EXPLANATION OF PLATE LVI.

Fig. 1.—Section from the Lung in a Case of Miliary Tuberculosis. *a*, Tubercle containing numerous bacilli; *b*, tubercle with fewer bacilli; *c* and *d*, tubercles with cheesy centre and containing no bacilli; *e*, cross-section of a blood-vessel surrounded by a deposit of pigment. Magnified 50 diameters.

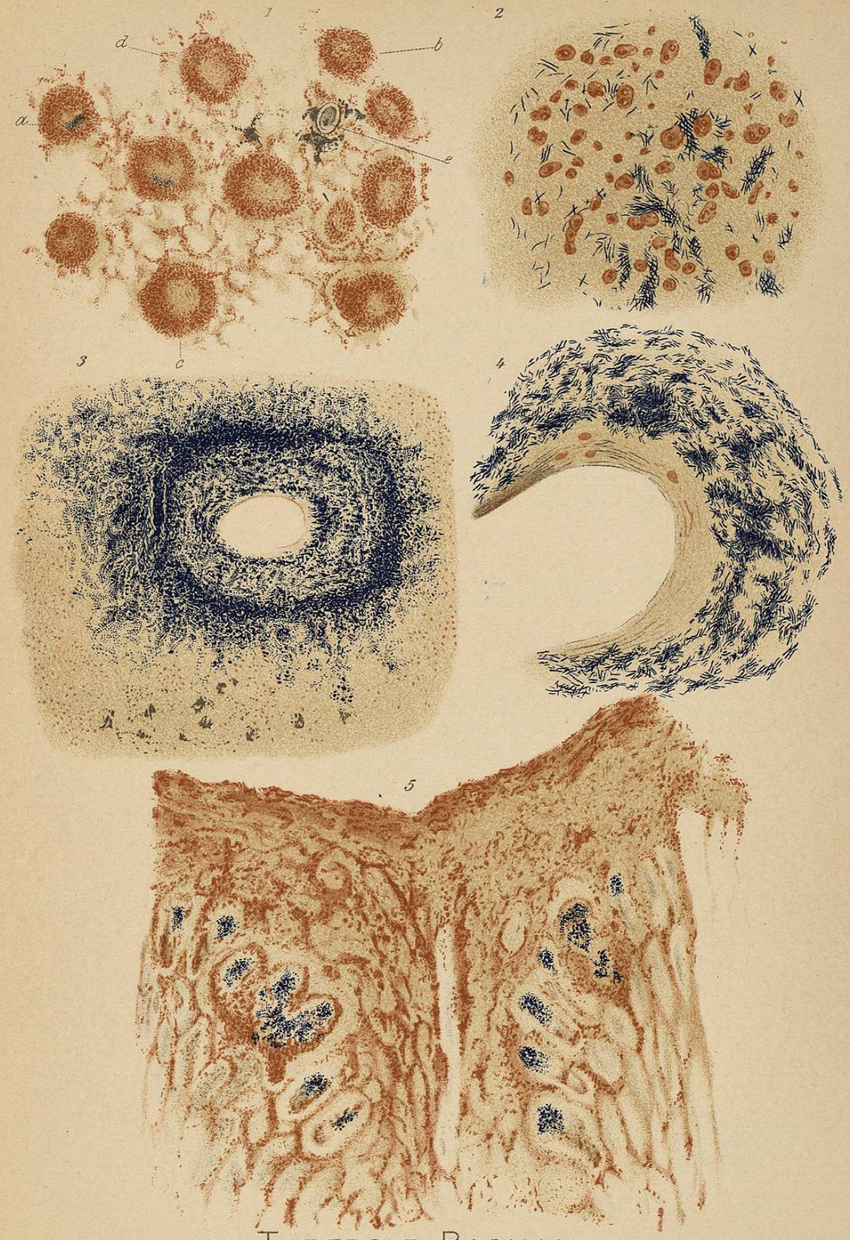
Fig. 2.—The Blue-stained Portion of the Tubercle *a* in Fig. 1. The tubercle bacilli are stained blue, the nuclei of the cells brown. Magnified 700 diameters.

