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Action of Sporogenic Aerobic Bacilli on Living and Dead Bacteria.

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The antagonistic action of sporogenic aerobic bacilli against various bacteria *in vitro* was studied by Nicolle,¹ Pringsheim,² Much and Sartorius,³ myself,⁴ and others. Recently Dubos⁵ isolated from a sporogenic aerobic soil bacillus a very potent extract capable of destroying Gram-positive bacteria but not affecting Gram-negative.

The purpose of this investigation was to compare the action of sporogenic bacilli on living with that on dead bacteria. The following technic was used.

Thirty-one strains of the *subtilis-mesentericus* group which were isolated in our laboratory at various times were tried against the following test-bacteria: 7 Gram-positive species—*Staphylococcus aureus*, *Sarcina flava*, air coccus, *Pneumococcus* type I, *Streptococcus hemolyticus*, *Streptococcus viridans*, *B. diphtheriae*; and 7 Gram-negative species—*B. coli*, *B. typhi*, *B. paratyphi* B, *B. dysenteriae* Shiga, *B. prodigiosus*, *B. proteus* × 19, *B. pyocyaneus*. The growth of 24-hour living agar culture of a test-bacterium was suspended in 5 cc of saline and one drop of the suspension was evenly spread with a swab over the surface of an agar plate. In the case of *S. hemolyticus*, *S. viridans* and *Pneumococcus*, the sediment of a 24-hour culture in tryptose phosphate broth (Difco) was used for inoculating the surface of a serum agar plate. Immediately thereafter, upon the surface of this implanted plate, was made a pin-point inoculation of the sporogenic strain. After 24 hours of incubation at 37°C the plates were examined. A clear transparent zone surrounding the colony of the sporogenic strain and contrasting with the good growth of the test bacteria over the remainder of the plate gave evidence of inhibition of growth. The action of sporogenic strains on dead bacteria was studied by means of plates of agar which were mixed with a thick suspension in saline of a culture of test bacteria boiled

¹ Nicolle, M., *Annales de l'Inst. Past.*, 1907, **21**, 613.

² Pringsheim, E. G., *Centralb. Bakt. u. Par.*, 2 *Abt.*, 1920, **51**, 72.

³ Much, H. u., Sartorius, F., *Med. Klin.*, 1924, **20**, 347.

⁴ Rosenthal, L., *Compt. Rend. Soc. biol.*, 1925, **92**, 7; *ibid.*, 472; *ibid.*, **93**, 1569; *ibid.*, 1926, **95**, 10.

⁵ Dubos, R. L., *J. Exp. Med.*, 1939, **70**, 17, 256; *Ann. Int. Med.*, 1940, **13**, 2025.

for 5 minutes. On the surface of such a turbid agar plate were made pin-point inoculations of various sporogenic strains. The formation, after 24 hours of incubation, of clear transparent zones around the sporogenic colonies gave evidence of lysis, and absence of clarification of the turbid agar showed that the dead bacteria were not affected by the corresponding sporogenic strain. With living bacteria the following results were obtained: Of the 31 sporogenic strains 7 were found to be inactive both for Gram-positive and Gram-negative species. Of the remaining 24 strains, 6 were active only against some Gram-positive bacteria but failed entirely to affect the Gram-negative group, and, finally, 18 affected organisms of both groups. In the Gram-positive group: *Sarcina flava* was inhibited in its growth by 24 strains, *Staphylococcus aureus* by 21 strains, *Air Coccus* by 21 strains, *B. diphtheriae* by 19 strains, *Pneumococcus* Type I by 11 strains, *Streptococcus hemolyticus* by 11 strains, *Streptococcus viridans* by 9 strains; in the Gram-negative group: *B. dysenteriae Shiga* by 14 strains, *B. prodigiosus* by 14 strains, *B. typhi* by 12 strains, *B. proteus* by 11 strains, *B. coli* by 3 strains, *B. paratyphi B* by 2 strains, *B. pyocyaneus* by no strains. In experiments with dead bacteria all the 31 sporogenic strains, including the 6 which were found to be deprived of any antagonistic properties against living bacteria, produced lysis of all 7 Gram-negative organisms, but failed to affect in any visible way the bacteria of the Gram-positive group.

Conclusion. The tested living Gram-positive organisms were more susceptible to the antagonistic action of sporogenic aerobic bacilli than the living Gram-negative. In contrast, all tested dead Gram-negative bacteria were susceptible to the action of all sporogenic strains, while dead Gram-positive remained unaffected.

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Biotin Synthesis by Microorganisms.

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It has been postulated that many identified growth factors, and others still unrecognized, are required by microorganisms in general, and that many types of organisms do not require these growth factors