced in milk. Saccharine nutrient media undergo fermentation. The prodigiosus will thrive also in the absence of oxy-

gen, but then without the development of red pigment. It is somewhat pathogenic, inoculated animals dying with toxic symptoms after introduction of large amounts. The name pro-digiosus is derived from the fact that the bloody appearance of the so-called miraculous holy wafers is attributed to infection with this microorganism.

17. Among other pigment-producing bacteria characterized only by the color of their cultures may be mentioned the *bacillus ruber balticus, bacillus ruber aquatilis, bacillus cœruleus, bacillus pavoninus, bacillus amethystinus.*

(b) **Nonchromogenic.**

18. Bacillus Liquefaciens.—This is one of the most wide-spread of the water-bacilli. It is an actively motile rod, often joined in short chains of four or more segments. It rapidly liquefies gelatin. In plates it assumes the form of a dish, upon the base of which lies a gray, bacterial mass. In stab-cultures the growth assumes the form of a stocking, with a dilated upper portion. The odor of the culture is highly disagreeable. The bacillus exhibits facultative anaerobiosis. In nutrient media containing nitrates it generates nitrous acid.

The following likewise are included among water-bacteria:

19. Bacillus Liquidis.—This is a short, plump, slightly motile bacillus, which also rapidly liquefies gelatin. In tubes the gray, liquefied gelatin becomes covered with a thin membrane, which sinks to the bottom on agitation.

20. Bacillus Aquatilis.—This is a slender rod, with spontane-ous motility. It liquefies gelatin slowly, and according to some observers not at all. It grows in gelatin upon the surface in the form of small, yellowish colonies, and upon potatoes with a scanty yellow deposit.

In the earth and in certain articles of food the following bacteria are always to be found, which are characterized by especial resistance of their spores:

21. Root-bacillus.—This is a large, thick bacillus, with rounded extremities, and possessing slight motility. It develops central spores, and growth takes place only in the presence of oxygen. The whitish-gray colonies that form consist of a network of fine, interlacing threads. They liquefy gelatin. In stab-cultures also filaments and processes form, and an appearance results re-sembling an inverted fir-tree. Upon agar-agar a network forms suggesting the ramifications of the roots of a tree.

22. Bacillus Subtilis (Hay-bacillus).—This is a large, deli-cate bacillus that often develops into long, straight threads. The bacillus subtilis is a strictly aerobic organism, and it quickly liquefies gelatin. The temperature-optimum is 30° C. (86° F.); the temperature-minimum, 10° C. (50° F.); the temperature-maximum, 45° C. (113° F.). Upon plates the bright, grayish-

white colony appears surrounded by a sparkling crown. Upon agar-agar the growth is peculiar, a stiff, wrinkled, readily detached deposit forming. The hay-bacillus forms central spores, which are somewhat thicker, but considerably shorter than the mother-cells. It is found in the air, water, dust, feces, hay, etc. In order to obtain the organism in pure culture hay is cut up into small pieces, which are covered with water in an Erlenmeyer flask, and exposed to a boiling temperature for fifteen minutes. In this way all of the germs are destroyed, with the exception of the resistant spores of the hay-bacillus. These then grow, and after two or three days they form a superficial membrane upon the hay-infusion.

23. *Potato-bacillus* (*Bacillus Mesentericus*).—Three varieties of this microorganism are distinguished : bacillus mesentericus vulgatus, fuscus, and ruber. The last, especially, which imparts a rose tint to potatoes upon which it grows, possesses permanent forms of extraordinary resistance, which withstand boiling for from five to six hours. In proportion to the cell the spore is quite large. The cultural peculiarities of the organism resemble those of the hay-bacillus. Upon potatoes the bacillus gives rise to a wrinkled coating.♦ Milk is coagulated and peptonized.

24. *Bacillus Spinosus*.—This is a strictly anaerobic, motile rod. Its colonies in gelatin form iridescent globules, with thorn-like processes. Gelatin is liquefied, with the formation of gas. Stab-cultures, before liquefaction takes place, present the appearance of a prickly caterpillar (Lüderitz). The bacillus spinosus grows both ·at room-temperature and at the temperature of the body. It forms central spores, the rod becoming at the same time spindle-shaped (clostridium). The bacillus is usually found in garden-earth.

II. MICROCOCCI.

1. NOT LIQUEFYING GELATIN.

(a) Chromogenic.

25. *Micrococcus Aurantiacus*.—This is a round or oval coccus, arranged in groups. The cultures are yellow, slimy, knob-shaped, and they do not extend greatly in width.

26. *Micrococcus Versicolor*.—This is a small coccus, arranged in groups or in pairs. It occurs with extraordinary frequency in the air. The colonies are irregular in shape, with a yellowish-green color. They exhibit, especially upon gelatin, a mother-of-pearl iridescence, and they cause fermentation in nutrient media containing glucose.

(b) Nonchromogenic.

27. Micrococcus Candicans.—This is a round coccus of moderate size. Its most distinctive feature is its growth in gelatin stab-cultures, in which a nail-shaped growth appears, with a porcelain-white, glistening head.

