Lecture.

THE TUBERCLE BACILLUS: ITS MORPHOLOGY, MODE OF DETECTION; ITS LIFE HISTORY. ITS RESULTS IN THE HUMAN ORGANISM. THE CULTURE OF THE TUBERCLE BACILLUS.¹

BY PROFESSOR GERMAIN SÉE,

Physician to the Hôtel Dieu, Paris, France.

AMONG the microphytes the one which interests us the most, because, although hardly discovered more than two years, it is now the best shown, the most clearly characterized, is the tubercle bacillus.

HISTORY.

Several years before the discovery of the bacillus, Klebs had already announced that he had succeeded in isolating the bacterium of tuberculosis, in cultivating this microbe in an albuminous liquid, and in producing, both by inoculation and intra-peritoneal injection, the same lesions as the primordial tubercle containing the same bacteria.

The researches of Klebs were verified in 1879; the same inoculations were performed by Schuller and Reinstalder in Germany, by Toussaint in France, and in 1881 by Deutschmann, who, however, did not succeed in reproducing phthisis, except by taking the precaution to add to the culture-liquid solid particles of tubercle; in reality all that these experiments really accomplished was the inoculation of common bacteria and the production of ordinary non-specific lesions having the appearance of tubercle. The bacteria with micrococci, indicated by Aufrecht, were not attended with any better success.

Zoöglœa.—At the same epoch Rindfleisch and the same Aufrecht believed that they had found, in the centre of tubercle, in the well-known segments known as giant cells, the microphytic masses which we have indicated under the name of *zoöglœa*. Renaut, of Lyons, attributed to them a certain importance in the composition of tubercle, and more recently Malassez and Vignal have cultivated and inoculated these *zoöglœa*. It was found that in the third generation of microphytes resulting from this injection of *zoöglœa*-liquids the living products of culture returned to the bacillus-state; they would then seem to be only a transitory form of the bacillus.

Bacilli.—Bacilli were indicated as causal by Baumgarten; he, however, did not know how to render them evident. It was Koch who first, by a special process of staining, succeeded in demonstrating these microphytes. In the month of April, 1882, he communicated to the Medical Society of Berlin the results of these researches, and proved in an unmistakable manner that in the tuberculous organs of man there exist certain rod-shaped corpuscles which, by their chemical and morphological properties, differ totally from all other known forms of microphytes; these are the bacilli of tuberculosis.

DESCRIPTIVE CHARACTERISTICS.

These bacilli are very thin rods, whose length scarcely equals the quarter or the half of that of a

red blood corpuscle; they resemble, principally by their form, the bacilli of lepra, which are rather finer and more pointed.

Among the bacilli spores are sometimes found; that is to say, fine, round, and refractive corpuscles. Along with them, moreover, you will sometimes see the gelatiniform masses known under the name of *zoöglœa*.²

TECHNICS.

1. *Koch's Method*.—The bacillus cannot be detected except by the methods of staining invented by Weigert and perfected by Koch, who has demonstrated that the bacillus, once colored by methylene blue, resists coloration by vesuvine, while other microphytes contained in the preparation lose their blue color to take on the brown tint of the second coloring agent.³

2. *Ehrlich's Method*.—Ehrlich proceeds quite differently. He had remarked that Koch's bacillus when impregnated with an aniline color resists the action of dilute nitric acid, while all the other elements, microbes, and cells are speedily decolorized. Ehrlich employs Weigert's solution, of which the composition is as follows: "Take of saturated aqueous solution of aniline oil 100 cc.; saturated alcoholic solution of fuchsine 11 cc. Mix for the coloring solution." "The tissue or sputum to be examined is spread in a very thin layer over a thin glass cover, and is then dried in the air. It is then passed a few times through the flame of a spirit-lamp to coagulate the albumen. The glass cover, thus prepared, is placed for twenty-four hours in a watch-glass containing the staining liquid. It is then washed with distilled water, then with a thirty per cent. solution of nitric acid till all the color comes away. Then it is again washed in distilled water, then dipped in an aqueous solution of methyl blue. The preparation is then dried and mounted in Canada balsam. It shows the bacilli of a brilliant red color, the cells with their nuclei, and the other microbes being stained blue."—(Talamon.)

