STUDIES ON STREPTOCOCCAL FIBRINOLYSIS

V. The in Vitro Production of Fibrinolysin by Various Groups and Types of Beta Hemolytic Streptococci; Relationship to Antifibrinolysin Production*

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There is considerable confusion concerning the relationship of streptococcal fibrinolysin to the pathogenesis of disease. Thus Madison (1) reported that 94 per cent of beta hemolytic streptococci isolated from patients with severe infections, such as pneumonia, septicemia, empyema, and meningitis, were fibrinolytic, whereas only 17 per cent of 123 strains isolated from superficial human tissues lysed fibrin clots. Dack, Woolpert, and Hoyne (2) observed that strains of streptococci isolated from complicated cases of scarlet fever were more actively fibrinolytic than those from uncomplicated cases. Tillett (3) found 154 of 157 streptococci from various infections to be actively fibrinolytic.

During the course of investigations of streptococcal fibrinolysis it was observed that there was a marked individual *variation* in the capacity of strains of streptococci to produce fibrinolysin. In contrast was the observation that strains of streptococci isolated during a food-borne epidemic of exudative tonsillitis exhibited a *uniform*, fibrinolytic ability (4). These data seemed to

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indicate that the amount of fibrinolysin produced was a characteristic property of this epidemic strain, and suggested that the fibrinolytic capacity might therefore be a peculiar property of the individual strain of streptococcus.

Before studying the relationship of streptococcal fibrinolysin to the disease state, it was first necessary to devise a more quantitative test for measuring fibrinolysin. The present investigation describes the technique which was developed and reports the results obtained in a study of various groups and types of streptococci isolated in different sections of the country, from patients with respiratory infections and from healthy carriers. In addition, the relationship of the fibrinolytic capacity of the beta hemolytic streptococcus to the ability of the homologous organism to stimulate antibody formation was investigated.

Materials and Methods

Source of Beta Hemolytic Streptococci.—All beta hemolytic streptococci, except where noted in the text, were isolated from pharyngeal cultures of well soldiers and patients with respiratory disease stationed at Fort Bragg, North Carolina. The technique of swabbing the throat and the inoculation of the blood-agar plates will be described elsewhere (5). After incubation at 37°C. for 18 to 24 hours, the culture plates were examined for colonies exhibiting beta hemolysis. A single colony was isolated and subcultured on a second blood-agar plate. When the identity of the organism had been confirmed, an entire blood-agar plate was streaked and incubated for 18 to 24 hours. This latter plate was stored in the ice box at 4°C. and was used as a source of organisms for grouping, typing, and tests for fibrinolysin production. These procedures were usually completed within 1 month after the original isolation.

Streptococcal Grouping and Typing.—The strains of beta hemolytic streptococci were grouped according to the method of Lancefield (6) and typing[1] was accomplished by means of the capillary tube-precipitin technique of Swift, Wilson, and Lancefield (7).

ibrinolysin Assay:

Fibrinogen.—The source of fibrinogen[2] was a lyophilized preparation obtained from human plasma and was supplied by Dr. E. J. Cohn and Dr. S. Howard Armstrong, Jr. A solution was prepared containing 0.6 gm. of fraction I in 100 ml. of buffered saline.

Buffered Saline.—A solution of 0.01 M phosphate in 0.85 per cent sodium chloride at pH 7.4 was employed throughout this study.

Thrombin.—A commercial rabbit thrombin was employed in a 1 to 8 dilution.

Bacteriological Media.—Two different media were used to culture the beta hemolytic streptococcus:

1. Beef heart infusion broth containing 0.2 per cent dextrose.
2. Fibrinolysin assay broth prepared from a *single lot* of tryptose phosphate broth (de-

[1] Type -specific rabbit serums were made available through the generosity of Dr. Homer F. Swift, Dr. Rebecca C. Lancefield, and Dr. Chester S. Keefer, Director, Commission on Streptococcal Infections, Army Epidemiological Board.

[2] The product employed was fraction I of the plasma proteins prepared by the Department of Physical Chemistry, Harvard Medical School, Boston, from blood collected by the American Red Cross, under a contract recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Harvard University.

hydrated).[3] To each liter of broth was added 1.6 gm. of sodium bicarbonate in 40 ml. of distilled water. The final pH was 8.4.