28. Micrococcus Concentricus.—This is characterized by the concentric extension of its colonies upon gelatin-plates and in stab-culture. The colonies present from a whitish-gray to a bluish-gray color, and are superficially serrated. The cocci themselves are small and are arranged like grapes.

29. Micrococcus Rosettaceus.—This is a coccus of moderate size. Its growth is mainly superficial. Roset-like deposits form, with irregular margins. The colonies are grayish-white in color, but darker in the center, up to brown.

30. Micrococcus Aquatilis.—The colonies are round, and possess a mother-of-pearl luster. The margins appear serrated; the color is light gray. Viewed with low powers of the microscope, the colony appears in the form of a berry.

2. LIQUEFYING GELATIN.

(a) Chromogenic.

31. Micrococcus Cremoides.—This is a small coccus, arranged in groups, and giving rise to a cream-colored pigment. At first, the colonies upon gelatin are from yellowish-white to brownish-gray, granular, circular; while later the discs appear eroded, and they lie in a liquefied excavation.

32. Sarcina Lutea (Yellow Sarcina).—This coccus, strictly aerobic, is arranged in so-called balls of twine. Upon gelatin-
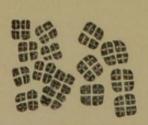
Fig. 87.—Sarcinæ; × 600 (Flügge).

plates it forms rounded, slightly granular, yellow colonies. In stab-cultures marked superficial growth takes place. The cultures generate a citron-yellow pigment. Liquefaction occurs quite late; then the clear, liquefied gelatin overlies the citron-yellow precipitate. In addition to the yellow sarcina there are also white, orange, and red sarcinæ, which are distinguished from that described only by the difference in color. The varieties of sarcinæ are present in the air.

33. Micrococcus Agilis (Ali Cohen).—This actively motile organism (flagella), cultivated from drinking-water, grows upon all nutrient media, with the formation of a rose-red pigment. It slowly liquefies gelatin.

(b) Nonchromogenic.

34. Micrococcus Radiatus.—This is a small coccus, without typical arrangement. Upon plates it forms colonies surrounded

by a sparkling crown. In stab-cultures the colonies, likewise, display horizontal radiation. Gelatin is slowly liquefied.

III. VIBRIOS.

Since the great epidemic of cholera at Hamburg in the year 1892, a large number of vibrios more or less closely resembling that of cholera have been described, which have been cultivated in part from river-water, in part from other sources, and of which the most important will be mentioned.

35. Vibrio Aquatilis Günther.—This organism is scarcely to be confounded with the comma-bacillus, on account of its circular, finely granular colonies, with smooth borders, even inde-

Fig. 88.—Spirillum aquatilis, from an agar-agar culture; × 1000 (Itzerott and Niemann).

pendently of the absence of the cholera-red reaction. It at first grew feebly in liquid nutrient media, but as a result of repeated inoculation it acquired, in consequence of adaptation to such media, the property of growing upon bouillon and peptone-water.

36. Vibrio Berolinensis.—This organism, which was cultivated by Neisser (1893) from the water-supply of Berlin, bears a close resemblance to the cholera-bacillus in regard to form and flagella. Upon gelatin-plates the border of the colonies is, however, mostly smooth. The colonies themselves exhibit a much more finely granular appearance than those of the comma-bacillus. Gelatin is slowly liquefied, and the cholera-red reac-

tion appears. Guinea-pigs died after intraperitoneal injection, with precisely the same symptom-complex as appeared after introduction of true comma-bacilli. Similar vibrios have been cultivated from Munich well-water by Weibel; from Peene water, by Löffler; from the harbor of Gröningen, by Fokker; and from the harbor of Altona, by Kiesling; and, further, from the Seine. In forming an opinion as to the identity of these and the following vibrios with the true exciting agents of Asiatic cholera, Pfeiffer's reaction (p. 187) and the agglutination-test must be given preeminent importance. Both tests yield negative results.

37. Vibrio Metschnikoff.—This was first cultivated in an epidemic among fowl, and subsequently from the water of the Spree.
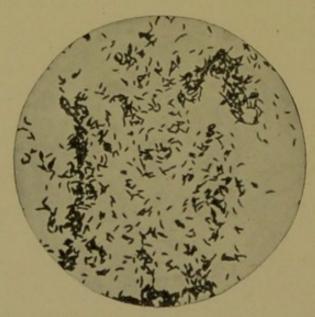
Fig. 89.—Spirillum berolinensis, from an agar-agar culture; × 1000 (Itzerott and Niemann).

It is somewhat thicker and shorter than the cholera-vibrio, and sometimes almost coccus-shaped. In hanging drop it exhibits active motility. The cultures resemble those of the cholera-bacillus, except that liquefaction is more marked. The nitroso-indol reaction appears within twenty-four hours. This vibrio, in contradistinction from that of cholera, is pathogenic for both pigeons and guinea-pigs.

38. Vibrio Gindha.—This organism was cultivated by von Pasquale from well-water in Gindha near Massowah. It is a rather long, slightly curved rod, actively motile (with a single terminal flagellum). It is but slightly pathogenic, and it does not yield the nitroso-indol reaction.

