average of 80 to 85% of the total 24-hour excretion occurred during the first 12 hours after administration of the test dose. When the latter was given intravenously, maximum excretion occurred during the 1st and 2nd hours. After oral administration, maximum excretion occurred during the 3rd, 4th, 5th, and 6th hours.

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Properties of Different Fibrinolysins Produced by Streptococci.

E. WITEBSKY AND E. NETER.

From the Bacteriological and Serological Laboratories of the Beth Israel Hospital, New York City.

Tillett and Garner[1] reported that hemolytic streptococci and some strains of staphylococci produce a fibrinolytic substance; this observation was corroborated by Schmidt.[2] But, Tunnicliff[3] found some strains of *S. viridans* to be fibrinolytic, and Madison[4] reported fibrinolysin-production by *B. pestis*. In a previous paper[5] the production of fibrinolysin by *S. hemolyticus* and *viridans, enterococcus, pneumococcus* and others was described. The discrepancy in these results may be explained by differences in the culture media employed: When 0.05% glucose-broth or 5% serum-broth was used, hemolytic streptococci only produced the fibrinolysin; when cultivated in 2% glucose-broth, however, various kinds of microörganisms proved to be fibrinolytic. The experiments to be reported are concerned with the question whether the fibrinolysins obtained in these media have different properties.

Technic. Infusion broths, containing 1% peptone, 0.05% and 2% glucose, respectively, with pH 7.4, were employed. Strains from human sources were incubated for 18 hours at 37° C. The supernatant fluid was tested for fibrinolytic activity according to the usual technic.[1]

Plasma was obtained by mixing 10 cc. of normal human blood with 1 cc. of 2% solution of potassium oxalate; it was stored in refrigeration not longer than 48 hours; supernatant fluid of a cul-

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

2 Schmidt, H., *Zeitschrift für Immunitaetsforschung*, 1936, **87**, 1.

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

4 Madison, R. R., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 301.

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

TABLE I.
Quantitative Titration of Fibrinolysins of *S. Hemolyticus*.

Decreasing amounts of supernatant fluid (volume 0.5 cc.)	Strain 693 in 2% glucose-broth				Strain 2327 in 0.05% glucose-broth			
	a	b	c	d	a	b	c	d
1. 0.5	++++	++++	++++	++++	—	++	++++	++++
2. 0.166	—	—	—	—	—	++++	++++	++++
3. 0.055	—	—	—	—	—	++++	++++	++++
4. 0.019	—	—	—	—	—	++	++++	++++
5. 0.006	—	—	—	—	—	—	+++	++++
6. 0.002	—	—	—	—	—	—	—	++++
7. 0	—	—	—	—	—	—	—	—

Read after a—10 minutes 37° C.
b—40 " "
c—90 " "
d—24 hours "
+ - ++++ = various degrees of fibrinolysis.
— = no fibrinolysis (coagulation).

ture (0.5 cc.) was mixed with 1 cc. of 1:5 dilution of human plasma; then 0.25 cc. of a 0.25% solution of calcium chloride in normal saline was added. The mixtures were shaken thoroughly, kept at 37° C., and the results read at various intervals.

Results. In the first experiment, 2 different strains of *S. hemolyticus,* one producing the fibrinolysin in 2% glucose-broth* but not in 0.05% glucose-broth, and the other in 0.05% glucose-broth, were incubated in 2% glucose-broth and 0.05% glucose-broth, respectively, for 18 hours at 37° C. The supernatant fluids of these cultures, in decreasing amounts (volume 0.5 cc.), were tested for fibrinolytic activity.

Table I shows that the 2% glucose-broth culture of strain 693 is fibrinolytic only when used undiluted, and that a dilution of 1:3 is ineffective; the 0.05% glucose-broth of strain 2327 is fibrinolytic up to a dilution of 1:81 after 90 minutes, and up to 1:243 after 24 hours. Further, strain 693, in 2% glucose-broth, inhibits the coagulation of plasma constantly, while with strain 2327, in 0.05% glucose-broth, clotting of the plasma occurs first, and lysis then follows.

Many strains of hemolytic streptococci produce fibrinolysin in 0.05% as well as in 2% glucose-broth. When such strains were tested for fibrinolytic activity, it was found that both produce potent fibrinolysin and cause secondary lysis of previously clotted plasma. The fibrinolysin produced by such strains in both media belongs to the 0.05% glucose-broth type.

Because Tillett[6] found that the fibrinolysin produced by hemolytic streptococci of human origin in 0.05% glucose-broth is effective toward human plasma only, it was of interest to determine whether the specificity for human plasma also applied to the 2% glucose-broth fibrinolysin. For this purpose, 2 strains of hemolytic streptococci—one (693) producing fibrinolysin in 2% glucose-broth only, the other (1755) in 0.05% glucose-broth—were incubated in the respective glucose-broths for 18 hours at 37° C. The supernatant fluid of these cultures was tested for fibrinolytic activity toward human as well as rabbit and guinea-pig plasma. Strain 693, in 2% glucose-broth, inhibited the coagulation of human, rabbit, and guinea-pig plasma. Strain 1755 in 0.05% glucose-broth dissolved human but not rabbit or guinea-pig clot. Strains of hem-

* The 2% glucose-broth was used as representative of broths with higher concentrations of glucose; as reported previously,[5] it is possible to reduce the glucose-concentration to 0.4% and still obtain fibrinolysin from these strains.

[6] Tillett, W. S., *J. Bact.*, 1935, **29**, 111.

olytic streptococci capable of producing fibrinolysin in 2% as well as in the 0.05% glucose-broth were found to be lytic for human plasma clots only.

