THE FIBRINOLYTIC ACTIVITY OF HEMOLYTIC STREPTOCOCCI IN RELATION TO THE SOURCE OF STRAINS AND TO CULTURAL REACTIONS

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The dissolving action, which beta hemolytic streptococci exert on solid human fibrin, is dependent upon an extracellular substance freely excreted by the living, growing bacterial cells (Tillett and Garner, 1933). Investigations of the lytic phenomenon have revealed certain special characteristics which pertain to the occurrence and nature of the reaction and to the specific immunological response of patients (Tillett, Edwards and Garner, 1934; Van Deventer and Reich, 1934; Hadfield, Magee and Perry, 1934; Dennis and Berberian, 1934; Madison, 1934; Garner and Tillett, 1934).

Recent biochemical studies (Garner and Tillett, 1934) indicate some of the properties of the streptococcal fibrinolysin, which, although enzymatic in its action, is not analogous to proteolytic ferments such as trypsin. For example, one difference between the streptococcal product and trypsin lies in the fact that the fibrinolysin, with the experimental procedures employed, produces no detectable changes in either casein, gelatin, or peptone. Furthermore, even when fibrin is used for the substrate, the special selectivity of the fibrinolysin is exemplified by the fact that the clot of normal human blood is highly susceptible, while the coagulum from the blood of small laboratory animals is not visibly affected. Van Deventer and Reich (1934) have reported the results of tests performed with the fibrin from several different animal species and found that only the blood of rhesus monkeys approximates that of man in susceptibility.

In addition to specificity with regard to substrate, the type of bacterium, which elaborates fibrinolysin, seems to be sharply limited. With the possible exception of the slow, irregular, liquefying effect of some strains of *Staphylococcus aureus*, fibrinolysis is a unique function of streptococci, and not demonstrable with other species of bacteria ordinarily associated with infection in man. A study has not been made of the many types of microorganisms occurring in animals or nature.

Concerning streptococci, only strains producing the *beta type of hemolysis*, as defined by Brown (1919), have been found to be active. Subsequent reference will be made to the importance of determining the type of hemolysis.

One other element of specificity, which will be elaborated in this paper, is brought out by the fact that, whereas beta hemolytic streptococci isolated from patients are usually highly fibrinolytic, strains of animal origin are often inert in this respect.

In pursuing studies of the fibrinolytic phenomenon, two hundred and seventeen strains have been acquired for bacteriological study. The strains were selected with particular attention to the source, and each one was of the beta hemolytic type. With a few exceptions, the human strains were isolated in this laboratory from patients in the wards of the hospital. The animal strains were given to us by Dr. J. Howard Brown of the Johns Hopkins Medical School, by Dr. Philip R. Edwards of the Kentucky Agricultural Experiment Station, and by Dr. R. C. Lancefield of the Hospital of the Rockefeller Institute. These investigators have also permitted us the use of results contained in published reports and in personal communications, thus insuring correct identification of the strains. It is a pleasure to acknowledge indebtedness to Dr. Brown, Dr. Edwards, and Dr. Lancefield for their generous assistance and suggestions.

The purpose of the investigation has been to correlate the fibrinolytic activity of the strains with other biological and biochemical properties of streptococci. The strains have been evaluated with respect to human pathogenicity on the basis of the presence or absence of fibrinolytic capacity in relation to the origin of the strain. In addition, the lytic potency of the several

active cultures has been determined with consideration of the type and severity of the infection produced in patients. The limitations, which restrict the interpretation of the results, will be referred to as the observations are described.

The investigation has been further extended to include, where applicable, a comparison of fibrinolytic activity with selected biochemical cultural characteristics which are commonly utilized in the bacteriological classification of hemolytic streptococci.

Although the serological classification, devised by Lancefield (1933), has not been carried out in this laboratory, Dr. Lancefield has very kindly permitted the incorporation of her published results in the tables of this report. Since many of the animal strains used by us were sent by Dr. Lancefield, the characterization of the strains is made more complete by tabulating all of the results together.

One of the striking features of the fibrinolytic phenomenon is the rapidity with which the fibrin may be dissolved. Under optimal experimental conditions, liquefaction occurs in a few minutes. However, the time necessary to effect complete lysis, is not the same with all strains (Tillett and Garner, 1933; Hadfield, Magee and Perry, 1934; Madison, 1934). The rate of dissolution is, therefore, an approximate quantitative measure of the amount of fibrinolysin produced by the test culture. For practical purposes, the lytic potency of cultures has been graded as follows:

++++ indicates complete lysis in less than 30 minutes
+++ indicates complete lysis in 30 to 60 minutes
++ indicates complete lysis in 1 to 3 hours
+ indicates complete lysis in 3 to 24 hours
− indicates no lysis in 24 hours

MATERIALS AND METHODS

The details of the method of demonstrating the fibrinolytic phenomenon have been described elsewhere. Briefly stated, it is as follows: 0.2 cc. of oxalated human plasma (0.02 gram potassium oxalate per 10 cc. of blood) + 0.8 cc. of physiological salt solution + 0.5 cc. of a sixteen- to twenty-hour broth culture

of the test organism + 0.25 cc. of 0.25 per cent $CaCl_2$ solution. The tubes are well shaken after the introduction of each additional constituent, and are placed in the water bath at 37.5°C. after the addition of $CaCl_2$. With potent cultures and susceptible plasma, coagulation occurs in six to fifteen minutes and complete dissolution in about six to ten minutes.

