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Fibrinolytic Activity of Hemolytic Streptococci and Other Microörganisms.

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The fibrinolytic activity of hemolytic streptococci has been extensively studied by Tillett and Garner.[1] According to these authors, only hemolytic streptococci, with the exception of some strains of staphylococci, produce fibrinolysin. Cultures of *Streptococcus viridans, Streptococcus non-hemolyticus,* enterococcus, pneumococcus, and other microörganisms proved to be ineffective. These findings were recently corroborated by Schmidt.[2] The object of our investigations was to ascertain the significance of the composition of the culture-medium for the production of the fibrinolysin.

The respective broths (with plain meat-infusion broth, pH 7.4 as a base), were inoculated and incubated at 37°C. for 18 hours. The cultures were centrifuged and the supernatant fluid tested for fibrinolytic activity according to the technique of Tillett and Garner.[1]

The plasma used for the experiments was prepared by mixing 10 cc. of human blood with 1 cc. of a 2% potassium oxalate solution. The blood was shaken thoroughly and centrifuged. For the demonstration of the fibrinolysin, the supernatant fluid of the culture, in the amount of 0.5 cc., was mixed with 1 cc. of a 1:5 dilution of human plasma; then 0.25 cc. of a 0.25% calcium chloride solution in normal saline was added, the tubes shaken thoroughly and kept at 37°C. The results were read at various intervals.

The influence of the glucose-concentration of the meat-infusion broth upon the production of the fibrinolytic substance by hemolytic streptococci was first studied. In Experiment I, 3 strains of hemolytic streptococci were incubated for 18 hr. at 37°C. in meat-infusion broth containing 2, 0.4, 0.08, and 0.016% glucose. The supernatant fluid of the respective cultures was tested for fibrinolytic activity.

As can be seen in Table I, strain 693 produced fibrinolysin in 2% and 0.4% glucose broth, but not in 0.08% and 0.016% glucose

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1 Tillett, W. S., and Garner, R. L., *J. Exp. Med.*, 1933, **58**, 485; Tillett, W. S., *J. Bact.*, 1935, **29**, 111.

2 Schmidt, H., *Z. f. Immunitætsf.*, 1936, **87**, 1.

TABLE I.

Fibrinolytic Activity of Hemolytic Streptococci in Meat-Infusion Broth Containing Various Amounts of Glucose.

	I Strain 693				II Strain 751 Glucose-Infusion Broth				III Strain 646			
Read after	2%	0.4%	0.08%	0.016%	2%	0.4%	0.08%	0.016%	2%	0.4%	0.08%	0.016%
30 min., 37°C.	++++	++++	—	—	++	—	—	—	++++	++++	—	—
3 hrs., ,,	++++	++++	—	—	++	—	++++	++++	—	—	—	—
24 ,, ,,	++++	++++	—	—	++++	++++	++++	++++	—	—	—	—

+, ++, ++++ = various degrees of fibrinolysis.
— = no fibrinolysis (coagulation).

TABLE II.

Fibrinolytic Activity of Hemolytic Streptococci in Meat-Infusion Broth Containing Various Carbohydrates.

	I Strain 69					II Strain 206				
Read after	Plain	Glucose	Mannit	Salicin	Lactose	Plain	Glucose	Mannit	Salicin	Lactose
	Infusion-Broths.									
15 min., 37°C	—	++++	++++	++++	++++	—	++++	—	++++	++++
1 hr., ,,	—	++++	—	++++	++++	—	++++	—	—	++++
24 ,, ,,	—	++++	—	++++	++++	++++	++++	++++	++++	++++

++++ = degree of fibrinolysis.
— = no fibrinolysis (coagulation).

broth. On the other hand, the formation of fibrinolysin by strain 751 occurred in broth containing glucose from 0.016% to 2%. Strain 646 was completely ineffective. It follows from this experiment that the production of fibrinolysin may depend upon the glucose-concentration of the broth employed, as well as upon the particular strain.

