A STUDY OF THE FIBRINOLYTIC PROPERTIES OF LANCEFIELD'S GROUP C STREPTOCOCCI

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A STUDY OF THE FIBRINOLYTIC PROPERTIES OF LANCEFIELD'S GROUP C STREPTOCOCCI

by

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A Study of the Fibrinolytic Properties of Lancefield's Group C Streptococci

Introduction

Since Lancefield (6) began to identify hemolytic streptococci serologically in 1933 there has been great interest in cultures which have fallen into her group C. The probable reason for this is that streptococci from both man and animals fall into this group so that it is of interest to the medical worker as well as the veterinarian. There have been various attempts to separate group C into "human" and "animal" types by biochemical and serological methods but these have not proved altogether successful. The present study was undertaken for the purpose of determining the possible use of the fibrinolytic test in separating "human" type and "animal" type strains of group C.

History

Much emphasis has been placed on the fermentation of sorbitol and trehalose as an aid in the differentiation of this important serological group. Lancefield (6) in 1933 found that of forty-nine strains which were classified serologically as group C and which were isolated from lower animals, forty-five fermented sorbitol but not trehalose and four strains fermented

neither of these carbohydrates. Her cultures were isolated from guinea pigs, cows, rabbits, horses, fowl, and foxes.

Dimock and Edwards (3) in 1933 demonstrated that Streptococcus equi, though serologically a member of Lancefield's group C, was a definite entity. They based their belief on the observation that Str. equi was the only member of group C which would produce "strangles" in horses. This organism has a biochemical pattern of its own in that it does not ferment lactose, sorbitol, or trehalose. Dimock and Edwards also proposed to divide streptococci of Lancefield's group C which were derived from animal sources into four types on the basis of their ability to ferment lactose, sorbitol and trehalose; namely, types A, B1, B2 and equi. Type A ferments lactose and sorbitol but not trehalose. Type B ferments trehalose only and type B2 ferments lactose and trehalose but not sorbitol. Str. equi does not attack any of these substances. This classification has been adopted by many but it has several shortcomings. In a follow up of this work, Edwards (4) in 1935 stated that these types set up by himself and Dimock fall into the same serological group and can be differentiated by biochemical reactions only. He also stated that their type A cultures were probably strictly animal parasites and that they had not been found in human disease. He found that streptococci isolated from animals might be

of the "human" type and cause human disease while human beings could harbor streptococci of the animal type.

Brown (1) in 1937 in an extensive study of the hemolytic streptococci found in milk, studied two hundred and fourteen strains of Lancefield's group C streptococci of animal origin and found that all fermented sorbitol but not trehalose. He stated that the animal strains of group C streptococci including Str. equi would not lyse fibrin. All of his fibrinolytic tests were made with human plasma. The strains that he isolated which were of human origin all lysed human plasma. In the summary of his paper he recommended the fibrinolytic test using human plasma to show which organisms are to be regarded as pathogenic for man.

In 1937 Plastridge and Hartsell (11) described a new species of streptococci found in bovine mastitis which was biochemically similiar to Lancefield's group B streptococci but which was serologically different. They named this species Streptococcus pseudo-agalactiae. They found that this organism represented about five percent of the cultures isolated from experimental herds being tested for chronic streptococcic mastitis. This organism usually produced an acute or mild mastitis of short duration. They showed that it yielded extracts which reacted serologically with Lancefield's group C antisera.

Sherman and Niven (12) in 1938 studied the hemo-

lytic streptococci of milk and applied the fibrinolytic test of Tillet and Garner in which they used human plasma. In their work they found that of eleven "animal pyogenes" five partially lysed the plasma clot in 24 hours while six did not. Of eight strains of "human" type group C streptococci, six caused complete lysis of the plasma and two only a partial lysis in twenty-four hours. Sherman and Niven incubated their fibrinolytic tests at 37°C. and also 53°C. and found that the "animal" type group C cultures would not lyse human fibrin at 53° C. while the "human" type group C cultures at 53°C. would give results comparable to those obtained at 37°C. and suggested this as a criterion for differentiating between these two types.

Tillet and Garner (15) in first describing the fibrinolytic test noted that three strains out of eighteen derived from animal sources were capable of lysing human plasma. One strain was isolated from a cow and the other two were from a rabbit and a guinea pig. The serological reaction of the cultures which they used at that time was not studied. They found that animal strains would not lyse rabbit plasma while some human strains would do so after twenty hours incubation.

