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Bacteriology of Deep Tissue Cultures from Chronic Cervicitis.

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This study of chronic cervicitis deals with the bacteriological examination of organisms in the depths of the ground tissue and also with the histopathological investigation of the sections. Chronic cervicitis is due to a persistent infection in the depths of the compound racemose glands, characterized pathologically by a periglandular round cell infiltration. The cervix, after the primary insult, either from a previous gonorrhoeal infection, the effects of childbirth, or other factors, forms a favorable habitat for the secondary invaders. Menge and Kronig,[1] Arnold and Brody,[2] Barrett, Lash and Pilot,[3] Moench,[4] Pilot and Kantor,[5] and Taylor and Wright[6] are agreed as to the various organisms found on the surface of the pathologic cervical canal; but no mention has been made of the bacterial flora in the depths of the glands.

The cervical tissue was enucleated by Sturmdorf's technique using ether, the electric cautery knife or scalpel. The sterilized enucleated cervix was immediately placed in a small sterile glass jar with a cork stopper and examined within one-half to 2 hours. The culture media was dextrose infusion broth 2%, Bacto dehydrated medium veal infusion pH 7.3, blood agar (human blood 10%), plain agar and egg yolk agar plates. At times 20% ascitic fluid was added to the veal infusion and to the blood agar plates. Ten cc. of veal infusion was used mainly, in test tubes, for inoculation from the surface, and small flasks containing 100 cc. for the ground tissue. Cultured aerobically and anaerobically at 37.5°C., 24 to 48 hours or longer. Smears from cultures were stained by Gram's method. The pyrogallic acid potassium hydrox method in a desiccator was used for anaerobiosis. The terminal hydrogen ion concentration was determined between 5 or 10 days' incubation.

Sugar fermentation reactions of 1% using brom cresol purple

[1] Menge and Kronig, *Bakteriologie des Weblichen Genitalkanales*, 1897, 1.

[2] Arnold and Brody, *J. Lab. and Clin. Med.*, 1925, **10**, 517.

[3] Barrett, Lash and Pilot, *Am. J. Obst. and Gynec.*, 1925, **9**, 797.

[4] Moench, L., *J. Lab. and Clin. Med.*, 1924, **9**, 289.

[5] Kantor and Pilot, *Surg. Gynec. and Obst.*, 1924, **7**, 96.

[6] Taylor and Wright, *J. Obst. and Gynec. Brit. Emp.*, 1930, **37**, 213.

TABLE I.—SUGAR FERMENTATIONS.

Strains	Dextrose	Lactose	Maltose	Litmus milk Coagulated	Mannite	Salicin	Nitrate	Glycerin	Gelatin	Arabinose	Saccharose	Raffinose	Inulin	Duleite	Hemolytic
34	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
42	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
44	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
41	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
47	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
39	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
51	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
57	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
43	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

indicator, were tested by utilizing Gordon's,[7] Andrewes and Horder's,[8] Holman's,[9] and Dible's[10] sugar fermentation classifications.

[7] Gordon, M. H., *Lancet*, 1905, **2**, 1400.

[8] Andrewes and Horder, *Lancet*, 1906, **2**, 708.

[9] Holman, *J. Med. Res.*, 1916, **34**, 388.

[10] Dible, J. H., *J. Path. and Bact.*, 1921, **34**, 3.

Pathogenicity was determined by injecting mice with ¼ cc., ½ cc. and 1 cc. of a 24-hour veal infusion culture. The sediment was observed in 100 cc. plain broth in a small flask. The tissue was ground under aseptic conditions described by Maryan.[11] The outer surface of the tissue was sterilized by searing it through the flame before grinding but thereafter the tissue was sterilized by holding it in boiling water for 15 seconds.

Experiments. From 51 cases, 41 strains of gram positive cocci were isolated and in the remaining 10 cases the cultures were sterile. These organisms were gram positive cocci and are facultative aerobes. Morphologically they occur predominately as lanceolate or oval diplococci surrounded by a halo and in short chains of 3 to 4 cocci but also associated with tetrads and small clusters. They appear pleomorphic. (We have also isolated a number of long chained cocci.) The cocci assume various forms and positions and appear swollen. They have no capsule.

Upon blood agar plates they appear as small moderately elevated pin point to pin head moist grayish white colonies, which grow luxuriantly with no discoloration. Four strains showed large whitish colonies with definite hemolysis, probably hemolytic enterococcus.[12] Upon plain agar they grow similarly with no difficulty. The colonies vary in size from 0.3 mm. to 0.7 mm. in diameter. Upon trans-illuminated light, they appear oval, granular, semi-opaque, and surrounded by a narrow clear zone, just within the outside margin of the colony. The sediment in a test tube of veal infusion appears granular and in streaks radiating downward from the surface. The sediment in a 100 cc. flask of plain broth appears white, stringy and mucoid. These cultures have been kept alive for about 4 months at 37.5°C. They are saccharolytic. From our study of the carbohydrate fermentation, our strains can be classified into a biologically active group, a biologically inactive group, and the variables. They are heat resistant withstanding 60°C. for one-half hour. Out of a series of 72 mice, 12 died, and the same organisms were recovered from the heart in all. The terminal hydrogen ion concentration, by the Hellige method, ranged from pH 4.3 to pH 6.7.

The organisms described correspond morphologically and biologically with the *Streptococcus fecalis* or enterococcus of Dible[11] or the enterococcus described by Arnold.[2] Therefore we call it enterococcus.

[11] Maryan, H. O., *J. Lab. Clin. Med.*, 1930, **16**, 64.

[12] Weatherall and Dible, *J. Path. and Bact.*, 1929, **4**, 413.

The findings of the histopathological sections will be reported at a future date.

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A Simple Continuous Standard for Colorimetric Determination of pH.

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Comparison of an unknown solution containing indicator with standards obtained by superimposing the extreme acid and alkaline colors of the indicator in varying proportions is used in several methods of pH determination. In the simplest of these the standards consist of a series of pairs of test tubes, one tube of each pair containing acid indicator and its mate alkaline indicator.[1] By partitioning a fixed quantity of indicator in different proportions in each pair of tubes a series of color standards is obtained whose pH values have been determined.

The mid-point of such a series is represented by a pair of tubes containing equal concentrations of the indicator. For our purposes they must also contain equal volumes. Starting with such a pair of tubes, if we add to the acid tube from a burette any volume of a solution of the indicator having a concentration twice that in the tube, and from another burette add to the alkaline tube an equal volume of water, the combined color of the tubes may be made to pass continuously through that portion of the indicator range which lies on the acid side of its mid-point. Similarly by adding indicator to the alkaline tube and water to the acid tube the other half of the range may be covered. The original mid-point pair of tubes may be titrated in this manner either to match a given unknown solution or to a color representing any desired pH. The amount of shift of the pH from the mid-point will be equal to log $\frac{A+X}{A}$, when A is one-half of the initial volume in the mid-point tubes, and X is the burette reading. Tables or graphs giving corresponding values of pH difference and burette reading may be constructed. It is apparent that the smaller the initial volumes in the mid-point tubes the more sensitive the end-point will be in the marginal portions of the indicator range.

[1] Gillespie, L. J., *Soil Science*, 1920, **9**, 115. Clark, W. Mansfield, The Determination of Hydrogen Ions, Edition 1928.