Fig. 3.—A Small Artery surrounded by a Mass of Tubercle Bacilli. From a bronchial gland in a case of miliary tuberculosis. Magnified 100 diameters.

Fig. 4.—A Portion of the Wall of the Artery shown in Fig. 3. Magnified 500 diameters.

Fig. 5.—Section from a Phthisical Lung, showing the Crowding of the Tubercle Bacilli into the Alveoli. Magnified 100 diameters.

Plate LVI.



TUBERCLE BACILLI.

FROM R. KOCH'S "DIE AETIOLOGIE DER TUBERCULOSE"
Mittheilungen aus dem Kaiserlichen Gesundheitsamte. Berlin: 1884.

AMERICAN LITHOGRAPHIC CO. N.Y.

Blood serum remains on the whole the best medium for the growth of the bacilli, especially for obtaining the primary growth. An important addition to the technique of their growth was made by the discovery that glycerin added to the culture media facilitated growth. The bacilli grow readily on agar and bouillon to which glycerin has been added. It is very difficult to obtain a pure growth of the bacillus from sputum, or even from the tissue lesions in man, owing to the liability of contamination with other organisms. Kitasato was the first to obtain a pure culture from sputum. The culture is usually obtained by inoculating an animal and using its tissues for cultivation. The advantage is that the bacilli are abundant, and if the animal has been killed there are no contaminating organisms. Smith has shown that it is better to use considerable portions of the tissues for inoculating the tubes, as the bacilli have become accustomed to growth in such tissue. Growth becomes more easy in every subculture as the bacilli become accustomed to a saprophytic existence. The bacilli in pure culture adhere together in masses which are difficult to separate, so that it is difficult to obtain a homogeneous mixture of them in a fluid. Hesse has strongly recommended, for cultivating the bacilli from a sputum, a medium containing a soluble albumen preparation known as Nährstoff Heyden. He obtained upon agar containing 0.5 per cent. of this substance along with three per cent. of glycerin a characteristic microscopic growth of the bacilli from tuberculous sputum in three days. Even when other bacteria are present, the commencement of the tubercle growth can be demonstrated in contact preparations in five or six hours. This is the best medium for obtaining a primary culture from sputum, though it is not the best medium for their continued growth.

The tubercle bacilli are not saprophytic under natural conditions. They grow only within narrow limits of temperature, and will not find favorable conditions for growth outside of the animal organism. The temperature limits of growth are 30°–42° C. Growth takes place best at a temperature of 39° C. Cultures will usually become sterile after twelve months. Bacilli in dried sputum kept in the dark may remain virulent for from six to ten months. The sputum does not lose its virulence by alternately moistening and drying. The dried sputum in a chamber, exposed to diffuse daylight, may retain its virulence for two months. Putrefaction does not destroy the bacilli, but they are rapidly killed by sunlight. Smith has shown that a temperature of 60° C., maintained for twenty minutes, will kill the bacilli in water, salt solution, bouillon, and milk, but that a membrane formed on the surface contained living bacilli after sixty minutes' exposure to this heat.

Fatty acids are important constituents of the tubercle bacilli, and Unna has supposed that the resistance of the stain to acids depended upon these fats. Aronson has shown that the body which gives the acid resistance is of the nature of a wax, and when this is removed the stained bacilli are no longer acid-proof. The best analysis of the tubercle bacilli is that of Rüppel. He finds in 100 gm. of dried tubercle bacilli: Fat and wax, 26.5; nucleic (tuberculinic) acid, 8.5; protamin, 24.5; nucleo-proteid, 23; mineral substances, 9.2; proteinoid substance, 8.3. According to Behring the nucleic acid possesses in high degree the specific properties of Koch's tuberculin.

Levene found differences in the composition of the bacilli dependent upon the medium on which they were grown.