This procedure, modified by Rindfleisch, who slightly heats the staining liquid to hasten the coloring of the bacilli, has received the approbation of Koch himself.⁴

Numerous variations have been introduced into the technics of the preparation, which concern especially one of the elements of the staining liquid. Thus, sulphates and chlorides of rosaniline have been proposed, and divers methyl violets, alone or

² Rindfleisch, Malassez, Renaut.

³ Koch's method is as follows: Dry a small piece of sputum on a glass slide, place it in a weak alkaline methylene-blue solution for twenty-four hours, and then wash the preparation with vesuvine.

—TRANSLATOR.
⁴ Gibbs, in the London Lancet, May 5, 1883, page 771, gives the following ready process, the great advantage of which consists in doing away with the use of nitric acid. The stain is made as follows: Take of rosanilin hydrochloride two grammes, methyl blue one gramme; rub them up in a glass mortar. Then dissolve anilin oil 3 cc. in rectified spirit 15 cc.; add the spirit slowly to the stains till all is dissolved, then add distilled water 15 cc.; keep in a stoppered bottle. To use the stain: The sputum having been dried on the cover-glass in the usual manner, a few drops of the stain are poured into a test-tube and warmed; as soon as steam rises pour into a watch-glass and place the cover-glass in the staining fluid. Allow it to remain four or five minutes, then wash in methylated spirit till no more color comes away; drain thoroughly and dry, either in the air or over a spirit-lamp. Mount in Canada balsam. The whole process after the sputum is dried need not take more than six or seven minutes. This process is also valuable for sections of tissue containing bacilli, as they can be doubly stained without the least trouble. In this way beautiful specimens have been made without the shrinkage which occurs in the nitric-acid process. (Braithwaite's Retrospect, Part 88, page 221.)—TRANSLATOR.

¹ Translated by E. P. Hurd, M.D.

mixed, always diluted in an alcoholic solution of aniline. Frankel has just added certain improvements; he stains a second time to render the coloring stronger and more durable.⁵ I have always adhered to the method of Ehrlich, which is the most employed and the most sure.

BIOLOGY OF THE BACILLUS. ITS PARASITE LIFE.

Under the name of tuberculous organisms I shall include only the specific bacilli; no other parasites produce the characteristic lesions.

Now the bacillus is found not only in true phthisis, but also in individuals called scrofulous, affected with divers bony, glandular, cutaneous diseases. It is also met with in a great number of tumors heretofore undetermined, which occupy the peripheral organs or even the viscera, in individuals to all appearance perfectly well and exempt from every scrofulous or tuberculous taint. It remains to mention the bacilli in connection with the disease which rages among cattle under the name of bovine phthisis.⁶ I shall return to this subject when I come to speak of the flesh and milk of these animals.

I. Tuberculosis. Divers Lesions.—The bacilli are found in the divers forms of the tuberculous process, whether this be localized in the lungs, or whether it be at the same time disseminated throughout other organs. When the principal if not only seat is the lungs, the bacillus characterizes the first as well as the more advanced periods of the disease. It is also found in the lesions called granulations, miliary tubercles, as well as in the destructive caseous and ulcerous stage. In all cases it has its seat of predilection, though not its exclusive seat, in the tubercular elements called epithelioid cells and giant cells, where, too, the necropsy reveals it.

Products of Secretion, Sputa.—During the life of the patient the microphyte is recognized with the same facility and with the same certainty in the products of expectoration as in the dead elements. The presence of the bacilli in the sputa constitutes, from the most insidious beginnings of the disease, from the first excretion of a formed sputum, the certain, irrefutable sign of the disease, as I shall show at some length when I come to treat of the signs and of the diagnosis of that form of phthisis which I shall call *latent* because it has no certain sign, and of that form which I shall designate under the name of *larvated* because it takes the mask (Latin, *larva*) of another disease.

Urine, Alvine Matters, Blood.—The sputa are the only excreted products which constantly contain the bacilli; the urine and fæcal matters contain them only in exceptional cases, where there is tuberculous disease of the genito-urinary organs or intestines. As for the blood, it does not constitute a habitual or favorable receptacle for the bacillus.

