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Cultivation of Ducrey Bacillus for Preparation of Vaccine.

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The preparation of Frei antigen for diagnosis of lymphogranuloma inguinale has emphasized the need of diagnostic procedures for ruling out the presence of chancroidal infection. Several culture methods have been described for determining the presence of active infection by the Ducrey bacillus,[1] but a skin sensitivity test (Dmelcos) is necessary for detecting previous or latent chancroidal infection. For the latter test it is desirable to obtain the Ducrey bacillus in pure culture and free from other organic matter.

Several methods have been described for the cultivation of the organism. Usually, a relatively large amount of pus from a "bubo" is added to blood agar, or blood media may be inoculated with a small amount of pus from the primary lesion. Teague and Deibert[2] described the use of clotted rabbit blood inactivated for a short time at 55°C., the organism being identified in pure or mixed cultures by the characteristic growth in chains of very small Gram-negative rods resembling streptococci.

The culture method of Teague and Deibert[2] is not suited for production of saline suspensions of the Ducrey organism, and subculture from blood or pus on the usual solid media was sparse or negative in our hands. Other workers have experienced similar difficulties.[3] Commercial preparations (saline suspensions of killed Ducrey bacillus) are available in Europe, but not generally obtainable in this country at the present time. Hence it was desirable to determine conditions which permit growth of the organism on solid media.

The following method gave satisfactory growth with all strains of Ducrey bacillus encountered in the laboratory of the City Hospital. It was found advantageous to employ tubes of clotted, inactivated blood as described by Teague and Deibert[2] for a preliminary culture medium, since such tubes were conveniently handled in the clinic. The tubes were inoculated with pus from chancroidal lesions and incubated for 24 to 48 hours at 37°C. Those tubes showing pure cultures or nearly pure cultures of Ducrey bacilli were selected for

1 Ducrey, A., *Monatch. f. prakt. Dermat.*, 1889, **9**, 387.

2 Teague, O., and Deibert, O., *J. Urol.*, 1920, **4**, 543.

3 DeWolf, H. F., and Van Cleve, J. V., *J. A. M. A.*, 1932, **99**, 1065.

use for subculture and isolation. A loopful of material from the blood tube was then spread on several blood agar slants (infusion agar as made by Wright,[4] 0.1% glucose and 3 to 5% whole blood), the wall of the test tube opposite the agar slant heated by passing through the Bunsen flame (usually 3 heatings of about one second each), the cotton stopper pushed into the tube and the tube tightly closed by a rubber stopper while the glass was still hot—a method of reducing the oxygen tension, described by Swartz[5] for the cultivation of gonococcus. The blood agar slants usually contained from 0.5 to 1.0 cc. of condensation water in the butt of the tube. Growth appeared promptly in most instances in the closed tube as typical colonies of tenacious consistency, with characteristic long chains of organisms in the water of condensation. Colonies transferred to new tubes grew well and were usually pure cultures. Suspensions were easily prepared by washing the growth from several slants, centrifugating and resuspending in saline. The growth was usually difficult to break into uniform suspensions, but this was accomplished by prolonged shaking, or agitation with a glass rod, or repeated aspiration in and out of a pipette with a narrow lumen at the tip.

The influence of reduced oxygen tension upon growth was marked in most instances. Blood agar slants similarly inoculated showed well developed colonies in the tubes that had been heated and stoppered, and no visible growth in heated but non-stoppered tubes. The growth of every strain was not enhanced to the same extent by this method, but all strains isolated showed some development in the closed tube in 48 hours. The strains isolated were not viable for long periods of time when stored at room temperature or in the ice-box. It was necessary to transfer cultures every 2 or 3 days.

Heat killed saline suspensions were prepared from 2 strains of the Ducrey bacillus and were tested by intracutaneous injections of the vaccines in patients with known chancroidal infection. These patients gave positive reactions with Dmelcos* vaccine,[6] negative reactions with Frei antigen; and Ducrey bacillus had been isolated from their primary lesions. Suspensions of both strains gave strongly positive skin reactions in these patients and no reactions in patients free from Ducrey infection.

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4 Wright, H. D., *J. Path. and Bact.*, 1933, **37**, 257.

5 Swartz, E. O., *J. Urol.*, 1920, **4**, 325.

*Dmelcos vaccine obtained through the kindness of May and Baker, Ltd., London.

6 Stannus, H. S., A Sixth Venereal Disease, Wm. Wood & Co., Baltimore, 1933.