EXPLANATION OF PLATE XI.

FIG. 1.—Bacillus Coli Communis. Agar culture. Stained with fuchsin. × 1,000. Photomicrograph from Bowhill's "Bacteriology" by permission.

FIG. 2.—Streptococcus Pyogenes (Longus). × 1,000. Photomicrograph from Sternberg's "Bacteriology" by permission.

FIG. 3.—Staphylococcus Pyogenes Aureus. × 1,000. Photomicrograph from Park's "Bacteriology" by permission.

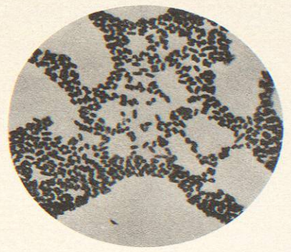
FIG. 4.—Micrococcus Tetragenus (Tetracoccus). × 1,000. Photomicrograph from Park's "Bacteriology" by permission.

FIG. 5.—Diplococcus Pneumoniæ (Fraenkel) in Sputum. × 1,000. Stained by Gram's method. Photomicrograph from Sternberg's "Bacteriology" by permission.

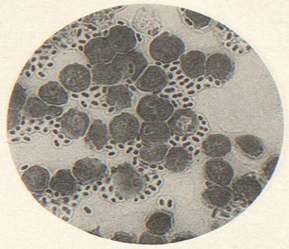
FIG. 6.—Diplococcus Pneumoniæ (Fraenkel) in Blood. × 1,000. Photomicrograph from Sternberg's "Bacteriology", by permission.

FIG. 7.—Micrococcus Gonorrhœæ (Gonococcus Neisser) in Urethral Pus. Stained with Loeffler's solution of methylene blue. × 1,000. Photomicrograph from Sternberg's "Bacteriology" by permission.

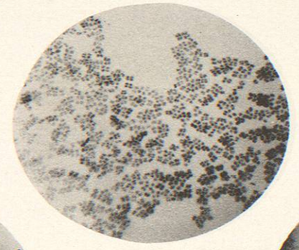
FIG. 8.—Diplococcus Intracellularis Meningitidis (Meningococcus). × 1,000. Photomicrograph from Park's "Bacteriology" by permission.



I.
Bacillus Coli Communis.



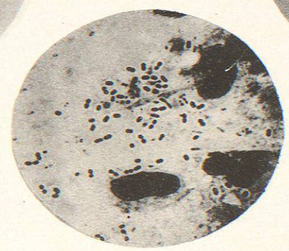
VI.
Diplococcus pneumoniæ
in blood.



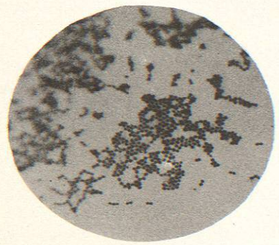
IV.
Tetracoccus.

II.
Streptolococcus pyogenes.

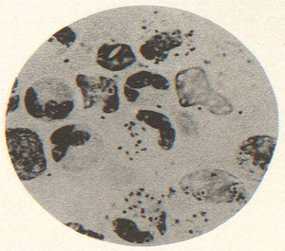
VII.
Gonococcus (Neisser).



V.
Diplococcus pneumoniæ in
sputum.



III.
Staphylococcus pyogenes
aureus



VIII.
Diplococcus Intracellular
meningitidis.

Pathogenic Bacteria.

are so great that these experiments are certainly worth continuing.

THE MENINGOCOCCUS (*Diplococcus Intracellularis Meningitidis*).—This organism was isolated by Weichselbaum (1887) from the exudate of cerebro-spinal meningitis, both when complicating pneumonia and in uncomplicated cases, and from its usual presence in the interior of pus cells he called it *diplococcus intracellularis*. It has since been found (1895) by Jäger and Scheurer in the nasal secretions and sputum of persons suffering from this affection during an epidemic. The frequency of its occurrence in and restriction to this disease afford sufficient evidence of its being concerned at times, at least, in the production of cerebro-spinal meningitis, though the pneumococcus is probably the most common cause.

Motility.—Non-motile.