Technique of Test.—Several colonies of beta hemolytic streptococci were picked from the blood-agar plate and inoculated into 5 ml. of the beef heart infusion medium containing several drops of mule blood.

Following incubation in a water bath at 37°C. for 18 to 24 hours, 0.1 ml. was transferred to 5 ml. of beef heart infusion broth containing 0.1 ml. of mule blood. After 8 hours' incubation, 0.1 ml. of the supernatant was transferred to 5 ml. of the assay broth, and at the same time a blood-agar plate was inoculated to test for purity. At the end of 18 hours' incubation in the water bath all cultures showing visual turbidity after shaking were centrifuged at 2500 R.P.M. for 20 minutes. One ml. of the supernatant broth was then removed to 4 ml. of buffered saline. Serial twofold dilutions in buffered saline were made in a series of nine chemically cleaned Wassermann tubes. The initial dilutions ranged from 1:5 to 1:1280 and were contained in a total volume of 1 ml. Automatic pipettes were then employed to add 0.5 ml. of the fibrinogen solution, followed by 0.2 ml. of thrombin. The racks containing the tubes were shaken immediately (gently). Clotting occurred regularly within 2 to 3 minutes. The tubes were then incubated in a water bath at 37°C. for 60 minutes. The titer of fibrinolysin was taken as the highest dilution which lysed the clot at the end of this period. A clot was considered lysed if it poured or slid readily when the tube was inverted.

Evaluation of Methods

Since interest in this study was centered not only on the presence or absence of fibrinolysin, but also on the quantitative production of fibrinolysin, it was first necessary to establish the reliability of the techniques employed. A definite but limited degree of variation was found in the technique of assay.

Early in the study two assays were made on each strain of beta hemolytic streptococcus isolated. The second assay was performed the day following the first and the source of the inoculum for the 8 hour culture was the 8 hour blood-broth culture which had been stored overnight at 4°C. Thus, the difference between the two assays was that the organisms used in the second determination had been passed through an additional blood-broth culture.

The results of such duplicate tests on 213 strains of group A beta hemolytic streptococci isolated from different subjects are presented in Table I. The amount of fibrinolysin produced by 83 strains (39 per cent) was identical in both assays, and in 92 additional strains (43 per cent) there was a difference of only one dilution increment. A difference of 2 dilution increments was shown by 25 streptococci (12 per cent), and in only 13 strains (6 per cent) was the variation greater than 2 dilutions. Thus, the assays were reproducible within 1 dilution increment in 82 per cent of the tests, and only 6 per cent of the strains exhibited considerable variation in their fibrinolytic titer.

In the first assay there were 8 strains which failed to lyse the fibrin clot in a dilution of 1:5. The following day, 4 of these 8 strains produced measurable amounts of fibrinolysin. In the second assay the total number of strains which failed to produce lysis was 9, but 5 of these were fibrinolytic in the first

[3] Difco.

test. A total of 71 strains (33 per cent) exhibited a greater fibrinolytic capacity in the first run as compared to the second test, and 59 (28 per cent) produced more fibrinolysin in the second test than in the first determination. These variations appear to be due to differences in the technique of testing, rather than to the number of passages in artificial media.

The data presented in Table I demonstrate the variations that may be expected when duplicate determinations are made on successive days on cultures of the same original colony of beta hemolytic streptococcus. They

TABLE I

Duplicate Fibrinolysin Assays of 213 Group A Streptococci

Titer of fibrinolysin 2nd Assay \ Titer of fibrinolysin 1st Assay	<5	5	10	20	40	80	160	320	640
<5	4	1		2		1	1		
5				1			1		
10	1		2		1				
20		1	1	5	5	3			
40	2	1	1	3	14	9	5	1	
80				5	7	17	18	2	
160	1	1		1	2	14	21	9	1
320				1		2	14	17	10
640								1	3

Figures refer to number of strains with the indicated titers.

do not indicate, however, the variations which may occur when multiple determinations of one strain are made on the same day.

