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Fibrinolytic Action of Gas Gangrene Anaerobes.*

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Tillett's[1] review of fibrinolysis indicates the importance of the reaction in hemolytic *Streptococcus* infections. It also makes it apparent that various other species of bacteria produce enzymes or toxins which, though lacking some of the characteristics of *Streptococcus* fibrinolytic substance, tend to break down coagulated plasma and therefore influence the course of infection to a significant degree.

The very rapid spread of gas gangrene infections in man and experimental animals suggests that fibrinolysis may be involved. Accordingly a series of preliminary trials has been carried out with the more important species associated with gas gangrene in man. This included some 77 cultures belonging to eleven species, as listed in Table I.

Cultures. The cultures used were the same as those recently described by Reed and Orr.[2] Purity of the cultures was established by repeated plating shortly before use. All gave characteristic reactions according to the identification procedure as described in the last-mentioned paper. A few cultures were recently isolated from gas gangrene in man, a few were isolated from experimental gas gangrene in guinea pigs, Reed and Orr,[3] the majority were laboratory cultures which had not been through animal passage for long periods.

Fibrinolytic Procedure. The fibrinolytic test procedure was that used by Tillett and Garner[4] in the case of *Streptococcus.* Fresh oxalated plasma was obtained from man, rabbit, guinea pig and sheep. One milliliter of 1:5 dilution of oxalated plasma was mixed with 0.5 ml of culture, 0.35 ml of 0.25% calcium chloride added (0.25 ml for human plasma) the tubes well shaken and placed in a 37° water bath. Fibrinolysis readings were made at half-hour intervals, after coagulation, for the first 7 hours and again after 24 hours.

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1 Tillett, W. S., *Bact. Rev.*, 1938, **2**, 161.

2 Reed, G. B., and Orr, J. H., *War Medicine*, 1941, in press.

3 Reed, G. B., and Orr, J. H., *Lancet*, 1941, March 22, 376.

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

TABLE I.
Fibrinolytic Activity of Gas Gangrene Bacteria.

Species	No. of cultures tested	Plasma	% of cultures producing solution of clot in	
			7 hr or less	24 hr or less
Cl. welchii	26	human	17	83
	33	rabbit	62	67
	5	guinea pig	80	100
	5	sheep	0	0
Cl. novyi	3	human	0	66
	3	rabbit	67	67
	3	guinea pig	0	67
	3	sheep	33	33
Cl. septicum	5	human	0	100
	5	rabbit	0	100
	3	guinea pig	100	100
	3	sheep	0	100
Cl. sordellii	4	human	0	80
	4	rabbit	0	0
	3	guinea pig	0	100
	3	sheep	0	0
Cl. fallax	1	human	0	0
	3	rabbit	0	0
	2	guinea pig	0	0
	2	sheep	0	0
Cl. tertium	1	human	0	0
	5	rabbit	0	0
	2	guinea pig	0	0
	2	sheep	0	0
Cl. chauvoei	2	human	0	50
	6	rabbit	0	17
	3	guinea pig	33	33
	3	sheep	0	33
Cl. aerofoetidum	1	rabbit	0	0
	1	guinea pig	0	0
	1	sheep	0	0
Cl. histolyticum	4	human	25	100
	7	rabbit	86	86
	4	guinea pig	75	100
	4	sheep	25	50
Cl. sporogenes	4	human	0	100
	8	rabbit	63	88
	3	guinea pig	0	100
	3	sheep	0	33
Cl. tyrosinogenes	1	human	100	100
	2	rabbit	0	100
	1	guinea pig	100	100
	1	sheep	100	100

Several media provided suitable cultures. Brewer's medium with 0.1% glucose was first used but it was found that continuous cultivation in this medium resulted in a gradual decrease in fibrinolytic power. Most consistent results were obtained with the supernatant fluid from 18-hour cultures in chopped meat medium prepared according to the method of Lepper and Martin.[5] This medium was used in all cases reported in the paper.

Fibrinolysis Results. Table I summarizes the results with the several species in plasma from man, rabbit, guinea pig and sheep. The figures in the table indicate the percentage of cultures tested which break down coagulated plasma in 7 hours or less and in 24 hours or less.

