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Fibrinolysis of Hemolytic Streptococci and Their Variability.

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Our interest in the above correlation takes origin as follows: For the past 8 or 9 years one of us (Mellon) has studied with encouraging results a new serum approach to the therapeusis of hemolytic streptococcal infections. Treatment of the cases has been conducted exclusively in this hospital. Accompanying the serum's use we have frequently observed an *in vivo* dissociation of smooth (or smooth-mucoid) cultures into more saprophytic colony types—sometimes characterized by rough-colonied hemolytic streptococci, sometimes by typical non-hemolytic diphtheroids, wholly avirulent.

Of special significance is the fact that such dissociation seems to be a direct sequence of a clinical crisis on the part of the severe cases, whose ultimate recovery is thereafter no longer in doubt. Obviously, a serum approach that would transform virulent organisms into their non-virulent phases, is rather different from the conventional bactericidal or antitoxic ones.

Only an occasional observer (Hadfield, *et al.*[1]) in the field of fibrinolysis has directed attention to a correlation of virulence (as indicated by colony type) and fibrinolysis. Recently Tunnicliff[2] has shown correlation in a general way between R and S streptococci with respect to the increasing lysis and virulence as one proceeds from R to S. Reich[3] has shown that serial animal passage transformed a fibrinolytic strain of human origin into a non-fibrinolytic one suggesting a bovine origin; (Lancefield classification[4]), and which on serial subculture returned to its original fibrinolytic state. Accordingly, we have wondered if this correlation might extend to certain variants such as the above; more particularly to diphtheroids *whose in vivo dissociation from the streptococci had been confirmed by their occurrence in vitro, under controlled conditions.*

As an example of such a strain we wish to direct attention to a small-colonied hemolytic streptococcus from a treated case of cellulitis (Stoddard, small, No. 1—See Table). From a large-colonied hemolytic dissociant of this strain, typical non-hemolytic diphthe-

1 Hadfield, G., Magee, V., and Perry, C. B., *Lancet*, 1934, **1**, 834.

2 Tunnicliff, R., *Arch. Path., Soc. Trans.*, 1935, **20**, 811.

3 Reich, T., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 639.

4 Lancefield, R. C., *J. Exp. Med.*, 1933, **57**, 571.

TABLE I.

The fibrinolytic activity of these cultures and of a strongly fibrinolytic control (Bloom, No. 9) was determined by the method of Tillett and Garner[5] against the plasma of a presumably normal individual. The figures in the horizontal columns represent the time of lysis of determinations run at different times. Figures in parenthesis show % of fibrinolysis at the end of 24 hours.

Strain No.	Culture and Source	Time (Given in minutes, unless otherwise indicated)						
1	Stoddard, small colonies. Cellulitis	90	35	40	60	105	60	50
2	Stoddard, large colonies. Diphtheroid variant		0	(10)	0	0	(10)	0
3	Stoddard, H. Large colonies. Variant	(90)	(90)	13 hr.	(90)	(90)	<22 hr.	<22 hr.
4	Bovine III. Diphtheroid TBC variant	(50)	(50)	(+)	(50)	(25)	(50)	(50)
5	Sporoid. Avian TBC variant	(75)	(75)	(90)	(50)	(75)	(75)	(50)
6	H. Streptococcus (Miller). Sepsis	75	90	120	75	75	105	105
7	Streptococcus (Miller), non-hemolytic variant	60	75	90	75	90	90	60
8	Diphtheroid (Miller). Variant	(25)	(10)	(50)	(25)	(25)	(25)	(+)
9 (Control)	H. Streptococcus (Bloom). Sepsis	8	30	30	9	10	22	60

roid bacilli, producing a still larger colony have been derived. (Stoddard, No. 2). They in turn have reverted to the original hemolytic, small-colony type, as well as to an intermediate type; that is to say, to a hemolytic diphtheroid of large colony type (Stoddard H, No. 3). These dissociants are being studied by Philip Hadley, who will report on them in detail in a future communication. Reference to the table will show that Strain No. 3 is intermediate in its fibrinolytic activity, when compared to the actively lysing small (virulent) colony and the rather inactively lysing diphtheroid colony (non-virulent).

Similar results were obtained with the dissociants of another culture known as the "Miller" strain, which was isolated from the blood of a patient in pure culture in 1931. This hemolytic streptococcus (No. 6) was dissociated *in vitro* on 6 separate occasions into a non-hemolytic diphtheroid (No. 8). The latter was reverted to a streptococcus resembling the original, *but lacking hemolysis* (No. 7). Nevertheless, it was identical fibrinolytically with the hemolytic strain; and also serologically by the agglutinin-adsorption test, proving rather conclusively that the diphtheroid (No. 8) was not a contaminant. The latter did not cross-agglutinate and its lytic pow-

ers, although definite, are negligible in comparison with those of the streptococcus. Although the examples given in the table are but a fraction of the diphtheroid strains studied, the low degree of fibrinolysis is characteristic for many of them.

The diphtheroids with acid-fast granules, Bovine III (No. 4), and Avian sporoid (No. 5), are known to be in the tubercle cycle, by reason of the inter-transformability existing between them and the tubercle group. Yet they are indistinguishable in their fibrinolytic ability from diphtheroids dissociated from streptococci. A greening streptococcus (Hamilton strain, not in table), and a diphtheroid dissociating from it, both gave the same reaction as the Avian sporoid No. 5. These results are not strictly in accord with the work of Tillett and Garner,[5] *et al.*, who limited the phenomenon of fibrinolysis to hemolytic streptococci and an occasional staphylococcus.

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Nonspecificity of the Chloride Impoverishing Mechanism of Small Intestine.

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Burns and Visscher[1] have shown that an isotonic solution of a mixture of sodium chloride and some other sodium salt with an indiffusible anion when placed in a loop of the small intestine becomes practically chloride-free in 1½ hours. This movement of sodium chloride, against a diffusion gradient into the blood through the intervention of the intestinal epithelium, resembles the removal of chloride by the kidney tubules from the glomerular filtrate. The intestinal preparation affords a convenient method of studying salt movement which may be of general importance.

As a step in elucidating this mechanism it was decided that the specificity of mechanism should be determined. Sodium bromide was chosen as an example of a salt similar chemically to sodium chloride but not a usual constituent of body fluid. It is the purpose of these experiments to see if, in the presence of an indiffusible

[5] Tillett, W. S., and Garner, R. L., *J. Exp. Med.*, 1933, **58**, 485.

[1] Burns, H. S., and Visscher, M. B., *Am. J. Physiol.*, 1934, **110**, 490.