A study of a type 5 food-borne outbreak of streptococcal sore throat showed that the organisms isolated from the throat exhibited a remarkably uniform ability to produce fibrinolysin (4). The assays in that study were all performed in a single test. Included in the assays were 20 strains obtained on the day the patients entered the hospital and 20 strains isolated from the same individuals 6 weeks later. These organisms had been stored in the frozen state (−70°C.) for a period of 6 months before testing. The results of these determinations are shown in Table II. The amount of fibrinolysin produced by 18 of the 20

strains isolated at the time of admission to the hospital, and 20 strains isolated 6 weeks later was identical. This demonstrates in a striking manner the uniform ability of 40 strains of type 5 streptococci (all originally from the same source), obtained 6 weeks apart from 20 patients, to produce fibrinolysin when determined by tests performed on 1 day.

In contrast with the lack of variation in the amount of fibrinolysin produced by 40 strains of type 5 streptococci, as determined in a single test, were the results obtained when tests were performed on different days. One such study is reported in Table III. For a period of 175 days daily cultures of the oropharynx were taken from a healthy volunteer who was a carrier of type 19 beta hemolytic streptococcus. During this period 76 type 19 strepto-

TABLE II

Results of Fibrinolysin Assays Performed in One Test on 40 Type 5 Streptococci Isolated from 20 Patients

Case No.	Fibrinolytic capacity of streptococci isolated at		Case No.	Fibrinolytic capacity of streptococci isolated at	
	Onset	6 wks.		Onset	6 wks.
	titer	*titer*		*titer*	*titer*
201	80	80	227	80	80
202	320	80	228	40	80
203	80	80	234	80	80
207	80	80	235	80	80
208	80	80	238	80	80
213	80	80	272	80	80
215	80	80	273	80	80
216	80	80	276	80	80
217	80	80	291	80	80
225	80	80	297	80	80

cocci were isolated and the fibrinolytic capacity of each strain determined shortly after the organism was identified. Although the majority of the strains produced lysis in a dilution of 1:20 to 1:80, in approximately 14 per cent the clots were lysed only in dilutions of 1:10 or less. These variations are of the same magnitude as observed in duplicate assays of the same original strains (Table I). They define the limits within which quantitative interpretation may be placed on the following data. About 3 per cent of the time a strain that presumably is a moderately good producer may be negative by this assay.

Fibrinolytic Ability of Different Groups of Beta Hemolytic Streptococci

Between October, 1944, and June, 1945, cultures were taken from approximately 1500 soldiers with respiratory infections one or more times during the

first 3 days of hospitalization. In addition, several cultures of the throat were made from 2500 soldiers on active duty. The fibrinolytic capacity of the various strains of beta hemolytic streptococci isolated from these two groups of soldiers was determined after the organisms had been grouped and typed. In all, over 2500 assays were done on streptococci isolated from approximately 800 subjects.

TABLE III

The Production of Fibrinolysin by 76 Strains of Type 19 Streptococci Isolated Intermittently from a Normal Carrier during a Period of 175 Days

Titer of fibrinolysin	No. of type 19 strains isolated on different days	Per cent of type 19 strains isolated
< 5	2	3
5	2	3
10	6	8
20	22	29
40	26	34
80	17	22
160	1	1
Totals.....	76	100

TABLE IV

*Fibrinolysin Titers of 766 Strains of Beta Hemolytic Streptococci According to Lancefield Group**

Beta hemolytic streptococcus group	Fibrinolysin titers										Total strains	Median titer
	<5	5	10	20	40	80	160	320	640	1280		
A (typable)	13‡	4	6	19	22	42	20	11	2	1	140	92
A (non-typable)	20	8	8	27	46	74	72	38	18	1	312	131
B	54	2	2			1					59	<5
C	22	3	9	20	29	22	20	10	2		137	61
G	38	3	12	9	15	17	7	3			104	20
F	11				2	1					14	<5

* Each strain was isolated from a different individual.

‡ Number of strains with the indicated fibrinolysin titer.