In testing many of the doubtful or negative strains, a fibrin clot composed of fibrinogen, chemically isolated from blood, coagulated with solutions of thrombin, also derived from blood, has been employed. The reason for this is based on the fact that the fibrinogen-thrombin coagulum is more susceptible to lysis than the clot of whole plasma. The method of obtaining fibrinogen and thrombin solutions has been previously described (Tillett and Garner, 1933).

Cultures. Plain, unbuffered, meat infusion broth, pH 7.6, containing 0.05 per cent glucose, has been the culture medium of choice. The cultures were allowed to incubate for sixteen to twenty-four hours and used promptly.

The strains, derived from patients, originally came from colonies picked from the surface or depths of blood agar cultures. The fibrinolytic test was, in the greatest number of instances, performed with the first broth subculture.

Strains received from other laboratories have been, in many instances, in artificial cultivation for several years. The data concerning these strains will be appropriately recorded.

Biochemical reactions. Sugar fermentations. The culture base consisted of plain meat infusion broth devoid of the 0.05 per cent glucose. Solutions of lactose, mannitol, salicin, sorbitol, and trehalose (0.5 per cent) were made in the broth. Trials with other media recommended for fermentative tests resulted in such great variations in the abundance of growth that the reactions were unsatisfactory. That the muscle sugar, which may be present in meat broth, was negligible, was indicated by appropriate controls.

After inoculation, the cultures were allowed to incubate for 5 days. Methyl red indicator was added after incubation.

pH in 1 per cent glucose. Glucose was added to meat infusion broth (pH 7.6). Fifty-cubic-centimeter centrifuge tubes, containing 25 cc. of medium, were inoculated and incubated for three days. The tubes were then centrifuged at high speed and the clear supernatant fluid was transferred to appropriate test tubes. Brom-cresyl-green indicator was employed. The standards, continually stored in the dark, were prepared within a month of use.

Hydrolysis of sodium hippurate. Cultures were allowed to grow for forty-eight hours. The usual procedure was employed.

SOME NOTES ON TECHNICAL PROCEDURES WHICH INFLUENCE FIBRINOLYSIS

Before proceeding with a description of the observations with which this report is primarily concerned, it is desirable to record briefly certain conditions which influence the fibrinolytic activity of cultures. Some of these factors are being given special study and the results will be presented in subsequent publications; others have not yet been investigated.

Identification of strains. As previously stated, fibrinolysis appears to be limited to hemolytic streptococci of the beta type. Frequently hemolytic colonies have been picked from the surface of solid media and found to be non-fibrinolytic. When these negative cultures were subcultured in poured blood agar, properly incubated, and finally chilled in the icebox for twenty-four hours, it was often found that they belonged to types classified by Brown as alpha or alpha prime (Brown, 1919), or as double-zoned (Brown, 1934). It is evident, therefore, from this experience that, when a strain of streptococcus proves incapable of liquefying fibrin, the type of hemolysis should be correctly established.

Culture medium. The rôle of environmental factors and culture media, in increasing or reducing the amount of fibrinolysin produced by streptococci, is being given special study. Although the results are incomplete, it is of interest to note, as one example, the effect which defibrinated rabbit's blood may have

upon a strain. Two drops of sterile rabbit's blood was added to 10 cc. of plain broth. Scarlet fever strain J was used:

	Dissolution time
1. First culture in plain broth	45 minutes
2. First culture in blood broth	15 minutes
3. Second culture plain broth (Transfer of No. 1)	1 hour 15 minutes
4. Second culture blood broth (Transfer of No. 2)	12 minutes
5. Third culture plain broth (Transfer of No. 3)	1 hour 40 minutes
6. Third culture blood broth (Transfer of No. 4)	16 minutes
7. Transfer of No. 5 to blood broth	50 minutes
8. Transfer of No. 7 to blood broth	30 minutes

The transfers were made at weekly intervals.

From the results it may be seen that the subcultures in plain broth in sequence tended to weaken in activity whereas those kept in blood broth maintained maximum potency. These same conditions do not, however, hold for all strains. With some, maximum activity is continually retained regardless of the type of culture media used. With other weakly lytic strains, no enhancement of activity has yet been accomplished. The lability in the function of some strains and the stability of others has been frequently observed.