The following experiments dealt with the rôle played by the particular type of carbohydrate of the culture-medium in the production of fibrinolysin. For this purpose, 2 strains of hemolytic streptococci were grown for 18 hr. at 37°C. in (a) plain meat-infusion broth, (b) 2% glucose broth, (c) 2% mannit broth, (d) 2% salicin broth and (e) 2% lactose broth. The cultures were centrifuged and the supernatant fluid tested for fibrinolytic potency. Table II shows that fibrinolysis varies with the type of carbohydrate used in the culture medium, as well as with the reading time. It is evident that glucose can be replaced by other carbohydrates. The fibrinolysin produced in some of the sugar-broths inhibits coagulation of the plasma continually; that of other carbohydrate-broths, however, is effective only temporarily inasmuch as fibrinolysis precedes or follows coagulation of the plasma. There seems to be no definite relationship between the growth of the respective strains and their fibrinolysin-production in the various sugar-broths. On the other hand, it was found that some strains of hemolytic streptococci produced the fibrinolytic substance after 48 but not after 18 hr. in the same medium. Further investigations will ascertain whether a parallelism exists between the production of fibrinolysin in the various sugar-broths and the fermentation of these carbohydrates.

The production of fibrinolysin has been described as a specific property of hemolytic streptococci. Since the above experiments showed the significance of the medium for the production of the fibrinolytic substance, the question of specificity was reconsidered. Various types of microörganisms in 2% glucose broth, were tested for fibrinolytic activity. Thirty-one of 40 strains of hemolytic streptococci produced fibrinolysin. Of 47 strains of *Streptococcus viridans,* 35 were found to be fibrinolytic. This result corroborates the observations of Tunnicliff,[3] who very recently reported fibrinolysin production by several strains of *Streptococcus viridans.* Thus far, 31 strains of enterococcus (streptococcus fermenting æsculin) were tested and all found to be fibrinolytic. Of 78 strains of pneumococci, 31 strains produced fibrinolysin, while 47 strains did not.

[3] Tunnicliff, R., *J. Inf. Dis.*, 1936, **58**, 92.

The distribution of fibrinolytic and non-fibrinolytic strains of pneumococci was about the same among all the types tested.

Preliminary studies with various kinds of bacilli such as *B. coli, B. lactis aerogenes, B. friedlænderi, B. pyocyaneus* and *B. proteus,* revealed that several strains of these microörganisms were able to produce fibrinolysin when cultured in meat-infusion broth containing 2% glucose.

It may be mentioned that sometimes it was possible to differentiate various strains of *Streptococcus viridans* obtained from one specimen of sputum by means of their fibrinolytic potency. Some of the colonies proved to be fibrinolytic, while others were not, when cultivated under identical conditions in 2% glucose meat-infusion broth.

Further experiments were concerned with the question whether enterococci, pneumococci and other microörganisms produce a fibrinolysin if the glucose of the medium is substituted by other carbohydrates, such as lactose, saccharose, maltose, mannit, galactose and xylose. As in the case of hemolytic streptococci, the production of the fibrinolysin varied with the particular carbohydrate and the strain.

It follows from our experiments that various types of microörganisms are capable of producing fibrinolysin. These findings seem to contradict the observations of Tillett and Garner that the production of fibrinolysin is specific for hemolytic streptococci. The following experiment may offer an explanation for the differences in the results obtained: One strain each of *Streptococcus hemolyticus, Streptococcus viridans* and enterococcus, was grown in (a) 2% glucose-broth, (b) 0.05% glucose-broth, for 18 hr. at 37°C. The supernatant fluid of the respective cultures was examined for fibrinolytic potency. Fibrinolysin was produced by *Streptococcus hemolyticus* in 0.05% glucose-broth, as well as in 2% glucose-broth. On the other hand, *Streptococcus viridans* and enterococcus did not show any fibrinolytic activity when cultured in 0.05% glucose-broth, but produced the fibrinolytic substance in 2% glucose-broth. The results of Tillett and Garner are corroborated in that only hemolytic streptococci produce the fibrinolysin in 0.05% glucose-broth. However, the fibrinolysin-production is not limited to hemolytic streptococci alone, if, for instance, the sugar-content of the culture-medium is increased.