The fibrinolytic capacity of 766 strains of beta hemolytic streptococci is shown in Table IV according to the Lancefield grouping. There were 452 group A strains of which 419 or 93 per cent lysed fibrin clots in a dilution of 1:5 or greater. Similarly, 84 per cent of group C, 73 per cent of group G, 21 per cent of group F, and 8 per cent of group B strains exhibited lysis in at least 1:5 dilution.

Not only were most of the group A organisms fibrinolytic, but they produced

more fibrinolysin than the other groups. This is indicated by the median titer of the various groups. For all group A streptococci the median titer was 117, for group C, 61, group G, 20, and groups F and B less than 5. It is thus apparent that although groups A, C, and G were generally fibrinolytic there was a considerable quantitative difference in their ability to produce this substance. It is also to be noted that a few strains of groups B and F streptococci were found to cause lysis of the fibrin clot.

Fibrinolysin Production by Various Types of Group A Streptococci

Of the 452 group A strains, 140 were typed and the remainder were non-typable with the sera employed.[4] The median titer of the non-typable group A streptococci was somewhat greater than the titer of the typable strains (Table IV). The fibrinolysin production of the typed strains is shown in Table V (Fort Bragg 2). Included also in this table are the results of tests on other typed group A organisms obtained from various sources. Each set of strains of a given type are presented separately since they not only were obtained from various parts of the country, but the organisms were handled differently following isolation.

Fort Bragg 1 strains were isolated in the late winter and spring of 1944 from patients with respiratory infections and from normal soldiers. These strains had been either lyophilized or frozen in milk and stored at −70°C. for a period of 6 months before testing. Each organism, therefore, had probably been passed through at least ten cultures before the fibrinolytic capacity was tested.

Fort Bragg 2 strains were isolated in 1944–1945 and had undergone a minimum of cultivation in the laboratory before testing. The method of handling these organisms was described above.

Fort Bragg 3 organisms were isolated during the month of March, 1946, from patients with scarlet fever and the fibrinolytic capacity was tested within 6 weeks after isolation. The type 30 organisms were responsible for a small outbreak of scarlet fever during this period.

Fort Lewis. These organisms were isolated in the winter and spring of 1945 at Fort Lewis, Washington, and were supplied by Dr. Morton Hamburger, Jr. About half of the strains were obtained from hospitalized soldiers, the remainder came from carriers. Most of the streptococcal infections occurred sporadically, although some of the strains of the same type may have come from a parent source.

The number of passages that these organisms had undergone prior to testing is not known but they had been stored on blood-agar slants for at least 4 to 6 months.

New York strains were received in the lyophilized state from Dr. Sidney Rothbard. The isolations had been made in 1941–1944 from patients with scarlet fever, rheumatic fever, or pharyngitis.

Boston. The organisms supplied by Dr. T. Duckett Jones were isolated from patients in the House of the Good Samaritan during the preceding 2 years, and had been stored in the lyophilized state.

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[4] The following typing sera were employed: types 1, 2, 3, 4, 5, 6, 8, 9, 11, 12, 13, 14, 15, 17, 18, 19, 22, 23, 24, 26, 28, 29, 30, 31, 33, 34, 36, 37, 38, 39, 40, 41, 42, 43, 44, 46, and 47.

TABLE V

Fibrinolytic Capacity of 372 Different Strains of Group A Streptococci According to Type*

Beta hemolytic streptococcus group A		Fibrinolysin titers									
Source	Type	<5	5	10	20	40	80	160	320	640	Total strains
Fort Bragg 1	1	4‡		1	1		2				8
Fort Bragg 2	1	1	1		2	3	4	4			15
Fort Lewis	1	1	1	1	6	3	1				13
Totals	1	6	2	2	9	6	7	4			36
Fort Bragg 1	3	16	1				4	7	4		32
Fort Bragg 2	3	3			1		1				5
Fort Bragg 3	3	4									4
Fort Lewis	3	5						2			7
New York	3	9				1					10
Totals	3	37	1		1	1	5	9	4		58
Fort Bragg 1	5				1	1	2	3			7
Fort Bragg 2	5			1			3		1		5
New York	5					4	2	1			7
Boston	5				1						1
Texas	5				1						1
Totals	5			1	3	5	7	4	1		21
Fort Bragg 1	6					3	8	4			15
Fort Bragg 2	6				4	4	8				16
Fort Lewis	6					5	6	1			12
Totals	6				4	12	22	5			43
Fort Bragg 1	12	1					1	3	5		10
Fort Bragg 2	12	5	1		1	1	2	7	3	1	21
Fort Lewis	12				1		2	2	7		12
Totals	12	6	1		2	1	5	12	15	1	43
Fort Bragg 2	14	1	1			1	1	2	1		7
Fort Lewis	14						2	6	4		12
Totals	14	1	1			1	3	8	5		19
Fort Bragg 3	17					4	2				6
Fort Lewis	17	1				1	8	3			13
New York	17					1					1
Totals	17	1				6	10	3			20