39. Vibrio Lissabon.—This has been cultivated in the course

of an extensive epidemic of cholerine in Lisbon, in which, however, but a single death occurred. On gelatin-plates cir-
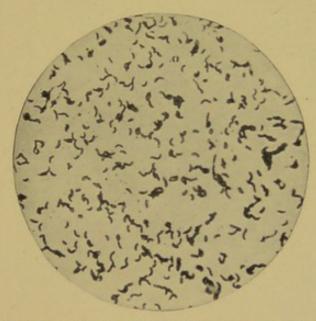
Fig. 90.—Spirillum Metschnikoff, from an agar-agar culture; ✕ 1000 (Itzerott and Niemann).
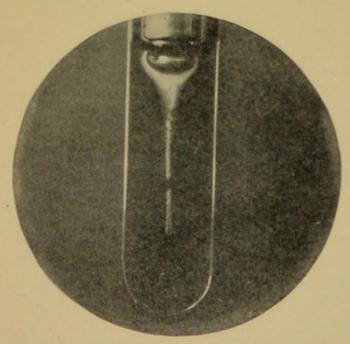
Fig. 91.—Spirillum Metschnikoff; stab-culture in gelatin forty-eight hours old (Fränkel and Pfeiffer).

cular, sharply circumscribed, whitish-yellow colonies form that cause little liquefaction. The culture does not yield the nitroso-indol reaction.

40. Vibrio Phosphorescens Dunbar.—This may be confounded with the cholera-bacillus on account of its morphologic and cultural appearances, but is distinguishable by its phosphorescence.

41. Vibrio Massowah.—This possesses from two to four flagella, whereas the comma-bacillus possesses but a single terminal flagellum. It yields the nitroso-indol reaction, and is quite pathogenic for pigeons, guinea-pigs, and rabbits.

Fig. 92.—Spirillum Dunbar, from agar-agar ; × 1000 (Itzerott and Niemann).

II. DISINFECTION.

To afford protection against the importation of epidemics, which mostly threaten Europe from the Orient, resort is had to supervision of maritime intercourse by means of *quarantine-stations*, the *International Sanitary Bureau*, and other official arrangements. *General hygienic efforts* also, through drainage of the soil, through the provision of a pure water-supply, through care for healthful dwellings, etc., to render the sanitary conditions of the country during periods free from pestilence such that epidemics, when imported, do not find the soil upon which they thrive, must be made by the public authorities. The practising physician can cooperate in these to only a slight degree. The conditions, however, are different when once the epidemic has been introduced ; then the relations of the physician with regard to it are identical with those that exist

with regard to endemic diseases, such as typhoid fever, whooping-cough, tuberculosis, etc. These also may attain epidemic distribution. The duty of *notification*, which devolves upon the physician, renders it possible for the authorities to have cognizance of every extraordinary increase in the prevalence of any infectious disease, and to recognize the epidemic at the outset, to trace its causes and eventually to remove them. An important part of the prophylactic measures against disease that already prevails lies within the control of the physician. He should endeavor to prevent the increase of an endemic disease to the proportions of an epidemic, and, when the disease is introduced from without, to prevent its further extension by suppressing the source of infection, by rendering the individual case harmless. *Disinfection*, the destruction of disease-germs, with which every case of disease threatens its immediate and remote environment, is an integral element of all disease-prophylaxis.

In the preceding chapters it has been pointed out in detail how the germs of disease pass over into the secretions and excretions, how they adhere to beds, to linen, and to sick-rooms, etc. It has also been pointed out how they become deposited upon articles of food and with these gain entrance into other organisms. All of these carriers of disease-germs should be subjected to disinfection in every case of disease.

The agents for disinfection act *mechanically* or *chemically*. Those of the first group are aimed at the removal of the disease-germs mechanically (by brushing, rubbing, washing, rinsing, scouring, etc.), or at their destruction directly (by drying, exposure to the sun, or to the action of heat). Of these mechanical means the first, washing with brushes and soap, etc., are in common employ. Exposure to the sun (airing of beds, etc.) is probably efficient, but can influence only the bacteria lying superficially. Heat may be employed in the form of *hot* or *boiling water*, of *hot air*, and of *steam*. Boiling water is an active disinfectant, destroying bacteria generally within a few seconds, and particularly resistant forms—apart from the spores of harmless potato-bacilli (mesentericus)—in from two to five minutes. Hot air is much less efficient. According to Koch and Wolffhügel, the most resistant of the spore-free bacteria are destroyed by exposure for one and a half hours to air

at a temperature of 80° C. (176° F.), and spores only after exposure for three hours at a temperature of from 140° C. (284° F.) to 160° C. (320° F.). Steam may be employed still or streaming, and further under tension and superheated. Live steam is most available for large disinfecting apparatus. These represent in principle nothing more than the Koch steam-chest. Into a large vessel or an airtight chamber, steam-vapor at a temperature of 100° C. (212° F.) is so introduced that it displaces all of the air and comes in contact with every portion of the articles to be disinfected. As in the steam-chest, in these larger apparatus also complete sterilization is effected in from fifteen to thirty or sixty minutes, in accordance with the size of the articles. Steam under pressure (120° C.—248° F.) is employed for purposes of disinfection, especially in France. With its aid sterilization is effected quickly and with absolute certainty when provision is made for the escape through a valve of all the air contained in the apparatus.

