7366 P

Dissociation of the Gonococcus.

CLARA RAVEN. (Introduced by A. A. Day.)

From the Department of Bacteriology, Northwestern University Medical School.

Dissociation of the gonococcus has been implied in the work of Herzog[1] and Warden[2] as early as 1913. However, very recently two contemporary schools of thought have appeared in the literature; the French (represented by Ramsine,[3] Milochevich,[4] and Lavrinowicz[5]) and the German (represented by Kandyba[6] and Delbanco-Lorentz[7]) who are convinced that changes in the morphology of the gonococcus exist *in vitro* and *in vivo,* while to the contrary, the Italian school (represented by Porcelli,[8] Sechi,[9] and Debiasi[10]) firmly believes that such changes have very little in common with the gonococcus and are due to errors in technique and interpretation. In view of the conflicting literature the present report, as well as more detailed results to appear later, may prove significant.

Dissociation was attempted and accomplished with 4 stock strains of the gonococcus. During the entire study a modification of Hitchen's semi-solid medium was found satisfactory. Dissociation was produced by ageing and the use of immune serum.

Primary changes through ageing were noted when a sparsely inoculated ascitic agar plate was sealed and incubated for varying periods of time. These changes were evidenced as secondary or papillated colonies superimposed on the original colony or streak. Briefly, the sequence of events was as follows: The secondary growth was yellowish and opaque and when stained showed a bead-like bacillus (as though cocci were strung together to form a closely beaded chain). This form through further ageing and cultivation developed 2 types of growth, namely, a large opaque smooth colony, 3 mm. in diameter, composed of typical biscuit-shaped diplococci

1 Herzog, H., *Virchow's Arch.*, 1913, **212**, 243.
2 Warden, C. C., *J. Inf. Dis.*, 1913, **5**, 12.
3 Ramsine and Milochevich, *Compt. rend. Soc. de Biol.*, 1928, **99**, 1261.
4 Milochevich, *Compt. rend. Soc. de Biol.*, 1929, **100**, 70.
5 Lavrinowicz, *Compt. rend. Soc. de Biol.*, 1925, **93**, 789.
6 Kandyba, *Ztschr. f. Hyg. u. Infektionskrankh.*, 1922, **96**, 347.
7 Delbanco-Lorentz, *Dermat. Woschr.*, 1923, 1137.
8 Porcelli, R., *Gior. ital. di Dermat. e Sifil.*, 1929, **70**, 1255.
9 Sechi, *Gior. ital. di Dermat. e Sifil.*, 1931, **12**, 1045.
10 Debiasi, *Clin. Ostet.*, Rome, 1930, **32**, 665.

but taking the Gram counterstain very deeply and a small colony, 0.5 mm. in diameter, composed of tiny gram negative micrococci.

The influence of immune serum was studied by making serial transplants through semi-solid agar containing immune serum in dilution of 1:50. In brief the results were as follows: Modifications in size were first noted in the fifth tube of the series. The cocci became greatly enlarged, took the counterstain more deeply, and in several instances the tube, next in series to the one composed of giant forms, contained a peculiar gossamer globoid body with tiny coccoid granules both within and without the cell. Most of the pleomorphic changes appeared in the seventh, eighth and ninth tubes of the series. At first the Gram negative coccus was accompanied by a Gram positive coccus, but in later tubes the Gram negative form disappeared, to be displaced by a variety of pleomorphic forms. The colony forms on ascitic agar included a grayish semi-translucent smooth colony with Gram negative diplococci, a gray opaque colony, consisting of giant cocci, and a yellowish opaque colony which contained either Gram positive cocci or granular rods or both cocci and rods. The yellowish opaque colony was the one which gave place to the greatest variety of forms on subculture; these included bacillo-coccoid forms, Gram positive diplococci, polar-body cocci, ring-forms (periphery staining dark and center light), beaded rods, and sometimes a peculiar sheath form which contained within it granules. Reversion to the original form was made possible through subculture in ordinary media occasionally through growth in immune serum, and occasionally by use of cystic or pericardial fluid.

During the course of this study, 3 distinct colony types were noted: Type I, the typical form, represented by a translucent grayish-green, slightly granular to smooth colony, 1 to 2 mm. in diameter, staining as a Gram negative diplococcus, and fermenting dextrose; Type II, represented by a large grayish to white opaque and hardier colony, 1.5 to 3.0 mm. in diameter, staining as a Gram positive or polar body diplococcus, and fermenting dextrose, maltose, sucrose, levulose, and occasionally lactose and other sugars; Type III, represented by a tiny colony (similar to Hadley's "G" colonies), 0.5 to 0.8 mm. in diameter, very rough, flat and adherent to the surface of the agar, growing sparsely, and consisting of a fusiform Gram negative granular bacillus (2.0 to 2.5 micron in length) and fermenting no sugars. Between these were interspersed other forms, some with chromogenic properties and varying in morphology such as beaded rods, dumbbell shapes, polar body diplococci, ring forms, globoid phantom forms, and tiny Gram negative micrococci. The pleo-

morphic forms and particularly the bacillary forms were least stable, while the Gram positive diplococcus and the large opaque colony form was most stable.

In regard to the fermentation of sugars, it was noted that the typical Gram negative diplococcus fermented dextrose; the extreme "R" or tiny colony form failed to ferment sugars, but as dissociation progressed, first dextrose alone, and then maltose, sucrose, levulose and lactose were utilized. The Gram negative micrococcus failed to ferment carbohydrates, but when the typical form was attained fermented dextrose. Therefore, reversion was accompanied by return to fermentation type.

The agglutination for the homologous and typical cyclostage attained a titer of 1:5120, while the titer for the opaque form (polar-body and Gram positive diplococcus) was 1:160 to 1:320. It was difficult to obtain a homogeneous suspension for the "R" form or the tiny Gram negative micrococcus, so that no determinations were made for these stages.

7367 C

Accumulation of Bacteriophage in Spleen and Liver Following Its Intravenous Inoculation.

W. J. NUNGESTER AND R. M. WATROUS.

From the Department of Bacteriology, Northwestern University Medical School.

The rôle of the reticulo-endothelial system in removing microorganisms from the blood stream has been generally recognized in recent years. The earlier work of Bull[1] in this field has been supported and extended by others, particularly Cannon[2, 3] and his coworkers. The question arose as to whether or not a readily filterable biological principle such as bacteriophage, which is probably particulate and of the general order of magnitude of the filterable viruses, would be removed from the blood stream by organs of the reticulo-endothelial system to a greater degree than by organs with few fixed phagocytic cells.

[1] Bull, Carroll G., *J. Exp. Med.*, 1915, **22**, 475, 484.

[2] Cannon, Paul R., Sullivan, F. L., and Neckermann, E. F., *J. Exp. Med.*, 1932, **55**, 121.

[3] Sullivan, F. L., Neckermann, E. F., and Cannon, Paul R., *J. Immunol.*, 1934, **26**, 49.