A substance called *tuberculin* is present in cultures of the tubercle bacilli and in the infected tissue. It may be extracted from the dead bacilli and represents the sum of their proteid substance. It is not known whether it is actually given off from the living bacilli as a secretion, analogous to diphtheria and tetanus toxin, or whether it is exclusively derived from their protoplasm. Koch first described this substance and its action at the International Medical Congress in Berlin in 1900. As first prepared it was known as crude tuberculin. The bacilli are grown in large dishes, which give a large surface to the growth. The culture medium used is bouillon containing one per cent. of peptone and from four to six per cent. of glycerin. The bacilli grow abundantly in this, forming a thick yellowish membrane on the surface. After six weeks growth ceases and the membrane begins to break up and sink to the bottom. The culture is then evaporated to one-tenth its volume on the water bath and the residue filtered through porcelain. Tuberculin as so prepared is a brownish fluid, the color being due to the concentrated color of the bouillon, and contains enough glycerin to preserve it indefinitely. It evidently represents a number of different things, including a concentration of the soluble substances of the bouillon. Later, Koch attempted to purify the product by extracting the pulverized bodies of the dead bacilli. He produced two substances, one soluble in water and one soluble in glycerin. The material soluble in water has the same action as the crude tuberculin. Koch was led to the therapeutic use of tuberculin by the difference which he found in the inoculation of a healthy and a tuberculous guinea-pig with a virulent culture of the tubercle bacilli. In the normal animal subcutaneous inoculation leads to the formation of a tuberculous ulcer, which persists until the animal dies from generalized tuberculosis. If a second inoculation is made five or six weeks after a primary, only induration and necrosis of the tissue at the site of inoculation are produced. The necrotic tissue is thrown off and the ulcer heals by cicatrization. He found the same results from the dead bacilli, and endeavored to obtain the chemical substance which was present in the body, and which caused the second inoculation to take a more favorable course. In his explanation of the action of the remedy he supposes that the bacilli produce substances injurious to the cells, causing them to undergo coagulation necrosis. Such necrotic tissue forms a bad culture medium for the bacilli and they die. A greater amount of this substance causing necrosis will extend the area and still more wall off the bacilli contained in it. The necrotic material may become detached and remove the bacilli contained in it outside of the body. The tuberculin contains the material which produces the necrosis, and when injected in sufficient amounts into a healthy man will cause fever and toxic symptoms. Very small doses injected into a tuberculous subject will cause fever and other symptoms. It also exerts a local action on tuberculous tissue, producing intense hyperæmia with leucocytic invasion and increasing the extent of the necrosis. It has this intense general and local action in the tuberculous subject by increasing the amount of tuberculin in the fluids and in the local lesions, some being always formed by the bacilli in the affected subject. This hardly explains its action because a severe general and local reaction takes place when the tuberculous lesions are so slight as scarcely to be manifest, and it can hardly be supposed that in such lesions, containing only a few hundred bacilli, an appreciable amount of tuberculin can be produced. It is generally considered that the action of tuberculin is specific, that its local and general action in very small doses, 0.1–1 mgm., is exerted only in tuberculous individuals. There are opposing opinions to this view of specific action. Roemer and Buchner have obtained the tuberculin reaction in tuberculous guinea-pigs with extracts of other organisms. Tuberculin reaction has been obtained in man in cases of syphilis and in other infections, and in individuals who were clinically in perfect health. In these cases it is impossible to say that an individual giving the tuberculin reaction has not some focus of latent tuberculosis. Koch regarded the remedy as directly curative in the early stages, the advance of the disease being stopped and the lesions cicatrized. The most enormous literature on tuberculin followed the publication of Koch. Every physician who could obtain any of the precious fluid published his results, however insignificant they might be. The remedy seemed to have a more decided and favorable action in tuberculosis of the skin than in any other forms. Portions of tissue here can be excised and the influence exerted on the lesions followed. Its general action was

seen to be the production of acute inflammation in the lesions with leucocytic infiltration and serous and fibrinous exudation. The general results were not favorable to the views of Koch. Numerous autopsies were made on cases of tuberculosis in which the remedy had been used. It was shown that it could be dangerous and even fatal, producing extensive inflammation around foci in important organs and leading to dissemination. The strongest possible reaction succeeded the enthusiasm with which the remedy was first greeted. At present it seems to be the opinion that tuberculin is of great importance in the diagnosis of tuberculosis in animals (cattle) and in man, being far more accurate than any other mode of diagnosis with the exception of the bacteriological and experimental; it is available when these methods are not. It is of therapeutic value in certain early cases, and when most judiciously used. It is a very dangerous agent when considered as a general remedy for the disease.