It is apparent from the table that the 4 species principally concerned in gas gangrene, *i. e., Cl. welchii, Cl. novyi, Cl. septicum* and *Cl. sordellii* produce active fibrinolysis. Guinea pig and rabbit plasma clots are slightly more readily broken down than human plasma while sheep plasma clots resisted action of *Cl. welchii* and sheep and rabbit plasma resisted *Cl. sordellii.* More than half the cultures of *Cl. chauvoei* were inactive on all 4 plasma.

It should be noted that cultures which break down plasma clots in 24 hours are regarded as fibrinolytically active. A considerable number produce complete solution of the clots, as shown in the table, in 7 hours or less. A few produce complete solution in one to 1½ hours. The most active were cultures of *Cl. welchii* recently isolated from acute cases of gas gangrene in man.

Three species occasionally found in gas gangrene wounds, *Cl. fallax, Cl. tertium, Cl. aerofoetidum* were completely inactive in all 4 plasma. These species produce little or no soluble toxin.

Three proteolytic species, *Cl. histolyticum, Cl. sporogenes* and *Cl. tyrosinogenes* were very active in the break-down of all 4 plasma. The former produces a low yield of toxin, the latter 2 no toxin.

The extent to which the fibrinolytic substances produced by these anaerobic species resemble the *Streptococcus* enzyme remains to be determined. It is quite possible, moreover, that the activity of the saccharolytic, toxin-forming species results from one type of enzyme and that the proteolytic, non-toxin-forming species break down plasma clots through the activity of a very different system. Regardless, however, of the resemblance of this digestive mechanism to that present in other species or the possible dissimilarity of mechanism in different groups of anaerobes, it is apparent that the gas gangrene species do rapidly destroy coagulated plasma. It is therefore

[5] Lepper, E., and Martin, C. J., *Brit. J. Exp. Path.*, 1924, **10**, 327.

highly probable that these reactions contribute to the spread of gas gangrene infections.

Anti-coagulation Factor. A few of the cultures from each fibrinolytic species regularly prevented clotting of plasma on the addition of $CaCl_2$; and a few additional cultures occasionally prevented clotting. This condition was observed with a few cultures of *Cl. welchii, Cl. novyi, Cl. septicum* and even with a few of the proteolytic species—*Cl. histolyticum* and *Cl. sporogenes.* A similar anti-coagulating factor has been observed by Dennis and Adham,[6] Tillett,[7] Christensen,[8] and others in certain cultures of hemolytic *Streptococcus.* Tillett[7] considers this to result primarily from pH changes. Where the reaction is more acid than pH 5.8, plasma coagulation is inhibited. In these 18-hour cultures of anaerobes in chopped meat the reaction never exceeds pH 6.0 and generally is from pH 6.2 to 6.4. Dennis and Adham,[6] on the other hand, consider that the anti-coagulation factor in *Streptococcus* cultures is a particular fatty acid which may be independent of pH.

Conclusion. 1. It is shown that most strains of the more important saccharolytic, toxin-forming gas gangrene species, *Cl. welchii, Cl. novyi, Cl. septicum* and *Cl. sordellii,* produce an active fibrinolytic substance. Certain other saccharolytic species, *Cl. tertium, Cl. fallax, Cl. aerofoetidum* fail to produce this substance. 2. The more common proteolytic gas gangrene species, *Cl. histolyticum, Cl. sporogenes* and *Cl. tyrosinogenes* produce an equally or more rapid destruction of coagulated plasma. 3. Most cultures which are active against plasma from one species will break coagulated human, guinea pig and rabbit plasma. Sheep plasma is less readily attacked and not attacked by any strains of *Cl. welchii* tested. 4. A few strains in both groups of gas gangrene anaerobes produce anti-coagulation factors which prevent $CaCl_2$ coagulation of plasma. It is shown that this is not a pH effect.

[6] Dennis, C. W., and Adham, L. D., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 84.

[7] Tillett, W. S., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 77.

[8] Christensen, L. R., *J. Inf. Dis.*, 1940, **66**, 278.