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A NEW AND RAPID METHOD FOR THE ISOLATION AND CULTIVATION OF TUBERCLE BACILLI DIRECTLY FROM THE SPUTUM AND FECES.*

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The object of this investigation was to devise a simple, practical, and reliable method for the isolation and cultivation of the tubercle bacillus from the sputum and feces. Most of the methods employed during the last twenty years do not give uniformly positive results. In the last few years the antiformin method has proved to be of value, but it has failed to give a rapid and uniform growth. Its disadvantage rests in the fact that repeated washing of the sputum with sterile water diminishes the number of organisms and increases the possibility of contamination. Passing a specimen through an animal may or may not modify the organism. All these difficulties made it impossible up to the present to make systematic studies of tubercle bacilli bearing on cultural characteristics, staining reactions, virulence, and other biological phenomena. The work of Churchman, Krumwiede, and Simons, on the effect of various dyes upon the growth of microörganisms, suggested the use of these dyes in the isolation of the tubercle bacillus.

At first an attempt was made to grow tubercle bacilli directly from the sputum on a medium containing gentian violet. Only a few Gram-positive streptococci and diplococci appeared in the tubes. These organisms were isolated and studied. A dilution of gentian violet 1 to 1,000 failed to inhibit their growth. For the elimination of some of them sodium hydrate proved to be of greater value than antiformin.

To determine the bactericidal action of sodium hydrate on the tubercle bacillus, a series of tubes containing 1, 2, 3, and 4 per cent.

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sodium hydrate were inoculated with tubercle bacilli and left in the incubator for twenty-four hours at 37° C. At the end of that time the tubes were neutralized to litmus with normal hydrochloric acid, centrifugalized, and the sediment containing the tubercle bacilli was inoculated into tubes. After incubation for some time they showed positive growths of tubercle bacilli.

The next step was to determine what stain and dilution were most favorable for the growth of tubercle bacilli. For this purpose a series of media containing gentian violet, methyl violet, methylene blue, crystal violet, and fuchsin in dilutions varying from 1 to 1,000 to 1 to 100,000 were used. Tubercle bacilli will grow well on egg-meat juice media containing all of the above stains even in a dilution as low as 1 to 5,000, with the exception of methylene blue which gave negative results below a dilution of 1 to 25,000. In media containing this stain the tubercle bacilli grow rather slowly, and the individual organisms are impregnated with the stain. From these results gentian violet was selected as the most favorable stain, on account of its inhibitory action on many organisms.

PREPARATION OF THE MEDIA.

After many attempts it was found that a medium containing whole egg, beef or, preferably, veal juice, and gentian violet gave uniformly positive results. This medium contains:

Two parts of egg (white and yolk).

One part of meat juice.

Gentian violet sufficient to the proportion of 1 to 10,000.

Meat Juice.—500 gm. of beef or veal are infused in 500 c.c. of a 15 per cent. solution of glycerin in water. Twenty-four hours later the meat is squeezed in a sterile meat press and collected in a sterile beaker.

Eggs.—Sterilize the shells of the eggs by immersion for ten minutes in 70 per cent. alcohol or by pouring hot water upon them. Break the eggs into a sterile beaker and after mixing the eggs well, filter through sterile gauze. Add one part by volume of meat juice.

Gentian Violet.—Add sufficient 1 per cent. alcoholic gentian violet to make a dilution of 1 to 10,000.

Tube about three cubic centimeters in each sterile test-tube and inspissate for three successive days: on the first day at 85° C., until all the medium is solidified, changing the places of the tubes if necessary; on the second and third days for not more than one hour at 75° C. For the bovine type omit the glycerin and infuse the meat for twenty-four hours in water. Bovine tubercle bacilli grow

in this medium even if it contains glycerin, but on account of the popular belief and the lack of data we used a medium without the glycerin.

