

ISOLATION, IDENTIFICATION and CULTURAL CHARACTERISTICS
OF
TUBERCLE BACILLUS.

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TUBERCLE BACILLUS.

Introduction. - Tuberculosis, one of the most wide spread of deadly diseases with which we have to deal, has been proved to be due to the presence of a specific micro-organism tubercle bacillus. Every year in the United States alone, a hundred thousand people die from this dread destroyer. The disease when once fairly established is extremely rebellious to the present mode of treatment and is treated by physicians with little hope of accomplishing a material improvement in the patient. Fortunes are spent every year by consumptives, in a vain attempt to find a cure for their disease. Tuberculin, creosote, cinnamic acid and various other materials have been used with more or less success in modifying the disease. Innumerable researches have been carried on with the object of finding the facts in the life history of this organism.

Sputum examination. - This is made for early detection of tuberculosis. Not only tubercle bacilli, but other bacteria, the possible cause of mixed infections must be sought. In view of these significant facts, the sputum of every suspicious case should be subjected to bacteriological examination, not alone to ascertain the absence or presence of tubercle bacilli but, the clinical findings must be carefully worked out, since it is only by the combination of clinical and bacteriological facts that diagnosis can be made.

Powers of Resistance. - The tubercle bacterium has considerable power of resistance to external influences and in various conditions can retain their life outside the human body. Tubercular sputum which has become dried has been found to contain virulent bacilli after several months. They also resist the action of putrefaction which is fatal to many organisms. Sputum has been found to contain

living tubercle bacilli after being allowed to putrefy for several weeks. By exposing the bacilli to the action of the gastric juice for six hours they are not killed, or at a temperature of 3°C for three hours they still retain life. They have been found to resist the temperature of 100°C for one hour when in a dried condition. However, they are destroyed, at this temperature, when exposed in the fluid or tissues.

Habitat. - This organism has been detected in the dust of rooms which was deposited from the air. Dry dusting and sweeping should be avoided especially in rooms which are occupied by consumptives and patients with other infectious diseases. The organism is principally found in the tissue of the animal body. Here it will grow and thrive as it will no place else.

Pathogenesis. - Man, cattle and monkeys are most subject to the disease naturally and many of the lower animals may become infected through inoculation. Guinea-pigs, field-mice, rabbits and cats are among the most susceptible, while dogs, rats and fowls may become infected through larger doses. Tuberculosis in man is no doubt the result of respiration of the susceptible individual, of air containing the bacilli in desiccated conditions. Infection may also occur through an open wound, or abrasion of the skin, as in the small circumscribed tumors which sometimes develop upon the hands of pathologists as a result of handling tuberculous tissue.

Human and Bovine Tubercle. - Koch called attention to certain differences that exist between the bacilli of human and bovine tuberculosis. The well known tuberculous disease of cattle was found to have lesions resembling those of human bacilli and stained similarly. It is not determined that there is any other difference between

the two bacilli than can be accounted for upon biological grounds, each organism being modified to accommodate itself to its environment. Some of the best authorities today are announcing the human and bovine organisms to be one and the same.

Occurance and Distribution. - We find the tubercle bacilli especially in the lungs and in the sputum in cases of pulmonary tuberculosis, further, in all tuberculous lesions. The blood contains the bacilli only in cases of general miliary tuberculosis, and then only in small numbers. In this respect the sputum is the most dangerous. Of less importance are the feces in cases of intestinal tuberculosis, and urine in cases of genito urinary tuberculosis, and the pus in cases of bone tuberculosis and suppurating tuberculous glands. By means of sputum, the tuberculous patient who, instead of using spittoon, expectorates upon the floor, the bacilli is disseminated in the environment of the patient. The micro-organism exhibits considerable resistance to drying. The powdered sputum carries its vitality and infectivity into the air, and in this way enters the air-passages of individuals and there gives rise to infection. A further source of infection is constituted by the milk of tubercular cows. The use of meat from tubercular cows probably gives rise to the development of intestinal tuberculosis.