Relation of lytic potency to duration of cultivation outside the body. It may be briefly stated that some strains have been found to be highly lytic when first obtained from a patient while after several weeks or months of repeated subculturing, the dissolution time is markedly slowed. The human strains used in this investigation were, with few exceptions, tested on the first or second subculture after isolation. This procedure was an attempt to approximate by the laboratory test the potential lytic capacity of the strain when causing infection.

Importance of time factor in preparing a test. This factor in the technical procedure refers to the effect on dissolution rate of the length of time plasma culture mixtures are allowed to remain liquid before the addition of $CaCl_2$. The following protocol illustrates the nature of the results.

	TIME OF INCUBATION BEFORE ADDITION OF CaCl	DISSOLUTION TIME	
		Strain Co	Strain St.
1	Immediate	7 minutes	50 minutes
2	15 minutes	6 minutes	1 hour
3	30 minutes	20 minutes	1 hour 50 minutes
4	45 minutes	38 minutes	5 hours
5	60 minutes	1 hour 30 minutes	Negative
6	2 hours	Negative	Negative

These results indicate that even in normal plasma there is a small amount of antifibrinolytic substance which, during incubation, gradually "neutralizes" the lytic principle. The inhibiting factor in normal blood was also indicated by previous experiments (Tillett and Garner, 1933) which demonstrated that the fibrin formed by clotting fibrinogen solutions with partially purified thrombin was dissolved more rapidly than the coagulum of whole plasma clotted with $CaCl_2$.

In view of the results just described, it has been the rule, in this laboratory, to prepare only ten or twelve tests at a time, thus minimizing the effect of the normal antifibrinolysin.

Selection of plasma. Oxalated plasma should be employed within forty-eight hours after removal from the body. In older samples coagulation with $CaCl_2$ is inhibited or very much delayed.

It is also important to use only plasma, the fibrin clot of which is known to be highly susceptible to lysis. Previous reports (Tillett, Edwards and Garner, 1934) have demonstrated that the blood of individuals convalescent from acute streptococcus infections are often highly resistant to fibrinolysis and that about 25 per cent of normal persons possess varying degrees of the same resistance. In view of this fact it is obviously possible to evaluate the lytic potency incorrectly by testing with resistant plasma.

LYTIC ACTIVITY OF STRAINS ISOLATED FROM PATIENTS

The results, which are summarized in table 1, were obtained with cultures isolated in this laboratory. The cultures were

promptly identified and fibrinolysis was determined with the first broth transplant. Since the individuals, from whom the streptococci were obtained, were patients in the hospital, it was possible to establish with considerable certainty the relationship of the organisms to the type of infection. The fulfillment of these conditions has been considered helpful in evaluating results which have, at best, the potential fallacies inherent in

TABLE 1

Summary of fibrinolytic test with human strains of different disease sources

SOURCE OF CULTURE	NUMBER OF CULTURES	++++ DISSOLUTION IN LESS THAN 30 MINUTES		+++ DISSOLUTION IN 30 MINUTES TO 1 HOUR		++ DISSOLUTION IN 1 HOUR TO 3 HOURS		+ DISSOLUTION IN LONGER THAN 3 HOURS		− NEGATIVE, 24 HOURS	
		Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
Septicemia	17	13	76.3	2	11.3	2	11.3	0	0	0	0
Acute suppurative diseases*	21	16	76.1	2	9.5	3	14.2	0	0	0	0
Erysipelas	9	5	55.5	1	11.1	3	33.3	0	0	0	0
Acute tonsillitis	22	9	40.9	8	36.3	5	22.7	0	0	0	0
Scarlet fever	31	10	32.2	10	32.2	9	29.0	1	3.2	1	3.2
Acute nephritis	8	8	100.0	0	0	0	0	0	0	0	0
Acute rheumatic fever	7	3	42.8	3	42.8	1	14.2	0	0	0	0
Chronic conditions, routine cultures and normals	25	6	24.0	4	16.0	10	40.0	5	20.0	0	0
Total	140	70	50.0	30	21.4	33	23.5	6	4.2	1	0.7

* This group consists of acute cases of meningitis, peritonitis, empyema, osteomyelitis, mastoiditis, and deep seated abscesses.

interpreting any test performed *in vitro* in terms of activity occurring *in vivo*.

The results presented in the table indicate that active fibrinolysin is characteristically elaborated by strains of beta hemolytic streptococci which are associated with human disease; one hundred and thirty-nine out of one hundred and forty strains were fibrinolytic. The one negative strain came from the throat culture of a case of scarlet fever. A single colony was picked from blood agar. In other cultural respects this strain

conforms to the human variety; it produces beta type of hemolysis, it belongs to the pyogenes group of Holman (1916), it ferments trehalose and not sorbitol (Edwards, 1932, 1933), it belongs to Group A in the serological classification of Lancefield (1933); toxin production has not yet been estimated.

