THE DEMONSTRATION OF BACTERIAL CAPSULES BY FONTANA'S STAINING PROCEDURE

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It has been discovered that, in addition to bacterial cells being stained a very dark brown or black color by Fontana's procedure, bacterial capsules are differentially stained a light brown color.

Fontana (Dermatol. Wochschr., 55, 1003, 1912 and Pathologica, Genova, 4, 582, 1912) described a method for staining Treponema pallidum and other spirochetes by the AgNO₃-NH₄OH method. A similar procedure was described by Tribondeau (Bull. soc. franc. dermatol. syphilig., 23, 474, 1912). The desirable features of both procedures appear to have been combined by Fontana (Pathologica, Genova, 5, 205, 1913) into the procedure which bears his name or sometimes that of Fontana-Tribondeau. Of 12 different staining techniques tried by Gilbert and Bartels (J. Lab. Clin. Med., 9, 273, 1923) on smears of syphilitic material, Fontana's stain demonstrated treponemae in the greatest number of smears. Modifications of the technique have been described by numerous authors; the more recent have been Rosahn and Freeman (Am. J. Syphilis, Gonorrhea, and Venereal Diseases, 36, 244, 1952). Throughout the years Fontana's technique or some modifica-

Figure 1. The demonstration of capsules by means of Fontana's stain. A. Coccioidoides immitis, B. Diplococcus pneumoniae, C and D, Klebsiella pneumoniae. Magnification, A, B, and C, 2,050 X; D, 3,465 X.
tion of it has demonstrated its worth for staining spirochetes and bacteria. The reduced silver is deposited on the surface of the organism, and under the microscope the organisms appear black or dark brown in color. After our observations Järvi and Levanto (Acta Pathol. Microbiol. Scand., 27, 473, 1950) reported the use of ammoniacal silver nitrate solution for detecting bacterial polycaccharides.

_Coccidioides immitis_ from mouse brain, _Diplococcus pneumoniae_ from mouse heart’s blood, and _Klebsiella pneumoniae_ recently passed through mice were employed for encapsulated organisms. Various nonencapsulated organisms were employed as controls. Smears were air dried and fixed with 3 or 4 changes of solution I (glacial acetic acid, 1 ml; formalin, 20 ml; water, 100 ml) of about one minute each. The slides then were flooded with 95 per cent alcohol, drained, and flamed. Subsequently, the smears were covered with solution II (tannic acid, 5 g; phenol, 1 g; water, 100 ml) warmed until steam arose and continued steaming for 30 seconds and washed with water. Then the smears were covered with solution III (5 per cent ammoniacal silver nitrate solution), warmed until steam arose and continued for 30 seconds, washed with water and dried. The results with encapsulated organisms are shown in figure 1. The capsules on _K. pneumoniae_ have been especially pronounced.

**THE MODIFYING EFFECT OF PHENOLIZED SALINE ON THE AGGLUTINATION OF A STRAIN OF _KLEBSIELLA PNEUMONIAE_**

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In the course of immunological studies on a type A _Klebsiella pneumoniae_, strain 108M, it was found that fresh suspensions of this strain prepared by harvesting 24 hour cells from beef extract agar slants into 0.5 per cent phenolized saline solution not only were agglutinated by type specific antiserum, but also were agglutinated by antiserum against a nonencapsulated (S) variant of this strain. Agglutination in S antiserum was flocculent in type. Previously, formalinized saline suspensions of strain 108M had been found to fail to be agglutinated by these S antiseras.

Supplementary experiments yielded the following results: Strain 108M cells cultured on beef extract agar and suspended in 0.5 per cent formalinized saline solution or in unpreserved saline solution were in no case agglutinated by the S antiseras. If, instead of beef extract agar, richer media such as tryptone-glucose agar or horse infusion agar were employed for cell growth, no agglutination by S antiseras occurred whether the cells were suspended in phenolized, formalinized, or unpreserved saline solution.

Thus, it appears that the phenol had a partial decapsulating effect on the cells, either dissolving part of the capsule or rendering the capsule more porous, making possible reaction between the cellular antigens and the somatic agglutinins. It seems probable that the inagglutinability of phenolized 108M suspensions prepared from cultures grown on richer media was due to the development by this strain of a heavier capsule when grown on these media, and that these more heavily encapsulated cells were relatively insensitive to the action of the phenol. In support of this explanation, the growth of strain 108M was more mucoid on the richer media. Hoogerheide (J. Bact., 38, 367, 1939) reported the size of the capsule of _Klebsiella pneumoniae_ to vary markedly on different culture media.