Isolation. - One of the best methods of isolation from the tissue is by means of Raux potato tubes. The bulb being filled with a 5% solution of glycerin. The potato is inoculated with the emulsion and incubated at a temperature of 37°C. Potato cultures of glycerine agar media are used to a good advantage if the organism has first been raised upon blood serum. Here they grow in colonies and the pure culture may be obtained with moderate ease. The safe and sure way of isolation is by means of inoculation into a susceptible animal. The

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Guinea-pig is used for this purpose. If the material to be tested is a fluid it is injected subcutaneously; if solid, it is placed in a pocket in the skin or it may be thoroughly broken up in sterile water and the emulsion injected. When proper lesions are manifestly shown, the animal is killed, and test tubes, containing blood serum, are inoculated from the spleen under strict aseptic precautions.

Identification. - After the organism has been successfully grown it is found to be a "non-motile aerobic and facultative anaerobic, strictly parasitic organism". Usually it occurs singly, occasionally linked in twos and threes so as to form short chains and under certain conditions, filamentous forms have been noticed. It grows best, as stated above under a different head, at 37°C and requires from two to three weeks for growth. It is a slender rod-shaped organism with rounded ends, often slightly curved and measuring from three to five microns in length. It is a single cell instead of a multiplicity of cells as is found in the higher plants. In stained preparations, one or more unstained intervals are often seen in the rods and these have been considered by some observers to be spores. Still this question has not yet been fully decided and many objections have been raised to this view.

Staining peculiarities. - Since the organism was discovered many methods of staining have been suggested. They, all alike, depend upon the remarkable property which the bacilli possess of staining with aniline dyes in alkaline solution, and retaining the dye after treatment with acid and alcohol. The 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. For this reason the simple aniline dyes do not suffice for tubercle bacilli without prolonged exposure. The most commonly

employed method, is to take a large loop full of sputum and transfer it to a wide cover glass thoroughly spreading it over the surface. It is then allowed to dry in the air, or by moving it to and fro over a low flame. The specimen is then covered with carbol fuchsin solution. It is warmed over a flame for two minutes. A drop or two of the stain should be added from time to time to prevent drying. The excess dye is taken off by washing in dilute nitric acid. After this it is washed with methylene blue. The latter is washed off and the specimen examined with a 1/12 inch oil immersion objective. It should show the bright red tubercle bacilli on a light blue back ground. The ordinary bacteria that may be present are stained blue also. It is evident that ordinary bacteria are readily decolorized by treatment with alcohol and acids and in this respect differs from the tubercle bacillus. This examination of sputum for tubercle bacilli is the routine procedure and is often of the greatest value in forming an early and correct diagnosis.

According to recent investigations made by Koch, tubercle bacilli contain two fatty acids, one of which is soluble in dilute alcohol and is saponified by sodium hydroxid; whereas the other is not saponified, and is soluble only by boiling in absolute alcohol. Both fatty acids take the stain of the tubercle bacilli but as one is soluble in alcohol, only the other remains after decolorization and this fixes the stain and must be looked upon as the medium of color reaction. According to Koch these fatty acids form a coherent layer within the bodies of the bacilli to which they afford protection against external influences.

Biological characters. - It is very difficult to obtain a pure culture of the tubercle bacillus because they grow extraordinarily slow, and require for their development an incubator temperature of -

maximum 41°, minimum 29°C. The isolation of the bacilli from the mixture of bacteria in sputum of tuberculous patients is difficult on account of the tubercle bacilli growing so slowly. The other bacterial colonies outgrow and overwhelm them easily.

The new Tuberculin. - For the purpose of combating this disease many remedies have been produced. However, none with better results than the new tuberculin prepared by Koch for this purpose. The tuberculin T R is prepared by pulverizing dried cultures of tubercle bacilli and then adding distilled water. The fluid removed is classed as tuberculin and is preserved by adding 20% glycerin. The most remarkable property of the new tuberculin is that by its previous use, in graduated doses, complete immunity can be conferred on such highly susceptible animals as the guinea-pig, experimentally inoculated with virulent cultures of tubercle bacilli.

Cultural characteristics. - The evidence produced by Cornet together with statistical evidence points to the lungs as the most common portal of natural infection for the human being. The bacilli will be found in the tissues which are in the active stage of the process. In the very initial stage of the disease the bacilli will be found fewer in number than later. At this time only here and there single rods may be found, later they will be found in the granulation zones in clumps and scattered about in large numbers. It is in the strict sense of the word a parasite and finds conditions entirely favorable to its development only in the animal body. On artificial media it is raised with success. In an effort to cultivate this organism directly from the animal the method by which is obtained the best results is that recommended by Koch. Viz. - cultivation on blood serum.