Madison (7) while working on the fibrinolytic property of streptococci derived from lower animals found that the majority of the strains which he tested would lyse horse plasma in preference to human plasma.

He suggested the specificity of the strain for the plasma of the type host from which the strain was isolated. Serological identification of the cultures he used was lacking.

Tillet (16), while doing further work on the fibrinolytic reaction and correlating the biochemical and serological reactions with the ability of streptococci to lyse human plasma, found only one out of twenty-one cultures of Lancefield group C cultures that was able to lyse human fibrin. He stated that the determination of the presence or absence of fibrinolysin in the cultures is a helpful procedure in separating human pathogenic strains from others which probably do not cause human disease. In a review of the literature, Tillet (17) stated that fibrinolysis is not necessarily associated with any biochemical characteristics but appears to be related to the disease producing capacity of the strain when isolated.

Alice Evans (5) in 1936 after studying Str. equi and related strains concluded that there were waves of human infections due to trehalose fermenting strains of group C organisms, thereby suggesting a possible connection between such infections and bovine mastitis caused by strains of this group.

Planet (9) in 1935 first found that strains of streptococci from horses would act on horse fibrin but not on human fibrin and also that a human strain would not act on horse fibrin.

Materials and Experimental Procedures

For Biochemical Work

Blood Broth: Blood broth was prepared by adding 5 percent sterile defibrinated ox blood to beef infusion proteose peptone broth containing 0.5 percent sodium chloride and adjusted to pH 7.3.

Litmus Milk: This was prepared by adding 2 percent of a saturated aqueous solution of litmus to fresh skim milk.

Carbohydrate Broths:*

Solution A

Serum (ox serum not sterile)	90 ml.
15 percent sodium hydroxide	10 ml.

Put in flask in boiling water bath for 20 minutes.

Solution B

Distilled water	900 ml.
Proteose peptone	10 grams
Sodium chloride	5 grams
Beef extract	3 grams

Dissolve ingredients, add solution A to B, add 10 ml. of Andrade's indicator and adjust reaction to pH 7.4. Divide the resulting broth into three portions, add 1 percent lactose, sorbitol, trehalose respectively, tube and sterilize by autoclaving at 10

* Formula developed in the Department of Animal Diseases, Storrs Agricultural Experiment Station, Storrs, Conn.

pounds pressure for 20 minutes.

Broth for the Determination of Final pH:

Difco Proteose Peptone	10 grams
Meat extract	4 grams
Sodium chloride	5 grams
Dextrose	10 grams
Distilled water	1000 ml.

pH 7.0

Tube in 5 ml. amounts and sterilize at 15 pounds pressure for 15 minutes.

Sodium Hippurate Broth: This was prepared according to the method of Coffey and Foley as published in the American Journal of Public Health Volume 27, number 10, pages 972 to 974, 1937.

Procedure for Fibrinolytic Tests

Media: The media found to be best suited for the production of fibrinolysin by streptococci of animal origin was 1 percent proteose peptone beef infusion broth containing 0.5 percent sodium chloride and adjusted to pH 7.2.

Anti-coagulant: A 2 percent solution of sodium oxalate was used as an anti-coagulant. One milliliter of the solution was mixed with 10 ml. of freshly drawn blood. This procedure was used for horse and human plasma. For rabbit plasma 1.5 ml. of the solution was used per 10 ml. of blood.

Coagulant: Coagulation was accomplished by adding

0.25 ml. of a 0.25 percent solution of calcium chloride in 0.85 percent saline to the plasma-culture mixture. This coagulated oxalated plasma within a period of 36 hours after it is drawn.

Method of Activation: After the cultures were incubated at 37°C. for 18-24 hours they were then immediately chilled and stored in a refrigerator for 5 hours at 4°C.

Description of the Test: To 0.1 ml. of oxalated plasma, 0.9 ml. of physiological saline and 0.5 ml. of "activated" broth culture were added. Then 0.25 ml. of the calcium chloride solution was added and the ingredients mixed well. The tubes were then placed in a water bath or incubator at 37°C. Under these conditions the clot will form in approximately 15 minutes and the cultures may begin lysing within a period of 15 minutes after they have clotted. The tests were arbitrarily terminated after 24 hours incubation.