Differences in Bacilli.—In his first work Koch held that there was but one species of tubercle bacillus, and that all forms of the disease in man and in animals were the same, and due to the same bacillus. Subsequent study has shown that this statement must be greatly modified, and that there are several distinct varieties or strains of the bacilli. These different strains are distinguished from one another less by their morphology and cultural characteristics than by the effects which they produce on inoculation. The general opinion seems to be that they cannot be made to pass one into the other either by cultures or by series of inoculations. Undoubtedly much confusion in the pathology arises from these variations. It will be necessary to repeat a great deal of the work which has already been done, holding in mind these differences and in every case determining the character of the organism which has been used in the experiments. These different strains of bacilli will be considered in the tuberculosis of animals.

Differences in Virulence.—Great differences are found in the clinical course of the disease and in the character of the lesions. In certain cases the disease runs its course, ending in death, in a few weeks or months; in others it takes a very chronic course, and may terminate fatally only after years. Autopsies show an extraordinary number of cases in which infection has been followed by cure or at least by complete quiescence of the disease. In the microscopic study of the lesions the same differences are seen. In one case little or no reparative action of the tissue; in another a formation of fibrous tissue, limiting the extension of the process. These differences are due to a number of factors: The anatomical structure of the organ attacked, which may or may not favor the dissemination of the bacilli or the chemical substances derived from them; the number of bacilli and their mode of entrance into the tissue; peculiar individual qualities of the tissue attacked or of the body generally.

There is undoubtedly a great difference in the organs and tissues attacked as regards their favoring or not the multiplication of the bacilli, this difference being due not to anatomical arrangement, but to peculiarities probably of a chemical nature. It is possible also that there may be differences in different individuals, the bacilli finding all or similar tissues in one more favorable for growth than in another. It is generally assumed also that there are differences in the virulence of the bacilli. There are certainly differences in the virulence of the different strains of the bacilli, the bovine being most virulent, the human coming next. The virulence of cultures also diminishes in the course of cultivation. There is some uncertainty as to the variation in virulence of primary cultures of the human and bovine, and whether we can refer differences in the lesions and in the clinical course of the disease to this. Variations in the virulence of human cultures may be due to infection with other than the human bacillus. Vagedes has studied the virulence in thirty different cultures of the bacilli. Three were from rabbits which had been inoculated, two were from cattle, and the others were obtained directly from the human sputum or from contents of cavities. He tested the virulence by inoculation with a suspension of the bacilli made by rubbing up weighed amounts of the pure culture in a given amount of water. He found three different degrees of virulence, four of the cultures derived from man being as virulent as the bovine. The most virulent culture came from a fifteen-year-old girl, both of whose parents had died of the disease. The autopsy of the girl showed advanced lesions of the lungs and intestines. The less virulent cultures came from the cases which clinically showed a less rapid course. There was very little difference in the character of the growth in the different cultures. One culture of medium virulence resembled the avian type of bacillus, and there was much similarity in the shape of the organisms between those from his most virulent case and the bovine.

Tuberculosis in Animals.—The disease never appears in animals in a wild state. In some it never appears spontaneously, though they may be highly susceptible to inoculation. The same rules for infection govern the disease in animals and in man, being dependent upon the situation of the lesions, this determining the ways by which the bacilli leave the body and the opportunity given by the life of the animal for the entry of the bacilli into the tissue. The disease in animals is of great importance, both from the enormous economic loss it entails and because animals play a certain part, the importance of which is probably overestimated, in the transmission of the disease to man. It is also of importance that the disease can be given to animals, because our knowledge of the disease has been chiefly obtained through animal experimentation. The unity of the disease could have been established only by animal inoculation. Modes of infection followed by preventive measures have been studied in the same manner.