Blood serum. - As was mentioned above this is one of the best media for obtaining pure cultures from tubercular sputum. In about

12 days, slightly raised circular or oval white nodules are seen. They never liquify the media. After the white colonies appear they should be transferred to glycerin agar, which is more favorable to their growth.

Glycerin Agar. - After about twelve to fifteen days small opaque, rounded nodules are visible, spreading out from the field of inoculation. The growth is at first white and nodulated; later on it becomes a light buff culture. The surface becomes wrinkled and foliated.

Veal Broth. - To which a five per cent. solution of glycerin has been added is a favorable media. The growth appears as a white scum which sinks to the bottom of the flask.

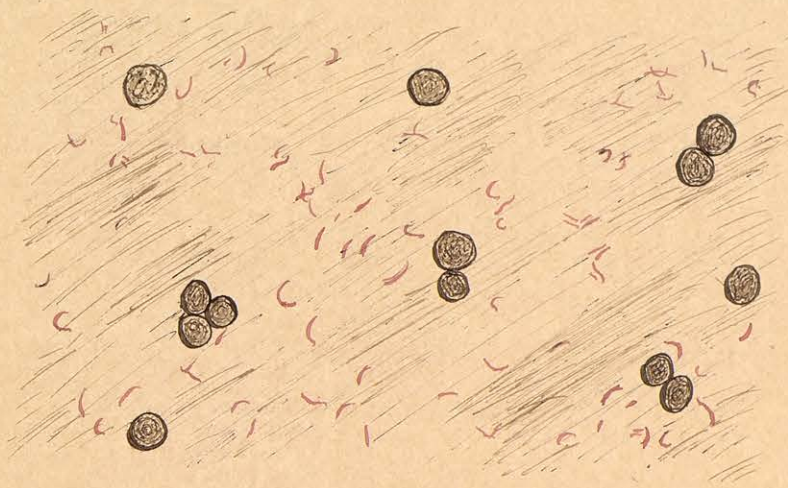
Potato media. - If made alkaline with a solution of sodium carbonate, potato culture shows white colonies in from two to three weeks. Observation. - Under original investigations probably the most surprising feature was the scarcity of the germ. We so often hear of the numerous cases of this dreaded disease, yet our high and dry climate aids in keeping us more or less free from infection.

I obtained the organism in sputum and after making slide preparations to insure the certainty of the presence of the tubercle bacilli I grew it upon the common medias and isolated the pure culture. The best results were obtained on veal broth (5% glycerin) which grew the recognized white scum at about the eleventh or twelfth day. (Incubated at 37°C.) On potato, glycerin agar, and blood serum the growth was well marked at the same length of time, yet much slower in development. The slides were obtained from these growths according to the double staining process. The slides show the organisms that were grown upon the artificial media to be much larger than those obtained origin-

ally from the tubercular sputum. On the following plate will be found the organism as it appears under the microscope after the double staining process has been followed out.

Conclusions. - From the facts treated above with regard to the conditions and growth of tubercle bacilli, their power of resistance, and the paths by which they can enter the body and produce disease, the manner by which tuberculosis is naturally transmitted can be readily understood. It is true that vegetable media will grow the bacilli at a summer temperature. It is a question whether all the conditions are present necessary for growth. At any rate, the great multiplying ground of the tubercle bacilli is the animal body, and tubercular tissue and secretion are the only means by which the bacilli are spread. The tubercle bacilli leaves the body in large numbers in sputum of tuberculous patients and when the sputum becomes dried and pulverized they are set free in the air. Every consumptive patient ought to be looked upon as a fruitful source of infection to those around, and should only expectorate on pieces of rags which may afterwards be burned.

As a final conclusion we may state that the two great modes of infection are by inhalation and by ingestion of tubercle bacilli. By the former method the tubercle will in most cases be derived from the human subject, in the latter probably from tuberculous cows. In both cases the tubercle bacilli may lodge about the pharynx infecting the pharangeal tissue, tonsils etc. Afterwards, other groups of glands, bones, or joints and internal organs.



Tubercle Bacilli from the Sputum.



Tubercle Bacilli from the Sputum.