Cold inhibits the development of, but scarcely destroys, resistant microorganisms. Anthrax-spores retain their vitality and virulence for more than twenty-four hours at a temperature of 130° C. (266° F.).

Of greater importance are *chemic disinfectants*. These consist of all sorts of chemic agents (acids, alkalies, salts, aromatic substances, etc.), and the large majority cause enfeeblement and inhibit the development of the bacteria, and in sufficient concentration effect their destruction.

As the chemic disinfectants are, in the nature of things, almost invariably employed in aqueous solution, recent investigations in the domain of physical chemistry with regard to the nature of the solutions have not only acquired significance for the comprehension of observed phenomena, but they have also yielded valuable aid in the selection and application of disinfectants. In the course of these observations, which were begun by Dreser, then continued especially by Paul and Krönig, and at the same time also by Scheurlen and Spiro, it has developed that the changes which the dissolved substances undergo in the solution through the action of the solvent are of decisive importance with regard to the mode of action. According to the views of Arrhenius, now almost generally accepted as correct, a dissociation (division) of the dissolved salts, acids,

and bases, into electropositive and electronegative constituents—into their ions—takes place in watery solutions. While in general the salts in watery · solution are much more actively dissociated than the acids and the bases, the degree of dissociation of the various salts of a metal is dependent upon the nature of the acid-ion, and that of the same salt in turn upon the dilution.

As the chemic and physical properties of solutions are dependent upon the degree and the character of the ionization, it has developed that also the disinfectant activity of equimolecular solutions is influenced by the dissociation, so that, for instance, solutions of mercurial salts are the more active the more mercury they contain, not by weight-percentage, but in the form of ions. For this reason solutions of mercuric chlorid exceed in activity solutions of all other mercurial salts.

In the course of their investigations Paul and Krönig discovered another series of laws. Thus, the acids act in general accordance with their electrolytic dissociation (except hydrofluoric acid, nitric acid, trichloracetic acid), and likewise the bases in accordance with the concentration of the hydroxyl-ions contained in the solution. It has also been shown that the oxidizing agents—nitric acid, chromic acid, chloric acid, persulphuric acid, and permanganic acid—are active in accordance with the position that they occupy in the scale of oxidizing agents by reason of their electric activity. The halogens—chlorin, bromin, iodin—whose disinfecting activity on the whole, in correspondence with their chemic activity, diminishes with increase in atomic weight, play a specific rôle.

Whereas ionization has thus proved itself an index for both the chemic and the disinfectant activity of salts, acids, and bases—in general, the inorganic disinfectants—there exists, according to Scheurlen and Spiro, another large group of disinfectants, whose activity is not dependent upon the action of ions, but upon "molecular action," and in connection with which the entire undissociated molecule must be taken into consideration. The mode of action of this class of substances, the principal representative of which is carbolic acid, can be explained, as Spiro has recently shown, by analogy with the laws for the division of a body between two solvent agencies: that is, the condition present is not, as in the first class of disinfectants, one

of true chemic reaction, but one of solution manifestations, similar to those better-known processes, of which, for instance, we have long had knowledge in a whole series of stains.

The distinction between the two classes of disinfectants is of significance also for practical purposes. It thus becomes clear why, on addition of other substances—for instance, acids—the action of the one class (chemic disinfectants) is usually lessened, while that of the other—for instance phenol—is increased, whereas addition of alcohol has the reverse effect. An explanation is afforded also for the practically significant phenomenon that the disinfection of organic or albuminous nutrient media (such as feces, sputum, etc.) is effected more readily with the second class of agents than with the first.

In order to determine the activity of a disinfecting agent, according to Koch's procedure highly resistant bacteria—anthrax-spores that have been dried upon short pieces of sterile silk thread are most commonly employed as test-objects—are introduced into a solution of the agent and from time to time it is noted in specimens that are removed and transferred to nutrient medium whether the bacteria have already been destroyed or not. Geppert has called attention to a possible source of error attending this mode of procedure. With the silk thread not alone the spores are transferred to the new nutrient medium, but always, even when the thread is rinsed with water after removal, also a certain amount of the disinfecting agent. This diffuses from the silk into the nutrient medium, and renders it unsuitable for the culture of the bacteria. Growth, therefore, may readily fail to take place, although all of the bacteria may not be destroyed.