Experimental

There appeared to be two ways in which cultures of group C streptococci might be induced to lyse fibrin; namely, by using media more suitable for growth, or by finding some means of making the test more sensitive. Our assumption was that all hemolytic streptococci might be fibrinolytic and that those which did not lyse fibrin failed to do so because they did not produce enough fibrinolysin to reveal a reaction or that the

History of Cultures

Table 1

Culture Number	Original Designation	Where Obtained	Source	Remarks
1	Str. scarlatinae	Dr.W.L.Kulp Univ.of Conn.	Human	Group A Low grade scarlet fever
2	Wilhelm	Dr.W.L.Kulp Univ.of Conn.	Human	Group A Skin lesion
3	Ottenhiemer	Dr.W.L.Kulp Univ.of Conn.	Human	Group A Human lesion
4	Elliot	Dr.W.L.Kulp Univ.of Conn.	Human	Group A Abcess
5	Str. hemolyticus	Dr.W.L.Kulp Univ.of Conn.	-	Group A Originally obtained-Lederle
6	Upton	Dr.W.L.Kulp Univ.of Conn.	Human	Group A Arm infection
7	Gatchell	Dr.W.L.Kulp Univ.of Conn.	Human	Group A Hand infection
8	Cleveland	Dr.W.L.Kulp Univ.of Conn.	Human	Group A Infection of human cervix
9	N49	Dr.A.W.Stableforth England	Bovine	Mastitis Group 1 type 1a*
10	S102	Dr.A.W.Stableforth England	Bovine	Mastitis Group 1 type 1b
11	G19	Dr.A.W.Stableforth England	Bovine	Mastitis Group 1 type 1c

History of Cultures

Table 1 (cont.)

Culture Number	Original Designation	Where Obtained	Source	Remarks
12	G2	Dr.A.W.Stable-forth England	Bovine	Mastitis Group 1 type 1d
13	S84	Dr.A.W.Stable-forth England	Bovine	Mastitis Group 1 type 2a
14	S101	Dr.A.W.Stable-forth England	Bovine	Mastitis Group 1 type 3a
15	S107	Dr.A.W.Stable-forth England	Bovine	Mastitis Group 1 type 3b
16	S104	Dr.A.W.Stable-forth England	Bovine	Mastitis Group 1 type 3c
17	090R	Dr.R.C.Lance-field Rockefeller I.	Human	Mastitis Group 1 type 4A
18	H36B	Dr.R.C.Lance-field Rockefeller I.	Human	Mastitis Group 1 type4b Blood of new born baby
19	G42	Dr.F.W.Stewart Australia	Bovine	Mastitis Group 1 type 4c
20	W-11-2	Univ. of Conn.	Bovine	Str. uberis type 6
21	2901	Dr.J.M.Sherman Cornell Univ.	Human	Group C Isolated from human feces
22	H46A	Dr.J.M.Sherman Cornell Univ.	-	Group C Originally from Dr. R.C. Lancefield

History of Cultures

Table 1 (cont.)

Culture Number	Original Designation	Where Obtained	Source	Remarks
23	Shirley	Univ. of Conn.	Bovine	Group C Mastitis
24	1	Dr.P.R.Edwards Univ. of Kty.	Equine	Group C Infection of Os uteri-mare
25	2	Dr.P.R.Edwards Univ. of Kty.	Equine	Group C Infection of Os uteri-mare
26	3	Dr.P.R.Edwards Univ. of Kty.	Equine	Group C Infection of Os uteri-mare
27	4	Dr.P.R.Edwards Univ. of Kty.	Equine	Group C Mare with lymphagitis -Str. equi
28	5	Dr.P.R.Edwards Univ. of Kty.	Equine	Group C hip joint of foal-died s. septicemia
29	Tuttle 33-3	Univ. of Conn.	Bovine	Group C Mastitis
30	Strick. 1-4	Univ. of Conn.	Bovine	Group C Mastitis
31	Sm. 67-4	Univ. of Conn.	Bovine	Group C No other evidence of Mastitis
32	Sm.75-2	Univ. of Conn.	Bovine	Group C Mastitis
33	Eck. 8-4	Univ. of Conn.	Bovine	Group C Mastitis

History of Cultures

Table 1 (cont.)