With respect to the potency of the cultures, the number of strains representing each type of infection is too few to justify statistical analysis. It is interesting to note, however, that among the strains, which were derived from conditions particularly characterized by bacterial invasion (septicaemia and acute suppurative processes), approximately 87 per cent belong to the more highly active groups (++++ and +++), whereas only 13 per cent are weakly lytic (++ and +). In contrast, the low grade disorders and normal carriers supplied strains, the majority of which (60 per cent) belong to the weakly lytic groups (++ and +) and 40 per cent to the more rapidly acting groups (++++ and +++).

Although all of the strains included in table 1 have not been tested for fermentative action on sugars, several examples of each group have been so used. Lactose and salicin were regularly fermented, but mannitol was unaffected. This selectivity is commonly encountered with human strains of hemolytic streptococci and is characteristic of strains designated *S. pyogenes* (Holman, 1916). Furthermore, each of these strains fermented trehalose but failed to attack sorbitol (Edwards, 1932, 1933). In order to determine whether or not there is an association between fibrinolytic activity and the sugar fermentation reactions used by Holman (1916) in the classification of streptococci, a few strains have been available, which, because of their special reactions, are separated from the pyogenes group. These cultures consist of two strains of *Str. alactosus*, three strains of *Str. infrequens*, four strains described as similar to *Str. equi*, and one strain of *Str. anginosus*. The organisms have been in artificial cultivation for periods of time varying from a few weeks to several years; all but two (Y. and Scott) were obtained at autopsy. Because of the interest in, and probable importance of, the source of the strain in relation to biological characteristics,

the origin of each culture is given in table 2 along with the results of biochemical reactions.

The results indicate that fibrinolysis is not necessarily associated with any particular pattern of fermentative action but does appear to be related to the disease-producing capacity of the strain when isolated. For example *Str. alactosus* strain S. H. caused acute peritonitis in the patient and is maximally fibrinolytic. *Str. alactosus* strain Y., on the other hand, had no demonstrable relationship with the endocarditis (*Streptococcus viridans*) from which the patient suffered. Similar circumstances hold for the four *Str. infrequens* strains. The negative strain, 13571–I, in this group was one of five species of bacteria obtained at autopsy performed thirty-four hours after death of the patient. The findings undoubtedly represent post-mortem invasion, since the patient's clinical condition was not that of infection.

Since the typical *Str. equi* fails to attack either sorbitol or trehalose, the three strains in table 2, because of their atypical action in these sugars, are designated as equi-like. The relation of the strains to disease is not entirely clear. The hemolytic streptococci were probably part of a mixed infection.

A third group of human strains, which are considered separately, consists of seven cultures of so-called *Streptococcus epidemicus*. The strains, received from Dr. Philip Edwards, were derived from patients suffering from septic sore throat, which occurred in milk-borne epidemics. The results of biochemical reactions and fibrinolytic tests are presented in table 3.

The high degree of fibrinolytic potency of this group of epidemic strains is striking. It is also interesting to note that the organisms have been in artificial laboratory cultivation for several years.

The observations, which have just been described, were made with one hundred and fifty-seven strains of beta hemolytic streptococci derived from human sources. The experiments were carried out with as strict adherence as possible to conditions which were outlined in the earlier part of this article and are deemed important. One hundred and fifty-four of the strains possessed fibrinolytic activity. Of the three negative cultures,

TABLE 2

Fibrinolytic activity and biochemical reactions with specially classified human strains

CLASSIFICATION OF STRAIN OF STREPTOCOCCI	STRAIN	SOURCE	DISEASE	FERMENTATION OF			pH in 1 per cent glucose	HYDROLYSIS OF SODIUM HIPPURATE	FERMENTATION OF		FIBRINOLYTIC TEST
				Lactose	Mannitol	Salicin			Sorbitol	Trehalose	
Alactosus	S.H.	Peritoneal	Peritonitis exudate	−	+	+	4.8	−	−	+	++++
	Y.	Extracted tooth	Endocarditis streptococcus viridans	−	+	+	4.7* 4.4†	−	+	+	Negative
Infrequens	8596	Blood. Autopsy	Meningitis	+	+	+	4.9	−	−	+	++++
	8606	Blood. Autopsy	Pneumonia, septicemia	+	+	+	4.9	−	−	+	++++
	Scott	Tonsil	Acute nephritis	+	+	+	4.9	−	−	+	++++
	13571–I	Blood. Autopsy	Renal rickets	+	+	+	4.6	−	+	+	Negative
Equi-like	12436	Blood. Autopsy	Congenital syphilis	−	−	+	4.5		−	+	+
	13384	Lung. Autopsy	Pneumonia type II	−	−	+	5.4	−	−	+	++
	13480	Bronchus. Autopsy	Pertussis	−	−	+	5.6	−	−	+	++
Anginosus	8003	Blood. Autopsy	Puerperal sepsis	+	−	−	5.1	−	−	+	++++

* Result obtained by Dr. J. Howard Brown.