Koch has himself studied the inhibition of growth in culture-media to which antiseptics were added in varying amount, and in which then silk threads with anthrax-spores were introduced. He found

DISTINCT RETARDATION OF GROWTH		COMPLETE CESSATION OF GROWTH
	ON ADDITION OF	
Mercuric chlorid in the strength of 1 : 1,600,000		1 : 300,000
Thymol " 1 : 80,000		
Oil of turpentine " 1 : 75,000		
Potassium-soap " 1 : 5000		1 : 1000

DISTINCT RETARDATION OF GROWTH			COMPLETE CESSATION OF GROWTH
	ON ADDITION OF		
Iodin	in the strength of	1 : 5000	
Salicylic acid	"	1 : 3300	1 : 1500
Hydrochloric acid	"	1 : 2500	1 : 1700
Camphor	"	1 : 2500	More than 1 : 1250
Borax	"	1 : 2000	1 : 700
Potassium permanganate	"	1 : 1400	
Boric acid	"	1 : 1250	1 : 800
Carbolic acid	"	1 : 1250	1 : 850
Quinin	"	1 : 830	1 : 625
Potassium chlorate	"	1 : 250	
Alcohol	"	1 : 100	1 : 12.5
Sodium chlorid	"	1 : 64	

From the foregoing it will appear that even quite slight additions of the disinfectant may nullify the results of the experiment when the method of investigation first named—of introducing the silk threads into the nutrient medium—is pursued. When Geppert, by means of dilute solutions of ammonium sulphate, removed completely the disinfectant from the anthrax-spores that had lain in mercuric-chlorid solution, 1 : 1000, before their transference to the nutrient medium, he still observed growth after exposure to the action of the mercuric chlorid for fifteen minutes, and also after exposure for five hours, and in one instance even after exposure for twenty-four hours. The spores were still infective. Animals that Geppert inoculated therewith frequently died of anthrax. Solutions of mercuric chlorid of 1 : 100 did not with certainty destroy the spores in from six to twelve minutes. From this it can be seen that the disinfecting activity of the agents tested by the method of Koch has probably been often placed too high. The figures obtained by Koch and numerous subsequent investigators still retain their value for practical purposes, as Koch himself endeavored to reduce to a minimum the sources of error indicated by making the silk threads as short, and using as large an amount of culture-medium, as possible, and rinsing in distilled water, alcohol, etc., the threads removed from the antiseptic solution before transference to the nutrient solution.
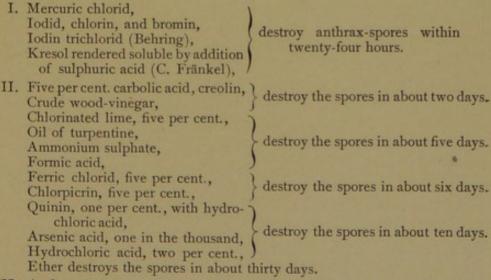
The following table from Flügge's " Text-book " (1897)

will give an idea of the utility of the better known antiseptics :

BACTERICIDAL AGENTS.	DESTROY			
	STREPTOCOCCI AND STAPHYLOCOCCI.	ANTHRAX-BACILLI, TYPHOID-BACILLI, CHOLERA-BACILLI.		ANTHRAX-SPORES.
	Within Five Minutes.	Within Five Minutes.	In from Two to Twenty-four Hours.	
Hydrogen dioxid .	Concentrated	1 : 200	1 : 500	1 : 100 after an hour.
Chlorin.	0.1 per cent.	0.1 per cent.	...	Fresh chlorin-water 0.2 per cent. in an hour.
Iodin trichlorid . .	1 : 200	1 : 1000	...	
Potassium iodid . .	...	...	1 : 10	
Sulphuric or hydrochloric acid . . .	1 : 10	1 : 100	1 : 1500 ; typhoid, 1:700	1 : 50 in ten days.
Sulphurous acid . .	...	...	1 : 300, gas 10 vol. per cent. (only superficially)	
Arsenic acid	...	...	...	1 : 1000 after ten days.
Boric acid	...	...	1 : 30	Concentrated after six days incompletely.
Potassium hydroxid .	1 : 5	...	1 : 300	
Ammonia.	...	...	1 : 300	
Soda	...	...	1 : 40	
Ammonium carbonate	...	...	1 : 100	
Calcium hydroxid .	...	...	1 : 1000	
Silver nitrate . . .	1 : 1000	...	1 : 4000	
Mercuric chlorid . .	1 : 10,000 −1 : 1000	1 : 2000	...	1 : 2000
Copper sulphate . .	...	...	...	1 : 20 (five days).
Potassium permanganate	1 : 200	...	...	1 : 20 (one day).
Potassium bichromate	...	...	...	1 : 1700
Chlorinated lime . .	...	1 : 500	...	1 : 20 (one hour).
Ferric chlorid . . .	...	...	...	1 : 20 (six days).
Alcohol.	80 per ct.	...	...	
Acetic acid, oxalic acid, etc.	...	...	1 : 200 or 300	
Chloroform	...	...	1 : 14	
Carbolic acid . . .	1 : 60	Cholera, 1 : 200; glanders, anthrax, 1 : 100; typhoid, 1 : 50	1 : 300	1 : 20 in from four to forty-five days.

BACTERICIDAL AGENTS.	DESTROY			
	STREPTO-COCCI AND STAPHYLO-COCCI.	ANTHRAX-BACILLI, TYPHOID-BACILLI, CHOLERA-BACILLI.		ANTHRAX-SPORES.
	Within Five Minutes.	Within Five Minutes.	In from Two to Twenty-four Hours.	
Salicylic acid . . .	I : 1000	...	...	
Creosote	...	I : 500	...	
Creosote sulphate .	I : 300	...	...	I : 20 in six hours.
Creolin	...	I : 100	I:3000; typhoid, I:250	
Aseptol	...	From 3 to 5 per cent.	...	10 per cent. in thirty minutes.
Quinin	...	...	...	I:100 after ten days.
Oil of turpentine . .	...	...	...	Concentrated in five days.