Staining Reactions.—Stains with the ordinary aniline colors, but best with Loeffler's alkaline methylene blue.

Microscopical Appearances.—Occurs as coffee-bean-shaped micrococci usually united in pairs (diplococci), but also in groups of four, and in small masses; sometimes solitary and smaller apparently degenerated forms are found. In the exudate it is generally found, like the gonococcus, to which it bears a close resemblance morphologically, in the interior of the pus cells and extra-nuclear. According to some authors it is sometimes indistinguishable in form from the pneumococcus, streptococcus pyogenes, and tetracoccus. (See Plate XI., Fig. 8.)

Biological Characters.—The meningococcus does not grow at room temperature but only in the incubator at 36°–37° C. Its development is usually scanty on the surface of agar, though sometimes a few colonies grow luxuriantly. It does not grow at all or very poorly in bouillon or bouillon mixed with one-third blood serum. It develops best on Loeffler's blood-serum mixture as used for diphtheria cultures.

When grown on *nutrient* or *glycerin agar*, at the end of forty-eight hours in the incubator a tolerably good growth develops, appearing as flat, grayish colonies, viscid and usually non-confluent. On *Loeffler's blood serum* the growth forms round, whitish, shining, viscid-looking colonies, with smooth, sharply defined outlines. The colonies tend to become confluent, but do not liquefy the serum.

Cultivated in artificial media the meningococcus soon loses its vitality (in six days), and must therefore be transplanted every two or three days to fresh media.

Pathogenesis.—Not very pathogenic for animals; most so for mice and guinea-pigs, less so for rabbits and dogs. Subcutaneous injections of animals give negative results; intrapleural or intraperitoneal inoculations in mice and guinea-pigs, in large doses, are generally successful. The animals usually fall sick and die within thirty-six to forty-eight hours, showing slight fibro-purulent exudation. In the blood and enlarged spleen diplococci are found in small numbers and mostly free; in the pleuritic exudate they are present in considerable quantities and then are found in the interior of the pus cells. Meningitis, corresponding to the disease as occurring in man, has been artificially produced in dogs by subdural inoculations of recent cultures.

Under natural conditions in the human subject the meningococci probably gain access to the brain and meninges by way of the nose, ear, and upper air passages. They have been found not only in meningeal pus but also in the nasal mucous secretions, the sputum and the urine of patients suffering from meningitis, and occasionally in the nares of healthy persons coming in contact with patients. A mixed infection of the meningococcus, pneumococcus, and streptococcus pyogenes is often met with.

A bacteriological diagnosis of cerebro-spinal meningitis may often be made by means of lumbar puncture to obtain a specimen of the fluid from the spinal canal, and microscopical examination and cultivation on Loeffler's blood serum. The clinical value of this is, that about forty per cent. of the cases due to the meningococcus recover, while almost all of those caused by the pneumococcus and streptococcus die.

THE GONOCOCCUS (*Micrococcus Gonorrhœæ*).—First observed by Neisser (1879) in gonorrhœal pus and described by him under the name of "gonococcus." It was obtained in pure culture by Bumm (1885), and its infective nature proved by inoculations into men.

Microscopical Appearances.—Micrococci usually united in pairs (diplococci) or groups of four. The bodies of the diplococci are shaped like coffee beans or a Vienna roll (Semel), having an unstained division or interspace, in stained preparations, between two flat surfaces facing one another. They are from 0.8 to 1.6 μ long and 0.6 to 0.8 μ broad. In gonorrhœal discharges the diplococci are found mostly in small, irregular groups in or upon the pus cells and extra-nuclear. Occasionally round, single, and undivided cells are observed, and again irregular forms, particularly in old cultures, and in chronic gonorrhœa of long standing. (See Plate XI., Fig. 7.)

Motility.—Non-motile.

Staining Reactions.—Stains readily with the basic aniline dyes, especially with methyl violet, gentian violet, and fuchsin; not so quickly with methylene blue, which, however, is the best staining agent for demonstrating its presence in pus. The gonococcus is decolorized by Gram's solution, which enables it to be distinguished from other pus cocci; but this method cannot always be depended on to differentiate it from all diplococci found in the urethra and vulvo-vaginal tract, some of which are morphologically similar to the gonococcus and are also decolorized by Gram's solution.