The fibrinolysin produced in 2% glucose-broth only, is not altered by being heated at 60° C. for an hour. The fibrinolysin of the 0.05% glucose-broth is also heat-stabile; this corroborates the findings of Garner and Tillett.[7] On the other hand, according to Schmidt,[3] the fibrinolysin obtained in 5% horse-serum broth is almost completely destroyed by this procedure. These results suggest the existence of even more than 2 types of fibrinolytic agents of bacterial origin.

Tillett and Garner,[1] Van Deventer,[8] and Schmidt[2] demonstrated that the fibrinolysins produced by hemolytic streptococci in 0.05% glucose-broth and 5% horse-serum broth may be neutralized by certain streptococcal antisera. Experiments were therefore undertaken to ascertain the influence of streptococcal antiserum upon the 2% glucose-broth fibrinolysin of hemolytic streptococci.

Two different strains of *S. hemolyticus*—one producing the fibrinolysin in 2% glucose-broth only, the other in 0.05% glucose-broth—were cultivated in the respective glucose-broths. A preliminary titration was performed with the supernatant fluids. In this experiment the 2% glucose-broth culture was used undiluted, the 0.05% in a dilution of 1:10. Both broths (volume 0.5 cc.) were mixed with decreasing amounts (0.5 cc.) of normal human spinal fluid, normal horse-serum and streptococcal antiserum;† the mixtures were incubated for an hour at 37° C. Then 1cc. of a 1:5 dilution of human plasma and 0.25 cc. of 0.25% calcium-chloride solution were added. The results are given in Table II.

Table II shows that the effectiveness of fibrinolysin produced by strain 693 in 2% glucose-broth is inhibited by undiluted spinal fluid, undiluted normal serum and streptococcal antiserum; the potency of the antiserum does not exceed that of normal serum. In contrast, the streptococcal antiserum has a very marked neutralizing effect upon the fibrinolysin produced in 0.05% glucose-broth, although the influence of normal spinal fluid and normal serum upon this type of fibrinolysin is almost negligible.

The fibrinolysins produced in 2% glucose-broth by *S. viridans, enterococcus* and *pneumococcus* inhibited constantly the clotting of both human and animal plasma, but only when undiluted culture

[7] Garner, R. L., and Tillett, W. S., *J. Exp. Med.*, 1934, **60**, 239.

[8] Van Deventer, J. K., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1117.

†Scarlet Fever Streptococcal Antitoxin, Lederle.

TABLE II.

Inhibitory Effect of Spinal Fluid, Normal Serum and Streptococcus Antiserum on Hemolytic Streptococcal Fibrinolysins.

Decreasing amounts of spinal fluid or of serum	Strain 693 in 2% glucose-broth						Strain 2327 in 0.05% glucose-broth					
	A Normal human spinal fluid		B Normal horse serum		C Streptococcal antiserum		A Normal human spinal fluid		B Normal horse serum		C Streptococcal antiserum	
	a	b	a	b	a	b	a	b	a	b	a	b
1. 0.5	—	—	—	—	—	—	—	++++	—	++	—	—
2. 0.1	++++	++++	++++	++++	++++	++++	—	++++	—	++++	—	—
3. 0.02	++++	++++	++++	++++	++++	++++	—	++++	—	++++	—	—
4. 0.004	++++	++++	++++	++++	++++	++++	—	++++	—	++++	—	+
5. 0.0008	++++	++++	++++	++++	++++	++++	—	++++	—	++++	—	+++
6. 0.	++++	++++	++++	++++	++++	++++	—	++++	—	++++	—	++++

Read after a—10 minutes 37° C.
b— 5 hours ''
+ − ++++ = various degrees of fibrinolysis.
— = no fibrinolysis (coagulation).

was used. Attempts to neutralize these fibrinolysins by means of specific antisera (especially with type-specific pneumococcal serum and its corresponding strain) thus far were not successful. The fibrinolysins of these microörganisms have the same properties as the fibrinolysin which is produced by hemolytic streptococci in 2% glucose-broth only.

Conclusions. 1. Hemolytic streptococci of human origin, when tested for fibrinolytic activity in 2% and 0.05% glucose-broths, may be classified in one of the following groups: (a) production in 2% only; (b) in 0.05% only; (c) in both 2% and 0.05%; and (d) no production in either.

Properties of the 2% glucose-broth fibrinolysin (a): This type of fibrinolysin inhibits clotting; it is effective toward human and animal plasma, but only when used undiluted; it is thermostable; and was not neutralized by specific streptococcal antisera.

The fibrinolysin of groups (b) and (c), even in high dilutions, lyses human plasma but not animal plasma; it is thermostable, and may be neutralized by streptococcal antisera.

Serum-broth fibrinolysin resembles 0.05% glucose-broth fibrinolysin, but is heat-labile.

2. *S. viridans, enterococcus,* and *pneumococcus,* when cultivated in 2% glucose-broth, may produce a fibrinolysin which has the same properties as the fibrinolysin produced by hemolytic streptococci in 2% glucose-broth.

TABLE III.
Properties of Different Fibrinolysins Produced by Streptococci of Human Origin in

I 2% glucose-broth	II 0.05% glucose-broth	III Serum-broth
Not specific for *Streptococcus hemolyticus*	Specific for *Streptococcus hemolyticus*	Specific for *Streptococcus hemolyticus*
Inhibition of plasma coagulation	Fibrinolysis	Fibrinolysis
Low titer	High titer	High titer
Lysis of human and animal plasma	Lysis of human plasma only	Lysis of human plasma only
Heat-resistant	Heat-resistant	Heat-labile
Not neutralized by streptococcal antiserum	Neutralized by streptococcal antiserum	Neutralized by streptococcal antiserum