

incident to the method of feeding, have emphasized the production of denticles in the incisors which has been noted by Wolbach and Howe³ in the more acute condition.

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Nature of the Anticlotting Activity of Streptococci *in vitro*.

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While studying factors affecting the formation of the fibrinous inflammatory barrier in acute local streptococcal inflammation, Dennis and Berberian¹ confirmed the observations of Tillett and Garner² on streptococcal fibrinolysin, and described the presence of an anticoagulant in filtrates of certain strains of hemolytic streptococci and of virulent viridans streptococci. Although certain strains of erysipelas streptococci produced both fibrinolytic and anticoagulant factors, sound evidence was offered that we were dealing with 2 distinctly different substances. The differences were: fibrinolysin was never produced by *Strep. viridans*; fibrinolysin acted only upon human plasma clot, while the anticoagulant prevented the clotting of both human and rabbit recalcified oxalated plasma; and fibrinolysin was thermolabile while the anticoagulant was thermostable. Anticlotting factor masked the action of fibrinolysin whenever the two occurred together. Tunnicliff³ confirmed our observations and correlated the anticlotting activity with the smooth (S) phase of greening streptococci. Neter and Witebsky,⁴ apparently unaware of our earlier observations, recently reported on the anticoagulant activity of streptococcal filtrates, but failed to recognize the fundamental difference between anticoagulant and fibrinolytic phenomena.

During the past 3 years we have extended our observations on

* Observations on purification of the anticlotting were made by one of us (E. W. D.) during tenure of a Rockefeller Research Fellowship in Bacteriology, at Harvard Medical School, 1934-35.

¹ Dennis, E. W., and Berberian, D. A., *J. Exp. Med.*, 1934, **60**, 581.

² Tillett, W. S., and Garner, R. L., *ibid.*, 1933, **58**, 485.

³ Tunnicliff, R., *J. Infect. Diseases*, 1936, **58**, 92.

⁴ Neter, E., and Witebsky, E., *PROC. SOC. EXP. BIOL. AND MED.*, 1936, **34**, 549, 858.

the nature of the anticlotting activity, using 45 strains of *Strep. hemolyticus* and 18 strains of *Strep. viridans*. We have used the original method of Tillett and Garner² throughout for the demonstration of fibrinolytic and anticlotting action.

Using filtrate of an E₁ strain of *S. hemolyticus* which was both fibrinolytic and anticoagulant, and various *S. viridans* filtrates which were anticoagulant only, an effort was made to isolate and identify the anticlotting factor. It was found that the anticoagulant is soluble in 75% alcohol, absolute alcohol, and ether. It is readily dialyzable when in a relatively pure state, and gives a strongly positive Kelling's test for lactic acid. We have concluded that the anticoagulant factor of streptococcal cultures is primarily lactic acid, perhaps with a minor admixture of other organic acids.

Having determined that it is not the lactate ion which is responsible for inhibition of coagulation, the relationship between anticlotting action and acid content of streptococcal filtrate was then carefully studied. Manifestation of anticlotting action is dependent upon (a) the amount of fermentable sugar in the broth, and (b) the degree of buffering of the medium. Anticlotting action was seldom shown if the medium contained less than 0.4% dextrose. Observations on the relative importance of pH of the filtrate and total acid content showed that the anticlotting activity is correlated with the total acid rather than any particular pH; the acidity of the plasma-clot test mixture is the determining factor.

We were not able to establish definite relationship between anticlotting activity and the dissociative phase of the organism, since certain apparently rough strains inhibited coagulation. However, rapid subculturing, with increased "smoothness" of the culture, was always paralleled by increased acid content and increased anticlotting activity of the filtrate.

The recent report by Dart⁵ on the solubility of the anticoagulant in alcohol, and on its thermostability, is entirely in harmony with our earlier observations and the results reported above.

⁵ Dart, E. E., *ibid.*, 1936, **35**, 285.