TABLE V—*Concluded*

Beta hemolytic streptococcus group A		Fibrinolysin titers									
Source	Type	<5	5	10	20	40	80	160	320	640	Total strains
Fort Bragg 2	18	1		4	1	5	6	1			18
Fort Bragg 1	19					2	15	4	6		27
Fort Bragg 2	19				3	5	7				15
Fort Bragg 3	19				2	4					6
Fort Lewis	19		1			3	6	1	1	1	13
New York	19					1					1
Boston	19	1			1	1					3
Atlanta	19					4					4
Totals	19	1	1		6	20	28	5	7	1	69
Fort Bragg 2	24	2	1		3	1	2		1		10
Fort Bragg 1	26						2	2	2		6
Fort Bragg 2	28							2	2		4
Fort Bragg 2	29			1	1	1	1				4
Fort Bragg 3	30					13	4				17
Fort Bragg 2	46				1		3				4

* 16 strains which were typed are omitted because less than 4 organisms were present in each type.

‡ Number of strains with the indicated fibrinolysin titer.

Fort Bragg 1, isolated in winter 1944.

Fort Bragg 2, isolated in winter 1944–1945.

Fort Bragg 3, obtained from cases of scarlet fever in March, 1946.

Texas. The type 5 streptococcus was isolated by Dr. R. Pike from a patient with a sore throat in March, 1945, and was stored on a blood-agar slant until tested in May, 1946.

Atlanta. Dr. Carl T. Nelson supplied these organisms which were recently isolated from hospitalized patients and had been stored on blood-agar slants.

It is apparent from the results shown in Table V that the distribution of the fibrinolytic capacities of a series of organisms of the same type was, in general, similar, irrespective of the year or part of the country in which the organism was isolated. Furthermore, lyophilization or the age of the culture apparently did not interfere markedly with the fibrinolytic capacity. For example, the type 3 streptococci isolated at Fort Bragg in the winter of 1944, 1944-1945, and in March, 1946, all exhibited a similar lack of fibrinolytic ability. Strains from New York and Fort Lewis were essentially similar. Two of the sets of type 3 organisms had been lyophilized, and yet a number were fibrinolytic, whereas none of 4 strains obtained in 1946 from patients with scarlet fever, and tested shortly after isolation, causes lysis of the fibrin clot.

The results obtained with the Type 6 organisms were striking. One group had been lyophilized in 1944 (Fort Bragg 1), one had been recently isolated (Fort Bragg 2), and the final group (Fort Lewis) had been stored for 6 months on blood-agar slants. These organisms all produced similar quantities of fibrinolysin.

There were some instances, however, where the fibrinolytic capacities varied somewhat within a given type, depending on the source of the organism. An example is shown in type 12 organisms, where 5 of 21 strains isolated at Fort Bragg in 1944–1945 failed to lyse the fibrin at a dilution of 1:5 and only 1 of

TABLE VI

Median Titers and Average Production of Fibrinolysin According to the Type of Group A Streptococcus

Type	Total strains	Median titer	Distribution by titer groups			
			Titer of less than 5	Titer of 5 to 39	Titer of 40 to 159	Titer of 160 or greater
			per cent	*per cent*	*per cent*	*per cent*
3	58	<5	64	3	10	22
24	10	33	20	40	30	10
1	36	38	17	36	36	11
18	18	64	5	28	61	5
30	17	66	0	0	100	0
5	21	97	0	19	57	24
19	69	99	1	10	70	19
6	43	100	0	9	79	12
17	20	104	5	0	80	15
14	19	230	5	5	21	68
12	43	283	14	7	14	65

10 strains isolated the year before and subsequently frozen failed to lyse the clot. Twelve type 12 strains from Fort Lewis were all fibrinolytic.