† Result obtained in this laboratory.

one was from a case of scarlet fever; in the other two instances, the organisms were not obtained from the sites of active infection and were, in all probability, not associated with illness in the patients.

Hadfield, Magee, and Perry (1934) tested twenty-nine human strains and divided them, according to fibrinolytic potency into three groups, the least active of which caused lysis slowly and incompletely in twenty-four hours. Eighteen of the strains belonged to the weakly active group. The more potent strains were from cases with severe infection. Recently Madison (1934)

TABLE 3

Fibrinolytic Activity and Biochemical Reactions with Human Strains Derived from Patients with Septic Sore Throat (Streptococcus Epidemicus)

STRAIN	LABORATORY	EPIDEMIC	FERMENTATION OF			pH IN 1 PER CENT GLUCOSE	HYDROLYSIS OF SODIUM HIPPURATE	FERMENTATION OF		FIBRINOLYTIC TEST
			Lactose	Mannitol	Salicin			Sorbitol	Trehalose	
E_{22}	Edwards	Baraboo, Wis.	+	−	+	4.9	−	−	+	++++
E_{23}	Edwards	Westerlo, N. Y.	+	−	+	5.0	−	−	+	++++
E_{34}	Edwards	Lee, Mass.	+	−	+	4.8	−	−	+	++
E_{59}	Edwards	Kirkland Lake, Ontario	+	−	+	5.0		−	+	++++
E_{60}	Edwards	Kirkland Lake, Ontario	+	−	+	5.1		−	+	++++
E_{64}	Edwards	Kirkland Lake, Ontario	+	−	+	4.9		−	+	++++
E_{65}	Edwards	Kirkland Lake, Ontario	+	−	+	4.9		−	+	++++

reported results obtained with one hundred and ninety-three strains of hemolytic streptococci, one hundred and fifty-five of which were designated as being from human sources. Madison further subdivided the human strains into those—32—from internal human tissues of which 94 per cent were lytic, and those —123—from superficial human tissues of which 17 per cent were lytic. Although the authors just cited found a much higher percentage of weakly active or negative strains among the cultures of human origin than have been encountered in this laboratory, both articles comment upon the high potency of streptococci obtained from severe infections.

LYTIC ACTIVITY OF ANIMAL STRAINS

The biological, biochemical, and serological characteristics, which distinguish animal strains of hemolytic streptococci, have been extensively investigated by others and reviews of the literature may be found in numerous articles (Lancefield, 1933; Edwards, 1932, 1933; Brown, Frost and Shaw, 1926). Final identification rests upon the results of several different biological and biochemical procedures, no one of which alone is adequate. The extensive studies of Lancefield (1933) on serological classification based on reactions of the somatic fraction C with immune rabbit sera offers a single test which separates human from animal strains and further subdivides the animal group. The observations of Lancefield have been recently confirmed by Edwards (1934).

In a previous report (Tillett and Garner, 1933) it was shown that only 3 out of 18 animal strains of beta hemolytic streptococcus were capable of dissolving human fibrin clot. Since the original report, additional strains have been obtained and studied in detail. The results given in table 4 were obtained with animal strains, exclusive of the bovine group.

From the table it may be seen that only two of the strains are capable of dissolving human fibrin clot. One of these (K158C) conforms in both biochemical reactions and in serological classification to a human strain. That fibrinolysis occurs, is, therefore, to be expected. The other active animal strain (K61) behaves in every way, except in the fibrinolytic test, as an animal strain and seems to be, therefore, an exception.

In view of the fact that a preponderant majority of human strains of hemolytic streptococci were found to possess the lytic function whereas animal strains were usually devoid of this activity, special attention has been given to strains derived from cows or milk. It is unnecessary to discuss the practical importance which is associated with these veterinary strains. Hemolytic streptococci of dairy origin present the problem of whether they may be pathogenic for men and cause epidemics of septic sore throat, or are probably harmless for man. The investiga-

tions of others, which need not be reviewed here, indicate that, by the determination of several biochemical reactions, the pathogenic significance of the organisms may be evaluated. If the strains are low-acid-producing, and incapable of hydrolyzing sodium hippurate, the tests for fermentation in trehalose and

TABLE 4

Fibrinolytic activity and biochemical reactions with animal strains (exclusive of bovine group)