Upon the basis of a comparison of the results of Koch's observations with those of numerous subsequent investigators Schimmelbusch arranges the disinfectants in accordance with the time in which they destroy anthrax-spores, in the following order :

I. Mercuric chlorid,
 Iodid, chlorin, and bromin,
 Iodin trichlorid (Behring),
 Kresol rendered soluble by addition
  of sulphuric acid (C. Fränkel),
 } destroy anthrax-spores within twenty-four hours.

II. Five per cent. carbolic acid, creolin,
 Crude wood-vinegar,
 } destroy the spores in about two days.

 Chlorinated lime, five per cent.,
 Oil of turpentine,
 Ammonium sulphate,
 Formic acid,
 } destroy the spores in about five days.

 Ferric chlorid, five per cent.,
 Chlorpicrin, five per cent.,
 } destroy the spores in about six days.

 Quinin, one per cent., with hydro-
  chloric acid,
 Arsenic acid, one in the thousand,
 Hydrochloric acid, two per cent.,
 } destroy the spores in about ten days.

 Ether destroys the spores in about thirty days.

III. Anthrax-spores are still not destroyed after the lapse of a month in absolute alcohol, distilled water, chloroform, glycerin, benzoic acid, ammonia, concentrated solution of sodium chlorid, five per cent. potassium chlorate, alum, borax.

The figures obtained experimentally, however, constitute no trustworthy index of the practical utility of an agent. For practical purposes the greater or lesser solubility of a

disinfectant, its capacity for penetrating the objects to be disinfected—for this reason oily solutions of even active antiseptics are almost ineffective, because the oil does not penetrate into the organisms—its chemic constitution (upon which is dependent the extent to which the object to be disinfected suffers in the process of disinfection), and much besides must be taken into consideration. The cost of a disinfecting agent is also not without bearing upon its practical applicability upon a large scale.

It would carry us too far to discuss individually the innumerable antiseptics that have been tested in recent years, and that have been recommended for one purpose or another. We shall limit ourselves to the following observations :

DISINFECTION OF THE HANDS.

Disinfection of the hands is practised at surgical clinics in the following manner, in accordance with the regulations of Fürbringer :   The hands are washed as clean as possible in tepid water with soap and vigorous brushing for five minutes, then rinsed in fresh water ; the nails are cleaned, especially the spaces beneath the nails, by means of a metallic nail-cleaner ; the hands are then rubbed in alcohol for three minutes ; next they are rinsed and rubbed for a minute in from ½ to 1 : 1000 solution of mercuric chlorid.   In the event of marked contamination of the skin, it is first rubbed with ether before the disinfectants are used, or the entire procedure is performed twice.

Before every operative procedure this process of rigid disinfection must be carried out completely at the site of operation, as well as on the hands of the operator. A simpler mode of disinfection of the hands—washing with soap and brushing, rinsing with mercuric chlorid or alcohol—should be practised by physicians and attendants always after contact with a patient, and especially before each meal.   In houses in which an infectious disease exists, especially in times of epidemic, disinfection of the hands before eating is a general duty.   Instead of mercuric chlorid, the following may also be employed : Carbolic acid, from 3 to 5 per cent. ; creolin, 3 per cent. ; lysol, 1½ per cent.   The substances last named, however, have, as has been pointed out, less disinfecting power than mercuric chlorid.

DISINFECTION OF MUCOUS MEMBRANES.

The disinfection of mucous membranes is far more difficult than that of the external integument. Simple irrigation with a disinfectant will not yield the desired result—quite apart from the danger of intoxication. Vigorous brushing, however, and alcohol and ether are naturally not applicable here in the same degree as upon the external integument. The greatest stress is, therefore, to be placed upon mechanical removal of the germs, and this may be effected by brushing and rubbing with the fingers or with swabs of cotton or of gauze. Irrigation of the loosened mucus and debris may be practised with simple warm water, or with nonirritant solutions (weak solutions of boric acid, potassium permanganate, aluminum sulphate, physiologic salt-solution, infusion of chamomile, etc.).

DISINFECTION OF INSTRUMENTS AND DRESSINGS.

Metallic instruments are boiled for five minutes in water, preferably after addition of one per cent. of soda, whereby rusting is avoided. Cotton, gauze bandages, and the like are sterilized in the steam-chest or the dry chamber.

DISINFECTION OF FECES AND CESSPOOLS.

Lime is best adapted for disinfection of the stools. In the bed-pan in which the infectious stool (especially in cases of typhoid fever and of cholera) is received, so much milk of lime is previously introduced as will just cover the bottom of the vessel. After defecation an amount of milk of lime is added equal to the bulk of the feces, and the mixture is vigorously agitated and permitted to stand for an hour. The mixture must be highly alkaline (Pfuhl). The milk of lime should always be freshly prepared: To unslaked lime in stone jars or wooden pails so much water is added as will be taken up. The slaked lime is diluted with four times the amount of water.

