ON CORPER'S NEW CULTURE METHOD FOR THE TUBERCLE BACILLUS.

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THE recent articles by Corper on a new method for the cultivation of the tubercle bacillus have stimulated interest in what has always been a difficult and tedious performance. Since cultural methods hitherto have not been much more delicate in the detection of tuberculosis than the microscopic examination of smear preparations of sputum and tissues, the latter has remained the popular choice, especially in ordinary hospitals. The guinea-pig inoculation method is unhandy and too expensive, and in any case detection of the bacilli in the inoculated animal has to be done by a microscopic smear preparation. The examination of smears of sputum and tissues seldom gives positive results in those cases where doubt exists. A negative result from a microscopic examination of a smear cannot be relied upon.

A new method, therefore, that is delicate and inexpensive and bears favourable comparison with the guinea-pig inoculation is of the greatest service. In mental hospitals the need for an accurate diagnostic method for tuberculosis has long been felt. Many patients either refuse or cannot give an account of their illness, neither can they cooperate in ordinary examinations. These are often the very patients who contract tuberculosis, so that a means of culturing the tubercle bacillus is of utmost importance.

For the past six months we have used Corper's method in this hospital with the greatest success. We have not once failed to cultivate the tubercle bacillus in a sputum that was known to be positive, while we have been successful in cases that were returned negative by the direct smear. The method(1) is as follows:

"The sulphuric-acid potato method for isolating or detecting tubercle bacilli consists essentially in taking a 1 c.c. specimen (whether sputum, urine or tissue), beating it to a homogeneous pulp and introducing it into a sterile 15 c.c. centrifuge tube with 1 c.c. of 6% sulphuric acid (prepared by cautiously adding 17 c.c. of 95–96% sulphuric acid, specific gravity 1·84, to distilled water

https://doi.org/10.1192/bjp.75.311.690 Published online by Cambridge University Press

of 500 c.c. final volume). After thorough mixing, the tube, stoppered with a sterile cork, is incubated at 37° C. for thirty minutes, being shaken frequently during this time, after which the contents are diluted with about 10 c.c. of sterile 0·9% NaCl solution, well mixed and centrifuged. The supernatant fluid is decanted and the residue seeded on the surface of the crystal violet potato medium, the culture-tube being capped with tinfoil after the cotton plug has been impregnated lightly with hot paraffin. The medium is prepared by cutting large clean peeled potatoes, free from surface defects, into cylinders about 3 in. long and $\frac{5}{8}$ in. diameter. The cylinders are halved longitudinally and immediately soaked in 1% sodium carbonate solution containing 1 : 75,000 or ·0013% crystal violet (the dye and sodium carbonate should be mixed just prior to use to prevent decolorizing) for from one to two hours. After this the cylinders are gently wiped off with a clean towel, and are introduced into a sterile culture tube (6 in. by $\frac{3}{4}$ in. in size), containing 1·5 c.c. of 5% glycerol broth, cotton plugged and sterilized in an autoclave at 15 lb. pressure for at least thirty minutes. Excessive or prolonged heating is to be avoided. After incubation on this medium for from two to six weeks a luxuriant elevated growth of tubercle bacilli becomes visible when positive."

A great advantage this method has over Petroff's is the short time taken for the tubercle bacillus to appear. In none of my cases has it been longer than three weeks; in some it has been as short as ten days. This is much in advance even of the guinea-pig inoculation method. Some of the tubes show a luxuriant growth, and for purposes of photography and museum specimens we have secured sub-cultures that are even more luxuriant.

We have also been successful in cultivating from infected supra-clavicular glands, from which carefully prepared smear preparations gave no positive results after long searching. Both tubes inoculated became positive on the twenty-first day. Never before have we detected the tubercle bacillus in such glands. The pus from a discharging hip sinus has also yielded positive results.

Mental hospital patients often refuse to use a sputum cup and swallow all the sputum coughed up. In these cases one must rely on methods devised for the detection of the bacillus in fæces. In this hospital we have used the direct smear method and the ligroin method for twelve months with excellent results. This latter method is used in pulmonary cases who are passing normal stools, and not only in those with diarrhœa of tubercular enteritis. Corper makes no mention of employing his medium for fæces, but we have tried it here with varying results. We recovered the tubercle bacillus from a specimen of fæces which had been mixed with a little

of a tubercular culture. In fæces, however, the contaminating organisms are multitudinous, so that the specimens require much longer treatment with the sulphuric acid. We have not yet had a tube that showed no contaminating organisms, although on a number of occasions isolated colonies of the tubercle bacillus have appeared on the potato. It is certain that slight modifications of the Corper method will give satisfactory results, and at present we are experimenting on these lines.

I am indebted to Dr. Clark, under whose supervision this work has taken place, for permission to publish the results.

Reference.—(1) Corper, H. J., "The Certified Diagnosis of Tuberculosis. Practical Evolution of a New Method for Cultivating Tubercle Bacilli for Diagnostic Purposes," *Journ. Amer. Med. Assoc.*, 1928, xci, No. 6.