STRAIN	LABORATORY	ANIMAL SOURCE	FERMENTATION OF			pH IN 1 PER CENT GLUCOSE	HYDROLYSIS OF SODIUM HIPPURATE	FERMENTATION OF		LANCEFIELD SEROLOGICAL CLASSIFICATION	FIBRINOLYTIC TEST
			Lactose	Mannitol	Salicin			Sorbitol	Trehalose		
R_{226}	Lancefield	Rabbit	+	−	+	4.7	−	+	−	Group C	−
K_{158A}	Lancefield	Rabbit	−	−	+	4.4	+	−	+	Group B	−
K_{158B}	Lancefield	Rabbit	+	−	+	4.8	−	+	−	Group C	−
K_{158C}	Lancefield	Rabbit	+	−	+	5.1	−	−	+	Group A	++++
K_{158D}	Lancefield	Rabbit	+	−	+	4.8	−	+	−	Group C	−
K_{158E}	Lancefield	Rabbit	+	−	+	4.8	−	+	−	Group C	−
K_{158F}	Lancefield	Rabbit	+	−	+	4.7	−	+	−	Group C	−
Br_1	Brown	Rabbit									−
K_{56}	Lancefield	Guinea pig	+	−	+	4.7	−	+	−	Group C	−
K_{61}	Lancefield	Guinea pig	+	−	+	4.8	−	+	−	Group C	+++
K_{44}	Lancefield	Guinea pig	+	−	+	4.8	−	+	−	Group C	−
K_{104}	Lancefield	Guinea pig	+	−	+	4.8	−	−	−	Group C	−
P_{454}	Lancefield	Guinea pig	+	−	+	4.8	−	+	−	Group C	−
J_{20}	Lancefield	Guinea pig	+	−	+	4.8		+	−		−
Br_2	Brown	Guinea pig									−
H_1	Own	Horse	+	−	+	4.6					−
H_2	Own	Horse	+	−	+	4.5		+	−		−
Reid	Brown	Turkey dressing	+	+	+	4.3		+	+		−

sorbitol, as devised by Edwards (1932, 1933), are of great value. The serological studies of Lancefield indicate that, with immune sera of the different groups, cultures may be definitely classified. With the results of others in evaluating the bovine strains available, studies on fibrinolysis have been made and the findings tabulated in connection with the identifying reactions.

Forty-three strains from either milk or cows have been investigated and divided into two groups: those belonging to the so-called epidemicus groups, and those considered harmless for man.

TABLE 5

Fibrinolytic activity and biochemical reactions with cultures from cows or milk, classified as human pathogenic strains

STRAIN	LABORATORY	EPIDEMIC	SOURCE	FERMENTATION OF			pH IN 1 PER CENT GLUCOSE	HYDROLYSIS OF SODIUM HIPPURATE	FERMENTATION OF		FIBRINOLYTIC TEST
				Lactose	Mannitol	Salicin			Sorbitol	Trehalose	
Lee 27A	Brown	Lee, Mass., 1928	Cow's udder	+	−	+	5.0	−	−	+	++++
C 108	Brown	Boston, Mass., 1917	Cow's udder	+	−	+	5.0	−	−	+	++++
C 2b	Brown	Canton, Mass., 1913	Milk	+	−	+	5.0	−	−	+	++++
1452	Brown	1927	Cow's udder	+	−	+	5.0	−	−	+	+++
W.Mkt.I	Brown	No epidemic	Milk	+	−	+	5.4* 4.9†	−	−	+	++++
V 10	Lancefield		Cow's udder	+	−	+	5.1	−	−	+	++++
E 56	Edwards	Kirkland, Lake Ont.	Cow's udder	+	−	+		−	−	+	++++

* Result reported by Brown, Frost and Shaw (1926).

† Result obtained in this laboratory.

The results, contained in table 5, were obtained with strains which were isolated from cows or milk and six of which were obtained at the time of human epidemics occurring in the same localities.

All of these strains possess fibrinolytic activity to a high degree. The organisms were considered by the investigators from whom they were received as being the cause of acute infection among

TABLE 6

Fibrinolytic activity and biochemical reactions with bovine cultures, not classified as human pathogenic strains