Culture Number	Original Designation	Where Obtained	Source	Remarks
34	Eck. 31-3	Univ. of Conn.	Bovine	Group C Mastitis
35	B938	Univ. of Conn.	Bovine	Group C No other evidence of Mastitis
36	A.S. 19-2	Univ. of Conn.	Bovine	Group C Mastitis
37	Horse	Univ. of Conn.	Equine	Group C Uterine exudate-foaled 5wks. early
38	Dys. I	Dr.K.Diernhoffer Germany	Bovine	Group C Str. dysgalactiae
39	Dys. II	Dr.K.Diernhoffer Germany	Bovine	Group C Str. dysgalactiae
40	C1	Dr.R.C.Lancefield Rockefeller I.	Cheese	Group D
41	C3	Dr.R.C.Lancefield Rockefeller I.	Cheese	Group D
42	Yale	Dr.W.L.Kulp Univ.of Conn.	Unknown	Group E History lost
43	Woodworth	Dr.W.L.Kulp Univ.of Conn.	Human	Group E Human infection
44	M. I.	Dr.W.L.Kulp Univ. of Conn.	Human	Group E Throat infection

History of Cultures

Table 1 (cont.)

Culture Number	Original Designation	Where Obtained	Source	Remarks
45	K129	Dr.R.C.Lancefield Rockefeller I.	Bovine	Group E From certified milk
46	K131	Dr.R.C.Lancefield Rockefeller I.	Bovine	Group E From certified milk
47	J148A	Dr.R.C.Lancefield Rockefeller I.	Monkey	Group G Normal throat
48	H46C	Dr.R.C.Lancefield Rockefeller I.	Human	Group G Human vaginal swab, normal
49	Turner	Unknown	Unknown	Group K From Queen Charlotte Hospital
50	Viridans 1	Dr.W.L.Kulp Univ.of Conn.	Unknown	Streptococcus viridans
51	Viridans 2	Dr.W.L.Kulp Univ.of Conn.	Unknown	Streptococcus viridans
52	Viridans 3	Dr.W.L.Kulp Univ.of Conn.	Unknown	Streptococcus viridans

* Mastitis Streptococci Group 1 as described by Stableforth (14) belong in Lancefield's Group B and may be designated as Str. agalactiae.

test was not sufficiently sensitive.

Influence of Composition of the Medium on Fibrinolysin Production

First an attempt was made to find a medium which would produce the largest amount of cells because it has been shown by Madison and Taranik (8) that the production of cells is connected with the production of lysin. Tillet (17) stated that, with strains which elaborate relatively small amounts of fibrinolysin, experience has shown that the extent of multiplication of streptococci - which may be limited in unfavorable media - and the age of the culture may be important factors in determining the results of fibrinolytic tests with individual strains. Madison and Taranik (8) have shown that the rate of fibrinolysin production and the rate of test tube proliferation of streptococci are parallel during the logarithmic phase of growth and that there is an apparent quantitative linkage between enzyme secretion and cell division. They further state that during the static phase of growth a fairly rapid destruction or inactivation of the lytic factor takes place.

The marked influence of the composition of the culture medium on the amount of growth and agglutinability of mastitis streptococci, and on the phase of streptococcus cultures was shown by Plastridge,Banfield

and Williams (10) and by Dawson, Hobby and Olmstead (2) respectively. These observations suggested that the different ingredients used in preparing medium might also influence the production of fibrinolysin.

Influence of Peptone: Peptone was the first ingredient studied and was found to be one of the two most important factors in the production of fibrinolysin. Table 2 shows the influence of different commercial peptones on the production of fibrinolysin.

Three different plasmas were used in making the fibrinolytic tests. One of these was highly susceptible, one partially so and the third was not attacked by streptofibrinolysin. It will be noted that Fairchild's Peptone, Bacto Peptone and Difco Proteose Peptone gave fairly uniform results, while Difco Neo-peptone and Bacto-Tryptone were the least favorable. While Witte's Peptone was satisfactory it seemed to contain some substances which tended to make the clot soft and to one not experienced in this technique it might be interpreted as partial lysis. Difco Proteose Peptone was chosen for use because uniform results were obtained upon repeated tests.

Influence of Dextrose: Tillet and Garner (15) added 0.05 percent dextrose to their medium to enhance the production of the fibrinolysin. They found that it materially aided fibrinolysin production by Lancefield group A streptococci. Dr. F. R. Smith, in personal

Effect of Peptone

Table 2

Culture	23			21			22		
Plasma	H[1]	R[2]	B[3]	H	R	B	H	R	B
Bacto Peptone	12[4]	p[5] 6	0	12	0	0	1	1	0
Difco Proteose Peptone	12	p 6	0	12	p 6	0	1	1	0
Difco Neo-peptone	24	0	0	24	0	0	1	1	0
Bacto Tryptone	24	p 6	0	12	0	0	1	24	0
Witte's Peptone	12	p 6	0	3	0	0	1	1	0
Armour's Peptone	24	0	0	12	0	0	1	24	0
Fair-child's Peptone	2	p 6	0	12	0	0	1	24	0

1- H-Human plasma
2- R-Rabbit plasma
3- B-Bovine plasma
4- Numbers-time required for lysis in hours
5- p-partial lysis in 24 hours

correspondence, remarked that he had found that dextrose could not be used in the culture medium because of the excess acid produced by his cultures. Although his paper (13) did not give the serological classification of the cultures with which he worked it would seem from his data that his cultures might have been Lancefield's group C.