A comparison of the fibrinolytic capacities of the separate types of group A streptococci showed considerable differences. This is shown in Table V and in summary form in Table VI where the median titer as well as the distribution of the fibrinolytic capacities of each series of organisms containing 10 or more strains of a given type is recorded. The median titer ranged from <5 in the group of type 3 streptococci to 283 for the type 12 organisms. Types 3, 24, and 1 produced small amounts, types 18, 30, 5, 19, 6, and 17, moderate amounts, and types 14 and 12 large amounts of fibrinolysin.

Not only was there a difference in the median titer of fibrinolysin of the

various types, but also the distribution varied according to type, thus, 64, 20, and 17 per cent of types 3, 24, and 1 streptococci, respectively, failed to lyse the clots in a 1:5 dilution. In the type 12 organisms, which had a high median titer, 14 per cent did not exhibit fibrinolysis but the majority of the remaining strains exhibited titers of over 160. Of the type 3 organisms which were fibrinolytic, two-thirds produced large amounts of fibrinolysin. In contrast are types 24 and 1 which produced low to moderate amounts when any fibrinolytic ability was exhibited.

The striking consistency of the fibrinolysin production by type 30 streptococci is probably explained by the fact that they were all isolated during an epidemic, and the organisms were tested on one day, which, as shown above, tends to decrease the variation in the test. The type 17 streptococci, on the other hand, were isolated from three widely separated parts of the country and were tested on different days, and yet 80 per cent of the strains produced fibrinolysin in titers of between 1:40 to 1:80.

Fibrinolysin Production of Streptococci Isolated from Cases and from Carriers

The fibrinolytic capacity of strains of streptococci of the same type did not appear to vary according to whether they were isolated from normal carriers or from patients with infection. For example, of the 15 type 6 strains isolated at Fort Bragg in the spring of 1944, 3 were obtained from patients with streptococcal infections, the remainder from carriers. Two of the 16 strains isolated in 1944–1945 were from streptococcal infections, and all but 3 of the Fort Lewis strains were isolated from hospitalized patients who presumably had streptococcal pharyngitis. Thus, strains of type 6, isolated from cases with infection and from carriers produced similar amounts of fibrinolysin.

Six of the type 19 streptococci (Fort Bragg 3) were from cultures from the throats of patients with scarlet fever, and most of the remainder of the strains isolated at this post were from oropharyngeal cultures of carriers. In both instances similar quantities of fibrinolysin were produced.

The type 3 streptococci varied in their fibrinolytic ability, but this variation could not be correlated with the source of the organism. Of the 16 strains that produced no fibrinolysin (Fort Bragg 1, Table V), 11 were isolated during the acute phase of streptococcal infections, and 5 were from carriers. Of the 16 strains (Fort Bragg 1) that produced fibrinolysin, 5 were from cases, and 11 from carriers.

The data presented in Table II are interesting in this regard, since half of the type 5 strains were isolated during the acute phase of illness, and half 6 weeks later. All except 2 strains produced similar amounts of fibrinolysin, demonstrating that the fibrinolytic capacity of organisms from convalescent carriers did not change. Half of the patients in this epidemic received sulfadiazine

for a period of 5 days. Such therapy, however, did not affect the production of fibrinolysin.

Relation of Fibrinolysin Production to the Ability of the Organism to Stimulate Antibody Formation

A preliminary report (8) has demonstrated that antifibrinolysin production following infection is related to the fibrinolytic capacity of the infecting organism. These observations were extended in the present study. Acute and convalescent phase serum specimens were available for antibody determination from approximately 90 per cent of the hospitalized subjects from whom beta hemolytic streptococci were isolated. A patient was considered to have a streptococcal infection if an increase in titer of 2 dilution increments of either

TABLE VII

The Antifibrinolysin Response in 56 Cases of Proved Streptococcal Infections According to the Fibrinolytic Ability of the Infecting Strain*

Fibrinolytic capacity of the infecting strain	Total No. cases	No. of cases with increase of antifibrinolysin	Per cent with increase of antifibrinolysin
Titer of 5 or less	26	6	23
Titer of 160 or greater	30	22	73

* An increase in antistreptolysin or antifibrinolysin titer of at least 2 dilution increments was demonstrated in the convalescent serum collected from each of these patients.

antifibrinolysin or antistreptolysin was demonstrated in the convalescent phase serum specimen (9).