STRAIN	LABORATORY	SOURCE	FERMENTATION OF			pH IN 1 PER CENT GLUCOSE	HYDROLYSIS OF SODIUM HIPPURATE	FERMENTATION OF		LANCEFIELD SERIOLOGICAL CLASSIFICATION	FIBRINOLYTIC TEST	
			Lactose	Mannitol	Salicin			Sorbitol	Trehalose		Fibrinogen*	Plasma†
K_{150G}	Lancefield	Normal	+	−	+	4.7	−	+	−	Group C	−	−
K_{150H}	Lancefield		+	−	+	4.8	−	+	−	Group C	−	−
K_{150J}	Lancefield		+	−	+	4.7	−	+	−	Group C	−	−
K_{106}	Lancefield	Mastitis	+	−	+	4.7	−	+	−	Group C	−	−
V_8	Lancefield		+	−	−	4.4	+	−	+	Group B	−	−
V_9	Lancefield		+	−	−	4.4	+	−	+	Group B	−	−
K_{107}	Lancefield	Mastitis	+	−	−	4.4	+	−	+	Group B	−	−
B_{63}	Lancefield	Acute mastitis	+	−	−	4.5	+	−	+	Group B	−	−
B_{112}	Lancefield	Mastitis	+	−	−	4.4	+	−	+	Group B	−	−
B_{115}	Lancefield	Mastitis	+	−	−	4.6	+	−	+	Group B	−	−
B_{116}	Lancefield	Mastitis	+	−	−	4.4	+	−	+	Group B	−	−
M_{216}	Lancefield		+	−	−	4.5	+	−	+	Group B	−	−
K_{127}	Lancefield		+	−	+	4.5	+	−	+	Group B	−	−
K_{128}	Lancefield	Certified milk	+	−	−	4.8	−	+	+	Group E	−	−
K_{129}	Lancefield	Certified milk	+	+	−	4.6	−	+	+	Group E	−	−
K_{131}	Lancefield	Certified milk	+	−	−	4.6	−	+	+	Group E	−	−
39	Brown	Milk	+	−	+	5.0	−	+	−		−	−
K.Gp.14a	Brown	Milk	+	+	+	4.7	− or ±	+	+		−	−
C_{18}	Brown	Milk	+	−	−	4.7	−	−	+		−	−
E_1	Edwards	Mastitis	+	−	+	5.0	−	+	−	Group C	−	−
E_2	Edwards	Mastitis	+	−	+			+	−	Group C	−	−
E_3	Edwards	Mastitis	+	−	+			+	−	Group C	−	−
E_5	Edwards	Mastitis	+	−	+	5.1	−	+	−		−	−
E_7	Edwards	Mastitis	+	−	+	4.7	−	+	−		−	−
E_8	Edwards	Mastitis	+	−	+			+	−		−	−
E_9	Edwards	Mastitis	+	−	+	4.7	−	+	−	Group C	−	−
E_{10}	Edwards	Mastitis	+	−	+			+	−		−	−
E_{13}	Edwards	Mastitis	+	−	+	4.7	−	+	−		−	−

* Human fibrinogen coagulated with human thrombin.

† Human plasma coagulated with $CaCl_2$.

TABLE 6—*Concluded*

STRAIN	LABORATORY	SOURCE	FERMENTATION OF			pH IN 1 PER CENT GLUCOSE	HYDROLYSIS OF SODIUM HIPPURATE	FERMENTATION OF		LANCEFIELD SERIOLOGICAL CLASSIFICATION	FIBRINOLYTIC TEST	
			Lactose	Mannitol	Salicin			Sorbitol	Trehalose		Fibrinogen*	Plasma†
25261	Edwards	Placenta abortion	+	−	+	4.7	−	+	−	Group C	−	−
25505	Edwards	Brain, calf septicemia	+	−	+	4.7	−	+	−	Group C	−	−
28117	Edwards	Brain, calf septicemia	+	−	+	4.7	−	+	−	Group C	−	−
Per	Edwards	Peritonitis fatal	+	−	+	4.8	−	+	−		+	+
126 Ut A	Edwards	Metritis fatal	+	−	+	4.7	−	+	−		+	+
E_{12}	Edwards	Mastitis	+	−	+	4.7	−	+	−		+	+
Beauty	Brown	Mastitis	+	−	+	4.9	−	+	−		+	+
Blackie	Brown	Mastitis	+	−	+	4.8	−	+	−		+	+

the population drinking the contaminated milk. It is interesting to note that strain C_2b has been in artificial cultivation for twenty-one years.

The results obtained with the remaining thirty-six bovine strains are given in table 6. These cultures act in biochemical reactions and in serological classification as animal strains.

From the table it may be noted that thirty-one of the thirty-six strains are incapable of causing fibrinolysis. Five of the strains are classified as weakly positive. Repeated tests have been performed with these cultures. Occasionally no liquefaction has occurred; at other times lysis was effected, either partially or completely, in from ten to twenty-four hours. The final conclusion has been, therefore, that the strains do elaborate a small amount of fibrinolysin. In thirty-eight strains from "veterinary tissues" Madison (1934) found thirty-five to be non-fibrinolytic and three to be weakly active.

At the present time it is not possible to interpret the results

obtained with the slowly acting cultures. Studies are now in progress concerning the effect of environmental factors in the production of fibrinolysin by the bacterial cells, and on the occurrence of variants among the culture population.

The data in tables 5 and 6 indicate that the presence of fibrinolytic activity coincides, in general, with the biochemical reaction of human strains of beta hemolytic streptococci, whereas the non-lytic cultures behave, in other respects, as animal strains. Lysis accompanies with great frequency the selective action on sorbitol or trehalose of the strains which fulfill the conditions defined by Edwards for classification. The representatives of the serological groups, which constitute the subdivisions of the animal strains, were inactive against fibrin regardless of whether they belonged to group B, C, or E.