As shown in table 3, the addition of 0.05 percent dextrose to the culture medium caused culture 20 to produce so much acid that the clot would not form upon the addition of calcium chloride. When the dextrose content reached 0.5 percent none of the culture-plasma mixtures would clot. Cultures grown in the presence of 2 percent dextrose and then neutralized to pH 6.6 would allow the culture-plasma mixture to clot. If the cultures produced fibrinolysin subsequent lysis occurred normally. The fibrinolytic test did not act more rapidly or seem more sensitive when the cultures were grown in the presence of 2 percent dextrose with subsequent neutralization than when grown without dextrose in the medium.

Influence of Buffer: Buffers were added to the medium containing 0.05 percent dextrose broth in the form of either dipotassium phosphate or calcium carbonate in quantities large enough to keep the pH within the critical range (pH 6.0-7.1) for clot formation, but the lysin was not appreciably more active than that which was

Effect of Sugar in Medium

Table 3

Concentration of Dextrose

	0%		0.05%		0.5%		1%		2%		2%*	
Strain	Clot	Lysis	Clot	Lysis	Clot	Lysis	Clot	Lysis	Clot	Lysis	Clot	Lysis
23	+	+	+	+	0	-	0	-	0	-	+	+
21	+	+	+	+	0	-	0	-	0	-	+	+
22	+	+	+	+	0	-	0	-	0	-	+	+
20	+	0	0	-	0	-	0	-	0	-	+	0

* Medium adjusted to pH 6.6 after growth of culture

produced in the absence of dextrose. Neither the presence of the phosphate nor the carbonate in the culture medium appeared to affect the fibrinolytic test.

Influence of Blood: The influence of salt upon the medium was not studied but 0.5 percent sodium chloride was added to the medium so that blood might be added and its effect studied. Defibrinated ox blood can be added to the medium and the hemolytic properties in blood broth determined at the same time that the culture is grown for the fibrinolytic test. The presence of hemolyzed blood cells in the medium did not interfere with the test.

The addition of blood to the medium in which the organisms were stored helped to retain the fibrinolytic properties of the cultures.

Influence of Incubation Time: Madison and Taranik (8) have shown that after the optimum growth period of 12 to 18 hours at 37°C. a rapid destruction or inactivation of the lytic principle takes place. In preliminary experiments the longer a culture was left at 37°C. after the 18-24 hour period of incubation the greater was the decrease in potency of the lysin. In the instance of one particularly potent strain no lysis could be demonstrated after 96 hours.

Comment: Due to the marked influence of the composition of the medium upon the fibrinolytic activity of the culture it was deemed advisable to use media of the

following composition. To a beef infusion base 1 percent Difco Proteose Peptone and 0.5 percent sodium chloride were added and the reaction was adjusted to pH 7.2. This medium was selected as best suited for fibrinolysin production by the group C organisms.

Attempts to Increase Sensitivity of the Fibrinolytic Test

The other possibility which lay open was finding a way to make the test itself more sensitive.

Physical Treatment of Cultures: Table 4 gives the results of a comparative study of the influence of activation, shaking, shaking and activation, and no physical treatment of the culture. Shaking was undertaken because of the possibility of increasing the metabolic products of the organism by shaking the culture during incubation. A shaking machine was placed in a 37°C. incubator and the medium was inoculated and immediately placed in the machine. The cultures were shaken for eight hours and at the end of that time taken out and tested for ability to lyse human and horse fibrin. These shaken cultures were then "activated" by rapid chilling and storage at 4°C. for 5 hours and another fibrinolytic test made. Activation of the cultures was the most successful physical treatment of those used to prepare streptococcus fibrinolysin from Lancefield group C organisms. Upon testing additional strains of Lancefield's group C streptococci for