Tuberculosis of cattle is the most common and the most important form of animal tuberculosis. The form of disease differs in many respects from that in man, and the identity of the two has been established only in the last twenty-five years. It is characterized chiefly by the presence of hard, tumor-like masses up to 20 cm. in diameter, on the serous surfaces or in the viscera. The masses are often flat on top and attached to the surface by a pedicle. Several such masses may be united together by small attachments, and hang from the serous surface like a bunch of grapes. The large masses are formed from the confluence of single tubercles, which give the surface an irregular character. The smaller nodules are hard and fibrous on section, later they become caseous, infiltrated with lime salts, and have a peculiar bright-yellowish color. They may undergo partial softening, becoming filled with a yellowish, greasy, gritty material of the consistence of mortar. Microscopic examination of the younger nodules shows a structure composed of single tubercle with epithelioid and giant cells containing bacilli. The lungs contain irregular solidified areas, circumscribed tumor-like masses similar to those first described, and cavities with smooth walls, filled with yellowish, thick, tenacious, often fetid material. The extension along the bronchi is very evident, the separated single lobules are often affected, the single small foci in these resembling the grapes on a bunch, the stems being represented by the bronchi filled with and surrounded by yellow caseous material. The lymph nodes are always infected, and may be the only seat of the disease. Infection seems to take place chiefly by inhalation and the bronchial and mediastinal nodes are those most often affected. The nodes may be enormously enlarged, forming hard caseous or calcareous masses with peculiar yellow pigmentation. The grayish tumor-like masses have given the disease the name "Perlsucht," or pearl disease. Acute general miliary tuberculosis as seen in man does not occur, though miliary tubercles harder and more transparent than in man may be found both on the serous surfaces and in the organs. Ulceration of mucous surfaces is not common, although in the trachea and larynx ulcers may be found which take the form of large projecting masses formed of conglome-

rate tubercles, and which seem to have burst through the mucous membrane. I have seen one case in which tuberculosis of the peritoneum took the form of a diffuse caseation of the surface with adhesions between the intestines which were filled with communicating ulcers, the whole forming a mass which it was impossible to disentangle. Tuberculosis of the mamma is common and important from its relation to the infection of milk. It may be primary or a part of the general process. It usually commences as a diffuse uniform thickening of the posterior part of the gland. The gland at this time may contain bacilli which are apparently growing in the ducts, since they can be squeezed from it through the udder. Later, the gland becomes filled with fibrous or caseo-calcareous masses, and increases greatly in size. The milk may present a normal appearance, even when there is advanced disease of the gland; later, it becomes thin, bluish, and on standing gives a sediment which contains numerous bacilli. The bones are rarely affected.

The disease ordinarily takes an extremely chronic course, and may remain latent for years. The animals may become fat, appear to be perfectly well, and yet the autopsy shows an advanced tuberculosis of the serous cavities. When the disease takes a more rapid course, cough begins, there are fever, discharge from the nose, and rapid emaciation. The disease is widely spread over the entire civilized world. It increases in a definite proportion with age, and is extremely rare in calves. Most cases are in cattle over six years of age. It is more frequent in cows than in oxen and bulls, which may in part be due to the more confined life of the cows and the greater demands made on their nutrition by pregnancy and lactation; but it is probably due rather to the fact that cows live longer. The disease spreads by contagion and one animal may infect a herd. The opportunities are good for the extension of the infection. The animals are usually in close proximity, they cough forcibly, and can project fine spray particles of sputum containing the bacilli for a considerable distance; they commonly use the same place for drinking, they lick away their nasal discharge, and by the habit of licking each other may transfer the bacilli. The disease is most common in stables where the animals are kept closely confined.

There is much difference in the susceptibility of the different breeds of cattle. The purest breeds where the selection of certain qualities economically valuable has been made are those most affected. The most susceptible races are the Alderneys and the Short Horns. Certain strains of these in England have been threatened with total extinction. Wilson estimates that in Great Britain there are 2,200,000 tuberculous cattle, and that the annual loss caused by the disease is £3,000,000 sterling.

The numbers of bacilli found in the lesions vary. They are about as numerous as in the human lesions, and they may be present in enormous numbers. The greatest number of bacilli I have ever seen in a disease was in a case of intestinal tuberculosis in a cow.