II. Tuberculosis: first Local, then General.—The phthisiogenous microphytes are found in all organs which are invaded by disseminated tubercle. Suppose, for instance, a case of pulmonary tuberculosis at first distinctly circumscribed; from this central focus the bacilli, undergoing multiplication, may penetrate by irruption the blood vascular or

lymphatic system, and produce general, so-called, miliary tuberculosis. Now in these miliary tubercles, found so often in the meninges, pleura, peritonæum, liver, kidneys, etc., you will discover the bacillus, though in less quantity than in the tuberculous products—more or less advanced—of the lungs.

III. Local Tuberculosis.—It is the same with localized tubercles which remain localized like those of the genital organs; the bacillus exists there, though relatively in small quantity. It is in bacillomas of this kind that you often note spontaneous and thorough cure.

IV. Scrofulas in General.—In the organisms of patients who are called scrofulous—neither the disease, however, nor the predisposition nor the constitution being well defined—you will often find the bacillus, whose identity with the tubercle bacillus proves also the identity of scrofula and tuberculosis.

Scrofulous Adenitis.—Also in indurated scrofulous glands you frequently observe the caseous degeneration which we are soon to describe in connection with tuberculous, called caseous, pneumonia. In the caseous mass the bacillus exists constantly, so that its very presence enables you to affirm the localized degeneration to be of tuberculous nature.

Scrofulous Osteitis.—In bones affected with osteitis, with periostitis, with white swelling, with chronic fungoid arthritis, the dominant lesion is tubercle, and the presence of the bacillus is constant.

Lupus.—Cornil has met with bacilli, few in number it is true, but constantly, in lupus, which ought henceforth to enter into the category of scrofulotuberculous affections. In fact, Besnier has with great sagacity shown that, in the Hôpital Saint Louis, lupus compromises life by the tubercles which it develops in the viscera, and especially in the lungs.

V. Circumscribed Tubercles of different Organs.—It is not necessary that the lesions above indicated should be grafted on a "scrofulous" stock; we often see localized and curable tuberculosis of the genital organs in healthy men and women. The affections of certain mucous membranes, such as the pharynx, mouth, the larynx, often manifest themselves by the lesions whose exact nature is not obvious till microscopic examination is made of their products; the presence of bacilli shows clearly their tuberculous nature.

VI. Local Tubercles becoming Infectant.—Do not forget that the circumscribed tuberculous foci of bones, of the genital organs, etc., may, as well as those of the lungs, become all at once the point of departure of a real infection which spreads to the important vital organs. After a long series of years of quiescence the tuberculous foci may, without known cause, discharge into the blood colonies of bacilli which invade and ravage the entire organism. This is the familiar history of those acute phthisical maladies which take their start in a bacillary centre almost or quite forgotten.

THE CULTURE OF THE MICROPHYTES, AND ESPECIALLY THE TUBERCLE BACILLI.

Culture-Liquids.—The specific microphytes develop with difficulty in the atmosphere, more readily

⁵ Society of Berlin, March, 1884.

⁶ Pommellère.

in inert substances, and still more readily in living organisms, their ordinary habitat. Where derived by the organism from without, it is by divers channels of absorption, especially by the respiratory mucous membrane and the alimentary canal, introduced by air and food, that they penetrate the economy. They then localize themselves and form a circumscribed focus, or multiply, carried along by the blood current in different parts of the organism. Now in these primitive foci, circumscribed or multiple, or in the blood itself, the microphytes can be obtained, transported to a nutrient liquid, and cultivated without losing their deleterious properties. It is only by a long series of cultures in nutrient liquids constantly renewed, or even by free access of air, that their pathogenic power diminishes.

Composition of Culture Liquids.—The most nutritive substances for the pathogenic parasites are not the albuminous groups, but the gelatinous compounds, glutine, chondrine, without doubt also mucine. You may advantageously use for cultures solutions of gelatine or decoctions of meat-containing gelatine. In other culture-fluids destined for the bacteria of putrefaction the pathogenic bacteria lose their properties and their forms.