Effect of Activation and Shaking on Lysis of Human and Horse Fibrin

Table 4

	Culture 23		Culture 21		Culture 22		Culture 35		Culture 38		Culture 39		Culture 37		Controls*	
	Human	Horse	Human	Horse	Human	Horse	Human	Horse	Human	Horse	Human	Horse	Human	Horse	Human	Horse
Unactivated	14 hrs	0	14 hrs	0	1½ hr	0	0	24 hrs	0	18 hrs	0	18 hrs	0	14 hrs	0	0
Activated	14 hrs	0	14 hrs	0	1½ hr	0	0	24 hrs	0	14 hrs	0	14 hrs	0	14 hrs	0	0
Shaken	0	0	14 hrs	0	1 hr	0	0	0	0	18 hrs	0	18 hrs	0	14 hrs	0	0
Shaken and Activated	24 hrs	0	24 hrs	0	2 hrs	0	0	Slight + 24 hrs	0	24 hrs	0	24 hrs	0	14 hrs	0	0

* Controls were uninoculated medium and culture 20, a streptococcus other than group C.

fibrinolysin production it was found in some instances that the only way in which the presence of fibrinolysin in the culture could be demonstrated was through activation. Shaking, and shaking and activation proved to be of no value.

Cultivation in the Presence of Fibrin: In preliminary work the effect of growing cultures of fibrinolytic streptococci in the presence of normal washed fibrin was studied. This was done by collecting sterile fibrin, washing and drying it. Shreds of the dried fibrin were dropped into the culture medium at the time of inoculation. No visible evidence of lysis of the fibrin took place.

Amounts of Fibrin in the Test: The next attempt to increase the sensitivity of the test was to reduce the amount of citrated plasma used. It was found that only 0.1 ml. of plasma was necessary for the test. The advantage of this was to make the same amount of plasma serve for twice as many tests as in the original procedure described by Tillet and Garner. This is especially helpful where the source of supply of human plasma is limited. By reducing the plasma content of the culture-plasma mixture it was hoped that a more rapid reaction would take place but this was not true. The use of less than 0.1 ml. of plasma in the test resulted in a clot that was not firm.

Source of Fibrin: Because Madison and others have

shown that some streptococci showed little or no evidence of the production of fibrinolysin when human fibrin was used, it seemed desirable to determine if some other suitable fibrin might be found to demonstrate fibrinolysin production by Lancefield's group C streptococci. Oxalated plasma from man, cow, horse, rabbit, sheep, chicken and goat was tried. Lancefield's group C streptococci would lyse only plasma from human, horse, and sometimes rabbit. Table 5 shows the fibrinolytic activity of various strains of streptococci in the presence of the three fibrins. The first group lysed human fibrin and under certain conditions also rabbit fibrin but upon repeated tests they have consistantly failed to attack horse fibrin.

The second group attacked only horse or rabbit plasma but never human. The strains which attacked human or horse plasma have never varied so this would seem to be a constant characteristic for this group. On the basis of these observations it would appear that the ability to lyse human or horse plasma might be a legitimate basis for dividing group C cultures into "animal" and "human" types.

The third group showed no evidence of attacking either human or animal plasma by the methods used.

Oxalated human and horse plasma, which has been dried while frozen and sealed in a vacuum, is suitable for this work. Plasma so stored has been usable for a

Comparison of Biochemical and Fibrinolytic Characteristics
of Group C Streptococci

Table 5

Culture No.	Blood Broth	Litmus Milk	Lactose	Sorbitol	Trehalose	pH1% Dex.	Origin	Fibrinolysis Horse	Human	Rabbit
21	+	A	A	0	A	6.5	Human	0	+	+
22	+	A	A	0	A	6.29	Human	0	+	+
23	+	A	A	0	A	6.55	Bovine	0	+	+
26	+	A	A	A	0	5.7	Equine	0	+	0
25	+	A	A	A	0	4.8	Equine	+	0	0
28	+	A	A	A	0	5.09	Equine	+	0	0
24	+	A	A	A	0	5.5	Equine	+	0	+
29	0	A	A	A	A	4.88	Bovine	+	0	0
30	0	A	A	A	A	5.08	Bovine	+	0	0
31	0	A	A	0	A	4.82	Bovine	+	0	0
39	+	A	A	0	A	4.9	Bovine	+	0	+
38	0	A	A	0	A	5.58	Bovine	+	0	+
35	0	A	A	0	A	4.95	Bovine	+	0	0
37	+	A	A	0	A	4.78	Equine	+	0	+
27	+	0	0	0	0	5.8	Equine	+	0	0
36	0	A	A	0	A	4.75	Bovine	0	0	0
32	0	A	A	0	A	4.82	Bovine	0	0	0
33	0	A	A	0	A	4.84	Bovine	0	0	0
34	0	A	A	A	A	5.05	Bovine	0	0	0

A - Acid

period of one year.