Biological Characters.—Aërobic and facultative anaërobic. Does not grow at room temperature, best at 37° C. Growth on ordinary culture media is so scanty that special media have been devised for its cultivation.

Human Placenta Serum Agar.—Wertheim has succeeded in developing luxuriant and virulent cultures to many generations on a mixture consisting of placenta blood serum and two per cent. peptone agar. His method is as follows: Several loops of gonorrhœal pus are diffused through liquid placental blood serum warmed to 40° C. in a test tube. Two dilutions are made from this, and an equal quantity of melted two-per-cent. peptone agar cooled to 40° C. is added to the three tubes, and the contents poured into Petri dishes. At the end of twenty-four hours in the incubator there will have developed on at least one of the plates distinct colonies, which are in appearance translucent and finely granular with scalloped margins. By transferring such a colony to slant cultures of serum agar, pure cultures of the gonococcus are obtained; these are somewhat shiny in appearance and of a grayish-white color.

Human Chest Serum Agar.—Heiman, and almost simultaneously, Kiefer and Menge, proposed a culture medium made from hydrothórax, ascitic or hydrocele fluid, obtained from the human subject. This medium as prepared by Heiman consists of a 2 per cent. agar + 2 per cent. peptone + 0.5 per cent. salt + 2 per cent. glucose; of this mixture two parts are added to one part of "chest serum," obtained from a patient suffering from hydrothorax, acute pleurisy, or hydrocele, which, if necessary, is sterilized. The chest serum agar should have a neutral reaction. The growth in this medium is thus described: "In plate cultures streaked on the surface, growth abundant, colonies circular in shape, edges somewhat irregular, shading off into yellowish white; texture finely granular in periphery, presenting punctuated spots of higher refraction in and around the centre of yellowish color."

Pig Serum Nutrose Agar.—Wassermann recommends a culture medium for the gonococcus consisting of 15 c.c. pig serum diluted with 30–35 c.c. water, to which is added 2–3 c.c. glycerin, and finally about 2 per cent. nutrose (casein sodium phosphate). This is thoroughly mixed and boiled and sterilized by the fractional method. To the mixture is now added an equal quantity of 2 per cent. agar cooled to 40° C., for the inoculation of cultures, and then poured into Petri dishes. The growth is favored

by admission of air, and is similar in appearance to that already described for plate and streak cultures.

Toxins.—Wassermann has obtained on his serum nutrose agar virulent cultures of the gonococcus, which after being killed still possessed toxic action. The gonotoxin produced was found to be very resistant to heat and the action of alcohol; it killed mice, and in rabbits gave rise to caseous infiltration often passing into necrosis, and in large doses produced general toxæmia. Injected into the human subject the gonotoxin seemed to produce no curative effect on an existing chronic gonorrhœa, the intense reaction caused not becoming less on repeated inoculations.

The production of gonotoxin would seem to account for the gonorrhœal secretion. It also renders more comprehensible several obscure points in the history of chronic gonorrhœa: for example, the fact that gonococci may be apparently absent from, or only isolated organisms present in, the gonorrhœal discharge, and yet a purulent secretion be kept up containing few bacteria; but if, owing to some injury to the tissues, the organisms increase in number an acute exacerbation of the disease is again set up and masses of gonococci are then found in the pus.

Vitality.—The gonococcus has but little resistant power against outside influences. It is killed by weak disinfecting solutions and by desiccation in thin layers. In comparatively thick layers, however, as when gonorrhœal pus is smeared on linen, it has lived for forty-nine days, and dried on glass for twenty-nine days (Heiman). No development takes place below 25° C. or above 39° C.; it is killed by a temperature over 42° C.

Pathogenesis.—Gonorrhœa as occurring in man is non-transmissible to dogs, monkeys, horses, and rabbits, whether inoculations be made into the urethral, vaginal, or other mucous membranes. Large doses of virulent cultures produce in animals toxic inflammations, similar to that produced by the gonotoxin, without any multiplication of cocci. Although animal inoculations have been thus followed by negative results, the etiological relation of the gonococcus to human gonorrhœa has been demonstrated beyond question by the infection of healthy men with the disease by inoculation of pure cultures by Bumm, Wertheim, Kiefer, and Heiman.