From a careful calculation it appears that if a single organism divides in two, it will take approximately from six to seven days to grow to a pin-point colony and be visible. To confirm this, five organisms were isolated by Barber's method and inoculated in a test-tube containing gentian violet-egg-meat juice media. Every twenty-four hours the tubes were examined. On the sixth day three pin-point colonies were visible. The strain of the tubercle bacilli used in this experiment was well adapted for growth outside the body, having been isolated two years previously. This experiment shows that under most favorable conditions it will take at least six days for a single tubercle bacillus to grow to a visible colony.

METHOD OF ISOLATING TUBERCLE BACILLI FROM THE SPUTUM.

Fresh sputum is advisable. Equal parts of sputum (about five cubic centimeters) and 3 per cent. sodium hydrate are well shaken and left in the incubator for twenty to thirty minutes until the sputum is fairly well digested. The sputum is then neutralized to sterile litmus paper with normal hydrochloric acid, centrifugalized, and the sediment inoculated into several test-tubes containing the media described above. Neutralization is not necessary, but it is advisable. In a few instances we added to the mixture of sputum and sodium hydrate a few drops of 5 per cent. litmus solution, but this method did not give satisfactory results.

To determine the value of this method and the medium, a parallel series was carried out with a medium which did not contain gentian violet. Fifteen out of twenty specimens of the plain medium were contaminated, while all twenty gentian violet-egg-meat juice medium specimens were free from contamination. This proved that if some of the organisms were not destroyed by the sodium hydrate their growth would be inhibited by the gentian violet. Smears were made from the sediment of each specimen with the object of comparing the original with that of the growth.

The appearance of the growths was not uniform. Some specimens gave positive growth in seven days, while others took from twelve to fourteen days, but never longer. All gave positive micro-

scopical findings upon the ninth day. There was a great variation also in cultural characteristics, some being small pin-point colonies and others large flat ones. Some of the types decolorized the medium while others picked up the dye and the colonies appeared violet. Morphologically they showed considerable variation, varying from cocci to long rods. Up to the present time we have isolated tubercle bacilli uniformly from sixty-nine consecutive specimens. The sediment from six specimens, after a careful microscopic examination, was negative for tubercle bacilli; the cultures, however, were positive. The value of this method for negative sputa is being studied.

METHOD OF ISOLATING TUBERCLE BACILLI FROM THE FECES.

To isolate tubercle bacilli directly from the feces is not easy. There are present many spore-forming bacteria which resist the action of the sodium hydrate. The concentration of the sodium hydrate seems not so important, because a careful study showed that the length of exposure is more important than concentration. Feces are collected in wide mouthed jars; a morning specimen gives the best results. The feces are diluted with about three volumes of water and mixed well, then filtered through several thicknesses of gauze to remove solid food particles. The filtrate is saturated with sodium chloride and left undisturbed for one half hour. At the end of that time all bacteria will be found floating. This floating film is then collected with a deflagration spoon in a wide mouthed bottle, and an equal volume of normal sodium hydrate is added. This is shaken well and left for digestion in the incubator at 37° C. for three hours, in the meantime being shaken every half hour, neutralized to sterile litmus paper with normal hydrochloric acid, centrifugalized, and the sediment inoculated into several test-tubes.

The growth from the feces appears much more slowly than that from the sputum. It takes on an average from two to three weeks for the growth to appear. In all probability many of the tubercle bacilli are dead or weakened when passed with the feces.

Of thirty-two specimens studied, nineteen were positive, six were contaminated, and seven were negative. Two of the positive cul-

tures were inoculated into guinea pigs, both of which developed tuberculosis.

SUMMARY.

Sixty-nine positive cultures were obtained from sixty-nine specimens of sputum from practically all stages of tuberculosis. Six of these specimens were negative by direct microscopic examination, but the cultures gave positive findings. These six specimens have been positive for tubercle bacilli at some time.

Nineteen positive cultures were isolated from thirty-two specimens of feces. All these thirty-two specimens, upon direct microscopical examination, gave positive findings, some showing only a few tubercle bacilli. Six specimens were not free from contaminating organisms, and the remaining seven were negative.

The method presented in this paper has proved very simple and accurate for the isolation of tubercle bacilli from sputum. The partial success in isolating tubercle bacilli from feces may be due to the fact that many of the bacilli may be dead.

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