Relation of Fibrinolytic Ability to Biochemical Characteristics

As shown in table 5 in attempting to correlate the biochemical characteristics of group C streptococci with their fibrinolytic activity, no definite biochemical pattern was found to be produced by any fibrinolytic type of organism. The only characteristic which showed a correlation with the fibrinolytic ability of the organism was the amount of acid produced in 1 percent dextrose broth. Those organisms which attacked human plasma produced an average pH of 6.26 in 1 percent dextrose broth while the final pH for the organisms that attacked horse plasma averaged 5.1.

No correlation between ability to ferment sorbitol and trehalose and fibrinolytic properties was found. Three cultures attacked both sorbitol and trehalose.

It was observed that the action of all group C streptococci cultures in blood broth was not similar. All of the cultures in group C produced at least a small zone of hemolysis on ox blood agar while some produced zones of hemolysis comparable to Lancefield's group A streptococci.

The results obtained indicate that if the Lancefield group C cultures are to be divided into "human" and "animal" types it would be better to divide them

on the basis of their fibrinolytic activity rather than by any other single characteristic. The "human" type should be designated by their ability to attack human fibrin only. The "animal" type should include both of the other two groups. It appears that group C organisms which produce little or no demonstrable lysin should be grouped with the "animal" type since the writer has never seen a culture which has produced partial lysis only of "human" fibrin in 24 hours. A further reason for classifying these organisms as "animal" type was the fact that they closely resembled this type in the amount of acid produced in 1 percent dextrose broth.

Fibrinolytic Properties of Hemolytic Streptococci From Different Sources

The last part of this study concerns itself with the fibrinolytic properties of hemolytic streptococci in relation to the serological type and to the source of the strain. The results are summarized in table 6.

Culture numbers 1 to 8 represent Lancefield's serological group A, and were taken from human infections. They were from patients affected with septicemia, scarlet fever or low grade skin infections. None of these cultures lysed either horse or rabbit fibrin. However all of the eight cultures lysed human fibrin. Several of the cultures were not particularly

Comparison of Serology and Fibrinolytic Activity

Table 6

Culture Number	Lancefield's Sero. group	Source	Fibrinolysis Horse	Rabbit	Human
1	A	Human	0	0	3hrs.
2	A	Human	0	0	18hrs.
3	A	Human	0	0	1hr.
4	A	Human	0	0	3hrs.
5	A	Human	0	0	3hrs.
6	A	Human	0	0	1hr.
7	A	Human	0	0	3hrs.
8	A	Human	0	0	24hrs.
9	B	Bovine	0	0	0
10	B	Bovine	0	0	0
11	B	Bovine	0	0	0
12	B	Bovine	0	0	0
13	B	Bovine	0	0	0
14	B	Bovine	0	0	0
15	B	Bovine	0	0	0
16	B	Bovine	0	0	0
17	B	Human	0	0	0
18	B	Human	0	0	0
19	B	Bovine	0	0	0
20	Str.uberis	Bovine	0	0	0
21	C	Human	0	24hrs.	18hrs.
22	C	-	0	3hrs.	1hr.
23	C	Bovine	0	0	18hrs.
24	C	Equine	18hrs.	18hrs.3+	0
25	C	Equine	18hrs.	0	0
26	C	Equine	0	0	24hrs.

Comparison of Serology and Fibrinolytic Activity

Table 6 (cont.)

Culture Number	Lancefield's Sero. group	Source	Fibrinolysis Horse	Rabbit	Human
27	C	Equine	24hrs.	0	0
28	C	Equine	18hrs.	0	0
29	C	Bovine	18hrs.	0	0
30	C	Bovine	24hrs.	0	0
31	C	Bovine	18hrs.	0	0
32	C	Bovine	0	0	0
33	C	Bovine	0	0	0
34	C	Bovine	0	0	0
35	C	Bovine	24hrs.	0	0
36	C	Bovine	0	0	0
37	C	Equine	14hrs.	24hrs.	0
38	C	Bovine	18hrs.	18hrs.3+	0
39	C	Bovine	18hrs.	18hrs.3+	0
40	D	Cheese	0	0	0
41	D	Cheese	0	0	0
42	E	Unknown	0	0	0
43	E	Human	0	0	0
44	E	Human	0	0	0
45	E	Bovine?	0	0	0
46	E	Bovine?	0	0	0
47	G	Monkey	0	3hrs.	3hrs.
48	G	Human	0	0	18hrs.
49	K	Unknown	0	0	0
50	Str.viridans	Unknown	0	0	0
51	Str.viridans	Unknown	0	0	0
52	Str.viridans	Unknown	0	0	0

active in lysing human fibrin and required from 18 to 24 hours to show evidence of lysis.