The gonococcus has never been found outside the body, except in articles of clothing, etc., which have become contaminated by those affected with the disease; nor has it ever been met with in healthy persons. In those suffering from gonorrhœa it has been found in the urethra and prostate of the male and in the urethra, vagina, and cervix uteri of the female, as the cause of the disease. Besides gonorrhœal urethritis and vaginitis, the gonococcus is the cause of certain cases of endometritis, metritis, salpingitis, oöphoritis, peritonitis, proctitis, cystitis, and probably also of epididymitis; also of gonorrhœal ophthalmia neonatorum, and rarely of diphtheritic conjunctivitis in children (Fraenkel). The gonococcus produces in adults severe conjunctivitis, seldom rhinitis and otitis. It is frequently the cause of gonorrhœal arthritis, also probably in some cases of pleuritis, malignant endocarditis, parotitis, periostitis, and bursitis.

In the local affection squamous epithelium protects better than cylindrical epithelium. The parasite penetrates gradually through the epithelium into the connective tissue. In travelling to distant organs of the body the gonococcus follows mainly the course of the lymphatics and produces inflammation which finally leads to fibrinous hypertrophy—stricture of the urethra, hypertrophy of the prostate, etc. There is no immunity produced after recovery from an infection.

In view of the fact that several non-specific forms of urethritis exist, and also that diplococci morphologically similar to the gonococcus Neisser are often found in the normal urethra and vulvo-vaginal tract, it becomes a matter of great importance to be able to detect gonococci when present and to differentiate these from the non-specific organisms. For the demonstration of gonococci, they must be found as diplococci lying in masses in the pus cells and extra-nuclear, when stained with methylene blue and decolorized by Gram's solution. Organisms having these characteristics microscopically may for all practical purposes be considered as certainly gonococci, if they are obtained from the urethral discharge and confirmed by examination on three successive days. But if there still remain any doubt, and especially if the organisms are obtained from the vulvo-vaginal tract, plate cultures should be made on one of the special media described (chest serum agar, etc.), on at least three consecutive days.

THE BACILLUS PYOCYANEUS (*Bacillus of Green and Blue Pus*).—This bacillus is found in green or blue colored pus which occasionally accompanies the discharges from open wounds, and is the cause of the pigmentation produced.

Microscopical Appearances.—Delicate, slender rods, about 0.4 μ broad and 1.5 to 6 μ long, often united in pairs or in chains of four to six elements, and occasionally growing into long threads.

Motility.—Actively motile, possessing only one flagellum.

Spore Formation.—Absent.

Staining Reactions.—Stains readily with the ordinary aniline colors; does not stain with Gram's method.

Biological Characters.—Aërobic and facultative anaërobic, but produces pigment only in the presence of oxygen. Grows readily on all artificial culture media at room temperature, but best at 37° C. On *gelatin plates* flat, irregular colonies with radiating borders are rapidly developed, imparting to the medium a fluorescent green color; liquefaction begins at the end of two or three days, and in five days the gelatin is completely liquefied. *In gelatin stab cultures* liquefaction takes place rapidly at first near the surface and gradually extends downward; a greenish color is produced in that portion in contact with the air. On *agar plates* a wrinkled, moist, whitish layer is developed, the surrounding medium being at first bright green, later darker in color, and finally blue green or almost black. In *bouillon* a green fluorescence is produced, the medium being clouded, and a flocculent sediment forms. *Milk* is coagulated and peptonized. On *potatoes* a greenish-yellow or brownish growth occurs, the surrounding surface being green.

The bacillus pyocyaneus produces two pigments—one of a fluorescent green (*bacterio-fluorescin*, soluble in water) and the other of a blue color (*pyocyanin*, soluble in chloroform) formed only in the presence of oxygen. A faint aromatic odor is produced in recent cultures; in old cultures a disagreeable ammoniacal odor. No indol or H_2S is formed by this bacillus, and very little acid from grape sugar; no gas. Nitrates and nitrites are converted into free nitrogen. The bacillus pyocyaneus produces poisons by its growth. It has but little resistance to outside influences. Drying kills it rapidly; exposure to the action of direct sunlight for four hours partly destroys its power of producing pigment.