For the present analysis, only those patients infected with organisms which produced fibrinolysin in a titer of 1:5 or less and those infected with streptococci which elaborated fibrinolysin in titers of 1:160 or greater were considered. As shown in Table VII, a total of 56 such patients had streptococcal infections caused by these organisms. Twenty-six patients were infected by strains producing little or no fibrinolysin, and only 6 or 23 per cent exhibited an increase in antifibrinolysin during convalescence. This is in contrast to 22, or 73 per cent of 30 patients who were infected by streptococci elaborating large amounts of fibrinolysin *in vitro* and who showed an increase in the antifibrinolysin titer of the serum following infection.

RECAPITULATION AND DISCUSSION

The studies reported in the present paper were undertaken in an attempt to clarify and extend the knowledge of the relationship of streptococcal fibrinolysis to infection. Progress in this field has been limited in the past by the lack

of a sufficiently quantitative method for measuring the fibrinolysin production of strains of beta hemolytic streptococci. Most assays have been made by incorporating the organisms in plasma clots and observing the time in which complete lysis occurred. The rapidity with which liquefaction occurred provided a rough index of the amount of fibrinolysin produced.

Recent studies of the mechanism of streptococcal fibrinolysis have facilitated the development of a more quantitative test (10, 11). It has been shown that fibrinolysin produced by the beta hemolytic streptococcus activates a substance, lytic factor, (12) which is normally present in human plasma. This activated protease is responsible for the liquefaction of the fibrin clots. By the use of a purified fibrinogen preparation which contained an adequate and constant amount of lytic factor it was possible to devise a simple method for testing fibrinolysin production. In this test the concentration of fibrinolysin was measured by determining the smallest amounts that liquefied the standard fibrin clot after 60 minutes' incubation.

By using this method, more reproducible results were obtained. Multiple tests performed in an identical manner on the same day yielded highly consistent results. Variations were still observed, however, from day to day, which presumably were related to uncontrollable differences in the beef heart infusion broth used to grow the streptococcus. In order to reduce these variations to a minimum, a standard assay broth was finally adopted, which consisted of one lot of dehydrated tryptose phosphate. By the use of a large inoculum of a rapidly growing culture of the test organisms, adequate growth in this broth was usually obtained. The amount of fibrinolysin produced in this medium was not maximal, but the variation from day to day was considerably reduced. By testing a large number of strains in duplicate, the limits of variation of this method were defined; and although there is need for further standardization of the technique, it is believed that the results of the present study are more quantitative than those which have been reported previously.

It has been observed previously (13) and also confirmed in this laboratory that strains of beta hemolytic streptococci occasionally lose their fibrinolytic ability upon prolonged passage in artificial media. In the present study, however, the fibrinolytic capacity did not appear to vary with the methods of storage nor with a limited number of passages of the test organisms. Thus, the fibrinolytic production of streptococci seems to be reasonably stable and, at least, not to vary as much as was previously thought.

Other studies have indicated that the majority of group A streptococci are fibrinolytic, (14, 15) and this observation was confirmed in the present study. Of the groups C and G strains, 84 and 63 per cent respectively produced fibrinolysin. This is essentially in agreement with the findings of others (14–17).

Fibrinolytic properties have not been observed in other Lancefield groups of beta hemolytic streptococci (18, 19). Five of 59 group B and three of 14 group F streptococci reported here produced small amounts of fibrinolysin.

Not only do most of group A organisms possess fibrinolytic properties, but, in general, they produce large amounts of the substance in comparison with the Lancefield groups C and G. The median titer for group A organisms was 117, group C, 61, and group G, 20.