The most important practical question relating to bovine tuberculosis is the part which it plays in the transmission of the disease to man. The danger comes through the use of the milk; the danger of infection through the use of the meat of tuberculous animals is remote. Tubercle bacilli have been repeatedly found in milk and in milk products. They are always present in large numbers in the milk in tuberculosis of the udder, and may be present in tuberculosis of internal organs when the udder is not affected. When we consider the enormous frequency of bovine tuberculosis and the facts that the milk coming from different farms is all mixed before distributing in the cities, and that the bacilli would certainly not be destroyed in the milk, it is evident that they must be frequently ingested. But a small proportion of the milk used in this country is boiled or even heated before it is used. Animals fed on milk containing bovine bacilli become infected, but the opinion is gaining that milk infection is an exceedingly rare source of tuberculosis in man.

Baumgarten (1882) was the first to point out differences in the transmission of the bovine and the human disease. He says that inoculation with material from bovine tuberculosis produced a disease which took a quicker course and led more rapidly to a general infection than inoculation with material from man or dog. He had no results from the inoculation of tuberculous material from chickens and pigeons.

The bacillus of bovine tuberculosis is the common cause of all tuberculosis in mammals with the exception of man. The morphological distinctions between the human and bovine bacilli are not sufficiently marked to enable us to distinguish them. The bovine bacillus is a little shorter than the human and the sizes are more constant. The cultural differences are more marked. The human bacilli from the start grow more vigorously than the bovine, though the bovine are less influenced by slight modifications of the culture media. There are marked differences in the character of the growth on the surface of glycerin bouillon, the growth of the bovine forming a more moist and translucent membrane with less tendency to plication. The inoculation differences are more striking. The bovine is more virulent for all animals and tends to more rapid generalization. When rabbits are inoculated in the ear vein with like amounts of pure cultures of the human and bovine bacilli the bovine inoculation produces general miliary tuberculosis and death in three weeks. With the human bacilli the animal lives longer and may survive the inoculation. A number of inoculations in cattle have been made with pure cultures of both bacilli and with somewhat varying results. But the general result shows that cattle are either completely immune to the human bacillus, or, if they acquire the disease at all, only slight local lesions are produced. The study of the bacilli shows that the characteristics of each form are retained after a series of cultures and animal inoculations. Those opposing the idea that the disease may be transmitted from cattle to man base their belief on a number of conditions. The types of human and bovine bacilli remain constant, and only the human type is found in pure cultures of the lesions in man. Bovine tuberculosis cannot be produced by inoculation of the human bacillus. Were infection by milk common, the lesions in man would show the primary foci in the intestinal canal. Experiments in feeding animals with tuberculous material produce a series of lesions pointing to infection from the alimentary canal. Primary intestinal tuberculosis or even primary tuberculosis of the mesenteric glands is rare in man, notwithstanding the opportunities for infection given in the extended use of milk. Baumgarten has recently reported a series of experiments made twenty years ago, in which inoculations of the bovine bacilli were made in man. Proceeding from the views of Rokitansky regarding the opposition between tuberculosis and cancer a physician inoculated a number of patients having advanced malignant tumors with pure cultures of bovine bacilli, with the hope of effecting a cure of the cancer. No influence was exerted on the cancer, of which the patients died, and at autopsy no effects from the injection of the bacilli could be found. Opposed to this there have been a number of cases reported of local and general tuberculosis in man due to infection with bovine tuberculosis, and some of them do not admit of any doubt. The matter of this mode of infection or the frequency of it is one which must be cleared up by further investigation. The permanence of type of the bacillus must be more accurately determined, and the bacillus must be isolated and studied in a large number of human cases, particularly in those in which infection seems to have come from the alimentary canal. The infrequency of primary tuberculosis of the alimentary canal is by no means opposed to the probability of food infection, because it is possible for the bacilli to enter the tissues without the production of a lesion at the point of entrance, or without any anatomical evidence of the lesion. No effective control of bovine tuberculosis is possible. To use the tuberculin test for its detection and to destroy all cattle which give the reaction would result in economic losses impossible for the