Pathogenesis.—Pathogenic for rabbits and guinea-pigs. Subcutaneous or intraperitoneal injections of 1 c.c. of a bouillon culture cause the death of these animals in from twenty-four to thirty-six hours, with the production of extensive inflammatory œdema and purulent infiltration of the tissues. The bacilli multiply in the body, and may be found in the serous or purulent fluid as well as in the blood and organs. Smaller amounts do not kill the animals, but render them immune to doses fatal to those not thus immunized. In rabbits inoculated with a culture of the bacillus anthracis a fatal result may be prevented by soon after inoculating the animal with a pure culture of the bacillus pyocyaneus. It has been suggested that the protective action is due to the chemical products of the growth of the bacillus, and not to an antagonistic effect of the living bacteria.

Though widely distributed in nature, the bacillus pyocyaneus has not so far been found outside the living body. It has been observed occasionally in the mouth and intestines of healthy individuals, on the unbroken skin and in the purulent discharges of open

wounds, also in bandages and dressings, at times epidemically in hospitals. Usually the organism appears only in association with the common pus cocci, coloring the pus blue or green. In some cases, however, it has been found alone in disease processes, as in otitis media, ophthalmia, broncho-pneumonia, pericarditis, etc., especially in children, so that we have reason to believe that this bacillus, although ordinarily non-pathogenic for man, may under certain conditions become a source of infection. In general its presence in wounds delays the process of repair and may give rise to a depression of the vital powers from the absorption of its toxic products.

THE BACILLUS PROTEUS VULGARIS.—This is the most important of a group of similar bacteria, known as the "Proteus group," which are among the commonest and most widely distributed putrefactive organisms. They were formerly included by the earlier observers under the name of "bacterium termo," which they applied to all minute motile organisms found in putrefying substances.

Microscopical Appearances.—Small, slender rods varying greatly in size, but on the average about 0.6 μ broad and 1.2 μ long, generally occurring in pairs but sometimes arranged in filaments, which may be more or less twisted. It is to its great variability in form that it was given the name of *proteus*.

Motility.—Actively motile.

Spore Formation.—Absent.

Staining Reactions.—Stains readily with aniline dyes, especially fuchsin or gentian violet; also stains with Gram's solution.

Biological Characters.—Aërobic and facultative anaërobic. Grows on almost all culture media, developing most rapidly at room temperature, but also in the ice box and in the incubator. Toxin production seems to be favored by admission of air.

The growth on *gelatin plates* containing five per cent. of gelatin is very characteristic. At the end of ten to twelve hours at room temperature, small, round, yellowish colonies with thick centres and irregular edges develop, from which brush-like offshoots are thrown out. Other colonies are surrounded by a zone of threads which, partly in circular, partly in irregular twisted figures, surround the central opaque mass. Straight and twisted offshoots, which frequently become detached from the parent colony, grow into the surrounding medium and continue moving about in the liquefied gelatin, sometimes called "swarming islands." When the consistency of the medium is more solid, as in ten-per-cent. gelatin, the liquefaction and migration of these surface colonies are more or less retarded. In *gelatin stab cultures* the growth is less characteristic—liquefaction takes place rapidly along the line of puncture, and soon the entire medium is liquefied. Upon *nutrient agar* a rapidly spreading, thin, moist, grayish-white coating appears, and migration of the colonies also occurs. *Milk* is coagulated with the production of acid. On *potato* a dirty grayish coating develops. *Bouillon* is uniformly clouded.

Culture media containing albumin or gelatin are decomposed by the proteus vulgaris with the production of a disagreeable putrefactive odor and alkaline reaction. It produces gas and acid from carbohydrates, thus giving off no odor. It also produces indol and H_2S. Urea is decomposed into carbonate of ammonia. It forms toxins, which may be obtained by filtration of the cultures through porcelain. The proteus vulgaris possesses considerable resistance toward chemical and thermic influences, but is killed at 60° C. in half a minute.