If chlorinated lime be employed, it must be added in an amount equal to one per cent. of the mixture of urine and feces. It may be added in the form of powder or of a paste

consisting of about twenty grams of chlorinated lime and 100 of water. After thorough admixture the stool need stand for only fifteen minutes.

For the disinfection of cesspools Pfuhl likewise recommends milk of lime. When the tun-system is used, 3 grams = 6 cu. cm. of pulverulent slaked lime, or, better, 30 cu. cm. of milk of lime, and in the case of pits 2 grams = 4 cu. cm. of pulverulent lime, or, better, 20 cu. cm. of milk of lime should be allowed daily for each person. The seat as well as the funnel-shaped basin and the discharge-pipe must be thoroughly irrigated with the disinfecting solution. If, in addition to feces, which may be estimated at 400 cu. cm. for each person daily, also the total amount of urine is thrown into the receptacle, the mass of material to be disinfected may be estimated at between 1500 and 2000 cu. cm., and four or five times as much of the disinfectant should be employed for each person. Under these conditions also disinfection is attained only when the privy-contents exhibit a distinctly alkaline reaction.

Of other disinfectants for water-closets, carbolic acid, sulphocarbolic acid, lysol, and saprol, may be mentioned. These must be introduced in solution, and in an amount equal to two per cent. of the material to be disinfected; about eight grams daily may, therefore, be estimated for each person. All of these substances are more expensive than milk of lime, without possessing especial advantages. For hospitals it may be recommended that the entire fecal accumulation be boiled in a suitable vessel with addition of deodorizing substances (potassium permanganate).

DISINFECTION OF BATH-WATER, WASH-WATER, ETC.

Bath-water, when contaminated by patients, is disinfected by means of milk of lime (six liters to a bath of 300 liters) or of carbolic acid. Mercuric chlorid is to be avoided when metallic tubs are used.

Wash-water is freed from typhoid-bacilli and cholera-bacilli, according to Pfuhl, within an hour if 1.5 to one thousand of calcium hydroxid be added, and if constant agitation is maintained.

Koch permitted milk of lime to be introduced into the drainage-fluid at Nietleben until the fluid appearing in the

main discharge-conduit at the lower end of the irrigation-field presented a marked alkaline reaction.

For the disinfection of water-conduits dilute milk of lime, carbolic acid, or a mineral acid, is employed. At Nietleben Koch disinfected the service by means of carbolic acid, permitting three per cent. carbolic acid to be driven from the pumping well into all divisions of the service, and to remain in the conduits for twenty-four hours. Then the pipes were irrigated with wholesome water. This procedure is attended with the objection that the water retains for a considerable time the taste of carbolic acid. On the other hand, however, it does not carry with it the danger of obstruction of the conduits that attends the employment of milk of lime.

DISINFECTION OF URINE.

The urine is usually disinfected in common with the feces. It is generally not so infective as the stools. If it is to be disinfected alone, this may be accomplished by addition of milk of lime, carbolic acid, or mercuric chlorid.

DISINFECTION OF SPUTUM.

The sputum must be received and preserved in a moist condition, preferably in sputum-cups containing a layer of water. So long as the sputum is contained within such cups it is relatively harmless. A greater danger resides in the conversion into dust and into spray of sputum evacuated into handkerchiefs, upon floors, etc. On emptying the vessel the sputum must be disinfected. Crude carbolic acid (from 5 to 10 per cent.) or mercuric chlorid (1 or 2 : 1000) would be suited for this purpose if the disinfectant penetrated the sputum. Generally, however, the albumin on the outer surface of the mass of sputum is coagulated, and the bacilli contained within do not come in contact with the antiseptic at all. For this reason the sputum must at least be thoroughly rubbed up in the disinfecting solution, and remain therein for a long time. Lysol (10 per cent.) and crude solutol (from 5 to 10 per cent.) do not coagulate the sputum, and are therefore better adapted for disinfection. It is further useful to disinfect the sputa by heat. If

they are not too abundant and are at the same time viscid, they can be simply burned in a stove. Otherwise they are introduced, together with the sputum-cup, into a specially constructed disinfector, resembling the steam-chest (Kirchner), in which they are exposed for half an hour to the action of steam at a temperature of 100° C. (212° F.). The apparatus has the disadvantage that a number of the cups will be broken, and in families it can not often be provided. Under such circumstances the sputa can be poured into a pot and boiled for half an hour in water. Usually, however, one will have to be satisfied with simply emptying them into the water-closets, where the pathogenic germs are simultaneously destroyed, in part with the disinfection of the feces and in part by the process of putrefaction. The sputum-cups should be sterilized by means of hot water and carbolic acid, and preferably likewise boiled.

DISINFECTION OF BODY-CLOTHING AND BED-LINEN.

Uncontaminated linen is boiled for half an hour in petroleum soap-water (two bucketfuls of water—about thirty liters—to 250 grams of soft soap and two spoonfuls of petroleum; then, after removal of the soap-water, rinsed in cold water; next washed with soap in clean hot water; then again rinsed in cold water, permitted to remain over night in clean water, and finally dried in the open air. Instead of this procedure the linen may also be disinfected in the steam disinfecting apparatus.