These gelatinous media present themselves naturally, under suitable conditions, in the soil and in stagnant waters. But cadavers and organic matters undergoing putrefaction at the surface of the earth, form a bad culture medium for the specific microbes, because these soon lose by access of air their pathogenic power, and in the second place because they are soon replaced by the septic forms.

Culture at a certain Temperature.—This substitution takes place the more quickly the less the surrounding temperature is favorable to the pathogenic microbes, and the better it suits the septic forms. Thus it is demonstrated that the specific microbes can only develop in a temperature above 25° C., from this up to 41° C., while the fungi of putrefaction vegetate between 16° and 20° C., and multiply at 15° C.

CULTURE-LIQUIDS OF THE BACILLUS TUBERCULOSIS.

At first Koch tried gelatine (extract of meat, peptone, and gelatine) without success, and this for the good reason that the bacillus cannot thrive at a temperature of 20° C., above which gelatine melts. He bethought himself then of coagulated and sterilized blood serum, which may be made to furnish thin transparent layers. You heat the blood (of beef or mutton) for six days, every day one hour at 58° C., then several hours at 65° C., till the serum remains coagulated. On this culture-field you sow bacilliferous-tubercle fragments, which are then exposed in a suitable covered receptacle to a constant heat of 37° C. About the tenth day there form on the surface of the liquid minute scales and little points; after several weeks the growth of the bacillary colony, often appearing contorted like the letter S, is finished. The bacilli thus nurtured are often passed through eight or ten cultures before serving for infection, inoculation, or inhalation experiments.

Original Articles.

PREMATURE BIRTH FOLLOWING THE SUCCESSFUL TREATMENT OF MORPHIA-POISONING BY ATROPIA.¹

BY EDWARD J. FORSTER, M.D.,

Visiting Physician to the Boston City Hospital.

THIS case is of interest to the therapist on account of the amount of atropia used to antagonize the morphia and to the obstetrician (which is my reason for reading it to this Society) on account of the premature delivery which followed, and also that a *living* child was born after the mother's system had been so thoroughly affected by the morphia as to reduce the respirations to two a minute.

Sunday, September 7, 1884, having finished my morning visit, I was about leaving the hospital at 1.10 P.M., when a young married woman of twenty years was brought in by her husband in an unconscious condition, with the statement that she had swallowed thirty grains of acetate of morphia. The patient was in a state of collapse, the pupils contracted to the merest pinholes, and the respirations very slow. By my order half a grain of sulphate of atropia was immediately given subcutaneously. A physical examination was then made. The patient was unconscious, the pupils contracted, not reacting to light. The heart, lungs, and abdominal organs were found to be normal. The uterus was found to be extending above the umbilicus one third the distance to the ensiform cartilage.

Dr. W. L. Richardson, who had accompanied me on my visit, was present, and was able to hear the foetal heart faintly, above the left groin, beating eighty times to the minute. The temperature was 98.6°, pulse 72, respirations 7. Another injection of one fourth of a grain of sulphate of atropia was then given, which was followed by dilation of the pupils.

The following history was obtained from the husband: The patient had been married two years and three months; had a child eighteen months old; last menstruation ended March 10th; not in the habit of taking either opium or alcohol. General health good, except morning sickness during pregnancy. Has seemed somewhat dispirited for last few days, but was singing happily when he left in the morning. At eleven o'clock he was sent for to come home to dinner, as his wife did not feel well; being the only clerk in a drug-store, he could not leave, but sent home a Seidlitz powder. Going home at 12.15 he found his wife lying on the sofa; she had a peculiar look, said she had taken something wrong, and told him to look on the table; there he found a two-drachm bottle labeled morphia, acetate, and poison. He thinks there must have been nearly a drachm in the bottle, as that amount had been put in and but little had been used. Thinks his wife must have mistaken it for a bottle of quinine of similar size and shape. She complained greatly of thirst, and was anxious to lie down. He walked her about for seven minutes, then gave her wine of antimony, which caused her to vomit slightly, afterward mustard-water, which made her vomit freely. She soon became unconscious, did not respond to slapping.

¹ Read before the Obstetrical Society of Boston, November 8, 1884.