Pathogenesis.—This bacillus is pathogenic for rabbits and guinea-pigs when injected intravenously, intraperitoneally, or subcutaneously in large quantities, death of the animal being produced with symptoms of intoxication. The effects are much more readily produced when other organisms, as the streptococcus, are introduced simultaneously into the body. Less virulent species of pathogenic bacteria (staphylococcus, streptococcus) also gain in virulence when they are injected along with living or dead proteus cultures.

The proteus vulgaris is found very commonly outside the body in putrid meat and other decaying substances, such as foul water, etc. It is found also in the digestive tract of healthy persons. In disease, it is the organism chiefly concerned in the production of cystitis with ammoniacal urine, either alone or in conjunction with the bacillus coli communis, and is also an etiological factor in many other genito-urinary affections. The *urobacillus liquefaciens septicus* of some authors is probably identical with the proteus vulgaris. Although this bacillus, however, occurs quite frequently, along with other bacteria in various diseases, it has seldom been positively shown to be the specific cause of infection. Booker, who has made extended investigations into the etiology of cholera infantum, concludes that the proteus vulgaris plays an important part in the production of this affection. He found the bacillus present in eighteen cases of cholera infantum examined by him, but not in the fæces of healthy infants. Levy believes that in so-called "meat or sausage poisoning" bacteria of this group are chiefly concerned, and that the pathogenic effects are due to toxic products evolved during their development; though others attribute this affection to an anaërobic organism, the *bacillus botulinus* of Van Ermengen, the symptoms being described as *botulism*. According to Jäger, certain forms of icterus accompanied with fever, pain in the muscles, and enlarged liver and spleen, known as "Weil's disease," are produced by the proteus. Thus it would seem that, though ordinarily a harmless parasite, the proteus vulgaris may at times become pathogenic to man. Considering the very wide distribution of this organism in nature, the wonder is that with its poisonous properties so few diseases apparently are produced by it.

THE BACILLUS OF MALIGNANT ŒDEMA (*Bacillus Œdematis Maligni; Vibrion Septique*).—This bacillus is widely distributed, being found in the superficial layers of the soil, especially in garden earth, manure, filth of all kinds, and house drains; also in the blood and intestines of animals. It was discovered by Pasteur (1877), and later carefully studied by Liborius and Koch.

Microscopical Appearances.—Rather large rods, similar morphologically to tetanus and symptomatic anthrax bacilli, but showing a greater tendency to grow out into long filaments; in size from 0.8 to 1 μ broad and 2 to 10 μ long.

Motility.—Motile, but not very actively so except the short forms, having three to twelve flagella attached to the ends and sides of the rods.

Spore Formation.—Forms spores generally in the middle of the rods and oval in shape.

Staining Reactions.—Stains readily with the ordinary aniline dyes, especially when obtained from the animal body; decolorized by Gram's method.

Biological Characters.—Strictly anaërobic, growing in all the usual culture media in the absence of oxygen. Development takes place at room temperature, but more rapidly and abundantly at 37° C.

This bacillus grows on *nutrient gelatin*, but more abundantly on glucose gelatin containing one to two per cent. of glucose. Gas is formed and the gelatin is liquefied.

On *agar plates* the colonies appear as dull, whitish points, irregular in outline, and when examined under a low power they are seen to be composed of a thick network of threads radiating irregularly from the centre to the periphery. *Blood serum* is rapidly liquefied, with the production of gas. *Bouillon* is clouded from the formation of gas. *Milk* is not coagulated. Cultures of the bacillus of malignant œdema give off a peculiar odor.

Pathogenesis.—Especially pathogenic for mice, guinea-pigs, and rabbits, although horses, cats, dogs, goats, sheep, calves, pigs, chickens, and pigeons are also susceptible, and occasionally man. Cattle are immune. A small quantity of a pure culture subcutaneously injected into a susceptible animal gives rise to general hemorrhagic œdema which extends over the entire surface of the abdomen and thorax and results in the death of the animal. There is no odor developed, and little, if any, gas. In infection with garden earth, owing to the presence of associated bacteria, gas is produced having a