Contaminated linen requires removal of feces, mucus, or pus before application of heat, as otherwise burned spots will appear. Such linen must immediately after removal be placed in a sheet moistened with mercuric chlorid, 1 : 2000, then in strong, moist sacks, and sent away for disinfection. The sacks, unopened, are introduced into the disinfecting fluid, three per cent. soft-soap solution, in which the linen is treated for three hours at a temperature of 50° C. (122° F.), and then remains for an additional forty-eight hours during the process of cooling; or mercuric-chlorid salt solution (of 0.5 to one thousand mercuric chlorid and 6 to one thousand sodium chlorid). After disinfection in this way the linen is further treated in the same manner as uncontaminated linen.

DISINFECTION OF BEDS AND CLOTHING.

Articles of clothing and beds are best disinfected in suitable apparatus in live steam. If such an apparatus is not available, exposure to air and sun must be resorted to or rubbing with three per cent. carbolic acid. To purify articles by exposure to air, they must be hung for days in a dry room and exposed as uniformly as possible upon all sides to the rays of the sun. Even then the disinfection is not trustworthy. The bedstead, articles of leather, and the like, are rubbed off with five per cent. carbolic acid. For the disinfection of clothing formalin (a forty per cent. solution of formaldehyd) has been warmly recommended. The clothing should be loosely packed in a chest, and between its layers are placed strips of goods saturated with formalin. From thirty to fifty grams of formalin are required for a suit of clothing. Disinfection is said to be completed within two hours, and the disagreeable odor may be removed by means of ammonia.

DISINFECTION OF ARTICLES OF FOOD.

The keeping clean of articles of food is especially a prophylactic measure. These should not be permitted to stand about in the sick-room, but they should be kept covered, etc. Portions of food left unused by the patient are burned, as well as articles of food that are known to have been infected in other ways. Milk and other liquids are drunk only boiled, as should also be drinking-water in times of cholera-epidemic.

DISINFECTION OF THE SICK-ROOM.

A sick-room should not contain pictures, curtains, etc., in fact any superfluous articles that only serve as dust-collectors. After the termination of the illness the room of the patient, and possibly the entire dwelling, should remain undisturbed for about ten hours, to permit the dust to settle, and then it should be thoroughly disinfected. In some large cities this is undertaken by special establishments. Such disinfecting stations should be established everywhere, for

only with their aid and through their trained attendants can thorough disinfection of dwellings be carried out. All portable articles that will withstand the action of moist steam are wrapped in cloths moistened with three per cent. carbolic acid or mercuric chlorid, 1 : 1000, and sent to the disinfecting station, where they are placed in a steam-apparatus. The walls and ceilings are thoroughly rubbed down with bread-crumbs, and these are eventually burned in the stove. Walls painted with oil-colors are washed with five per cent. carbolic acid or painted with milk of lime. White-washed walls are given a new coat. The furniture is rubbed off with three per cent. carbolic acid and then rubbed dry. Polished articles can be rubbed off with bread. Upholstered furniture, when possible, is sterilized in the steam-apparatus ; otherwise rubbed down with carbolic acid and brushed. Articles of leather, metal, glass, and the like can be vigorously rubbed with carbolic acid. Mantels, the upper surfaces of stoves, etc., are first freed of dust by means of moist cloths, then soaped and rubbed with three per cent. carbolic acid. Finally, the floor is disinfected by scouring with warm water and soap and then with carbolic acid. Of late formaldehyd, which has already been mentioned as a disinfectant for clothing, has also been employed for the disinfection of dwellings. The following mode of procedure is pursued : A forty per cent. aqueous solution of formaldehyd (also known as formalin or formol) is mixed with chlorinated lime and water—to each liter of formalin 200 grams of chlorinated lime in 400 cu. cm. of water ; the mixture is designated formochloral—and the mixture is evaporated in a Trillat autoclave, with four atmospheres of pressure. One liter of formochloral is sufficient for 200 cubic meters of air-space. The disinfection is, however, essentially only superficial, the bacteria in thick articles, such as beds, mattresses, clothing, etc., not being destroyed thereby. After disinfection has been completed the vapor of formaldehyd is removed by means of a spray of ammonia and exposure to air. Greater advantages than the Trillat apparatus, whose manipulation is always difficult, are possessed by the formalin-lamp placed upon the market by Schering,* with the aid of which the disinfecting vapor is generated in a most simple manner by the burning of

* Numerous forms of serviceable apparatus can now be obtained.

formalin-pastils. Two pastils, each of one gram, are employed for every cubic meter of space. According to our experience, trustworthy superficial disinfection can be effected by this means for all bacteria without spores.

DISINFECTION OF SHIPS, VEHICLES, RAILWAY-CARS, ETC.

Entire ships, cars, etc., are disinfected in a manner similar to that employed in disinfection of the sick-room. The burning of sulphur, which was formerly much practised (20 grams of sulphur to each cubic meter of space, moistened with alcohol before ignition), has been shown by Koch's investigations to be relatively useless, and injures many articles rather seriously.

INDEX.

G.

H.