It is interesting to note in passing that, of the ten group B strains, eight fermented lactose, but failed to attack mannitol or salicin. This selectivity in fermentative reactions conforms to the *anginosus* group of Holman (1916).

Strains V 9 and B 112 were double zoned hemolytic streptococci (Brown, 1934).

The final results with the two hundred and seventeen strains of hemolytic streptococci, when tabulated with respect only to origin and fibrinolytic activity, are as follows:

Human strains	157
Fibrinolytic	154 (98.1 per cent)
Negative	3 (1.9 per cent)
Animal strains	53
Fibrinolytic	6 (11 per cent)
Negative	47 (89 per cent)

When the test was applied to the problem relating to the human pathogenicity of hemolytic streptococci isolated from milk or cows, the results were as follows:

Strains of Streptococcus epidemicus (human pathogens)	7
Fibrinolytic	7 (100 per cent)
Average time required for dissolution: 18 minutes	

Strains bovine type	36
Negative	31 (86.1 per cent)
Fibrinolytic	5 (13.9 per cent)

Average time required for dissolution: 12 hours

DISCUSSION

The results, presented in this communication, represent an attempt to correlate the fibrinolytic activity with other biological characteristics and biochemical reactions of beta hemolytic streptococci. With the selected strains, which were employed, and under the experimental conditions, which have been described in detail, the capacity of the organisms to attack human fibrin bore a definite relationship to human pathogenicity. The preponderant majority of strains isolated from cases of infection were fibrinolytic. Furthermore, streptococci from patients having severe, spreading infections often had maximal lytic potency, whereas strains from milder disorders tended to be weaker. The non-fibrinolytic strains consisted of a group which were not associated with illness or were derived from animals. These strains are, in all probability, not pathogenic for man.

Although Hadfield, Magee, and Perry (1934), and Madison (1934) did not find fibrinolysis to be as constantly associated with human strains as has been the experience in this laboratory, the authors just mentioned did report that the more potent strains came from the more serious disorders, and that the animal strains, which they tested, were inactive or very slowly lytic.

The identification of cultures of hemolytic streptococci, which are either an actual or potential menace to man, and their separation from other streptococci, which come from a variety of sources and are probably not capable of producing human disease, is a well-recognized problem. A review of the literature reveals the advances which have come from the investigations of those who have analyzed the biological and biochemical characteristics of streptococci. However, with the exception of the serological classification recently devised by Lancefield, no single test seems adequate in the evaluation of the culture brought into question. Furthermore, the properties of bacteria which endow them with

virulence consist of a complicated mosaic of attributes which include insusceptibility to phagocytosis (Hare, 1934), capacities to survive and multiply in the tissues, production of toxin, and other methods of causing tissue damage. It becomes necessary, therefore, in studying the mechanism of bacterial pathogenicity to consider both qualitatively and quantitatively, the several properties possessed by the organisms and to evaluate them with respect to the type and degree of host immunity. The particular activity of hemolytic streptococci, to which this presentation is limited, concerns a single specific bacterial property, which acts upon and alters a particular type of tissue, namely fibrin.

CONCLUSIONS

There is a relationship between the infectivity of beta hemolytic streptococci for man and the fibrinolytic property of the cultures.

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.

BIBLIOGRAPHY

BROWN, J. H. 1919 The use of blood agar for the study of streptococci. Monograph of the Rockefeller Institute for Medical Research, No. 9, New York.

BROWN, J. H. 1934 Jour. Bact., **27**, 104.

BROWN, J. H., FROST, W. D., AND SHAW, M. 1926 Jour. Inf. Dis., **38**, 381.

DENNIS, E. W., AND BERBERIAN, D. A. 1934 Proc. Soc. Exp. Biol. and Med., **31**, 976.

EDWARDS, P. R. 1932 Jour. Bact., **23**, 259.

EDWARDS, P. R. 1933 Jour. Bact., **25**, 527.

EDWARDS, P. R. 1934 Jour. Bact., **27**, 527.

GARNER, R. L., AND TILLETT, W. S. 1934 Jour. Exp. Med., **60**, 239 and 255.

HADFIELD, G., MAGEE, V., AND PERRY, C. B. 1934 Lancet, **226**, 834.

HARE, R. 1934 Jour. Path. and Bact., **38**, 129 and 143.

HOLMAN, W. L. 1916 Jour. Med. Res., **34**, 377.

LANCEFIELD, R. C. 1933 Jour. Exp. Med., **57**, 571.

MADISON, R. R. 1934 Proc. Soc. Exp. Biol. and Med., **31**, 1018.

TILLETT, W. S., EDWARDS, L. B., AND GARNER, R. L. 1934 Jour. Clin. Inves., **13**, 47.

TILLETT, W. S., AND GARNER, R. L. 1933 Jour. Exp. Med., **58**, 485.

VAN DEVENTER, J. K., AND REICH, T. 1934 Proc. Soc. Exp. Biol. and Med., **31**, 821.