The Lancefield group B cultures (numbers 9 to 19) were the serological types of Streptococcus agalactiae described by Stableforth (14) in 1937. None of these cultures attacked any of the three plasmas used. It was further noted that, although Madison (7) suggested that there might be a specificity of the organism for the plasma of the host from which the organism was isolated, bovine mastitis streptococci including Str. agalactiae, group C streptococci of bovine origin and Str. uberis all failed to attack ox fibrin.

The Lancefield group C cultures lend themselves to three divisions discussed in detail previously.

Group D, E, and K cultures and also three strains of Streptococcus viridans failed to attack any of the three fibrins used.

Group G cultures reacted similarly to the "human" type group A and group C cultures which attacked only human or rabbit fibrin and not that from horses. These cultures required a longer time to lyse human plasma than did the group A cultures.

It may be seen from table 6 that the only serological type of hemolytic streptococcus cultures which attacked horse plasma was Lancefield group C.

Discussion

The fibrinolytic test definitely has its place in

the routine testing of Lancefield's group C streptococci. It is the only test used which gave an indication as to which of these cultures might affect the health of human beings. In making fibrinolytic tests it is necessary to use a suitable medium for growth of the culture because various ingredients used in the medium affect the production of fibrinolysin, the most important of these are the basic broth, the peptone, and fermentable carbohydrate content. In making fibrinolytic tests on group C streptococci it was necessary to omit the carbohydrate since it resulted in the production of so much acid that the pH of the inoculum lowered the pH of the plasma sufficiently, unless it was neutralized, to prevent coagulation upon addition of the normal amount of calcium chloride. It appears that this factor led many of the earlier investigators of this problem to believe that certain cultures were lytic when actually they produced little or no lysin.

Horse fibrin was the only fibrin used which the "animal" type group C cultures lysed consistently. The group C cultures which lysed neither horse nor human fibrin appear to be of the "animal" type because of their biochemical properties and because the "animal" type in general produced a weaker lysin than the "human" type of this group. It is possible that these cultures did not produce enough lysin to yield a reaction, by the methods used.

Physical variations of the original Tillet and Garner technique were tried such as adding sterile fibrin to the media in which the cultures were grown. Test cultures were grown while being agitated because it was thought that this might increase the amount of metabolic products produced by certain strains of organisms. Neither of these procedures was of any value in the production of fibrinolysin. "Activation" by rapid chilling the culture after growth and storing at 4°C. for five hours was the only physical treatment which gave a more rapid and clear cut reaction.

In testing various types of streptococci it was found that the "animal" types of group C were the only ones which would attack horse plasma. There was no indication by either the group A or group G cultures, which produce a large amount of lysin for human fibrin, that this lysin would attack horse fibrin. It would seem logical then that organisms which belong in the "animal" type of group C produce a different type of lysin than is produced by group A, the "human" type of group C, and group G. Ability of group C cultures to lyse human and horse fibrin would seem to offer a more rational basis for division of the group C cultures than any method suggested previously.

Summary

1. The lytic properties of group C streptococci were enhanced more by growing in a medium containing Difco Proteose Peptone than in any of the other six commercial peptones studied.

2. The presence of dextrose in the culture medium resulted in the production of too much acidity for this to be used in the fibrinolytic test unless the culture was first neutralized.

3. It would seem advisable to regard "human" type group C cultures as those which attack human fibrin and to regard as "animal" type group C those which attack either horse fibrin or neither human nor horse fibrin.

4. It was found necessary to run fibrinolytic tests with both human and horse fibrin to differentiate the types of Lancefield's group C streptococci.

5. Biochemical reactions were of no help in separating those cultures which lysed human fibrin from those which lysed horse fibrin or those which lysed neither, except in determining the final pH in a 1 percent dextrose broth.

6. Horse fibrin was lysed by cultures of Lancefield's group C of the "animal" type only, and not by group A or group G cultures.

7. Bovine mastitis streptococci, that is Str. agalactiae, Str. dysgalactiae, and Str. uberis, failed to lyse human, horse, rabbit, sheep, goat, ox, or

chicken fibrin.

8. No simple method other than "activation" was found which would aid in making the fibrinolytic test more sensitive or easier to interpret.

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