In a preliminary study, evidence was obtained that fibrinolysin production was also related to the type of group A streptococcus (8). In the present study, therefore, strains of the various types were collected over a 3 year period at Fort Bragg and also from other sections of the country. In each instance the strain was isolated from a different individual. Many of the typed streptococci at Fort Bragg were obtained from recruits within 1 week after their arrival at this army post from various induction stations situated east of the Mississippi River. Practically all strains at Fort Bragg were isolated from recruits who remained there for less than 4 months. It would appear, therefore, that the majority of the streptococci within a given type were not derived from an immediate common source. This factor is of some importance, since, as expressed above, organisms derived from a common source exhibit similar fibrinolytic properties. To establish the relationship of the serological types to fibrinolysin production, therefore, a number of strains were obtained from multiple sources.

Although the majority of group A strains of beta hemolytic streptococci produce moderate amounts of fibrinolysin the data (Table V) suggest that fibrinolysin production is related to the type of streptococcus. Perhaps the most striking example was the production of fibrinolysin by the type 6 organisms. Fifteen strains collected at Fort Bragg in the late winter of 1944, 16 strains isolated during 1944–1945 at the same post, and 12 strains obtained from Fort Lewis, all lysed fibrin clots in a dilution of from 1:20 to 1:160. Thirty-four of the 43 strains lysed the fibrin clots in a dilution of 1:40 or 1:80. Type 5 streptococci from North Carolina, New York, Massachusetts, and Texas produced moderate amounts of fibrinolysin. In marked contrast were the results with the type 3 organism. In this series, the majority of the strains isolated over a 3 year period at Fort Bragg, and most of the organisms from New York and Fort Lewis, failed to cause lysis in a dilution of 1:5.

Although a rather consistent pattern of fibrinolysin production was exhibited by series of organisms from various sources within a given type, it is to be emphasized that some types are apparently characterized by organisms that produced amounts of fibrinolysin within a narrow range, while others exhibit a wide range. Thus types 6 and 17 streptococci were remarkably consistent in their fibrinolytic capacity.

In types 1, 12, 14, 18, and 24 the streptococci varied tremendously in their

ability to produce fibrinolysin. Further studies are required, however, before such variations may be established as a characteristic of these types.

By the use of median titers, the general differences between the types of streptococci were shown in a striking fashion. Thus 58 type 3 organisms had a median titer of <5, whereas a median titer of 283 was exhibited by 43 type 12 organisms. On this basis, then, types 1, 3, and 24 produced small amounts, types 5, 6, 17, 18, 19, and 30 moderate amounts, and types 12 and 14 large amounts (Table VI).

These studies clarify to some extent the results reported in the past concerning the relationship of steptococcal fibrinolysis to infection (18). Several of these reports have indicated that streptococci obtained from patients with severe or suppurative infections were markedly fibrinolytic, whereas organisms isolated from mild infections produced little or no fibrinolysin. The results reported with strains isolated from infectious processes, however, have not been consistent. Thus, Tillett (3) reports his observations on 157 strains of beta hemolytic streptococci isolated from various diseases and found 154 were fibrinolytic. Madison (1), in contrast, found fibrinolytic properties in only 17 per cent of 123 strains from patients with a variety of infections. It has been suggested that these differences may be due to absence of lytic properties in the strains from minor infections or they may have been caused by laboratory cultivation of the organism prior to the time to testing (18).

On the basis of the present studies, it appears that the variations described previously may have been due to the type of organism studied, and also to the assay medium used to cultivate the streptococci. The majority of the group A strains were isolated from healthy subjects and most of them were found to exhibit fibrinolytic properties when tested. Some of the type 6 organisms were obtained from proved streptococcal infections, others from carriers, and yet they all produced similar quantities of fibrinolysin. Six of the type 19 streptococci (Fort Bragg 3) were from cultures from the throats of patients with scarlet fever, and most of the remainder of the strains isolated at this post were obtained from cultures from well soldiers. In both instances, similar quantities of fibrinolysin were produced. These data, though limited, suggest that fibrinolysin production is not necessarily related to the disease-producing capacity of the organism.

Other studies have indicated that there is a relationship between the production of antifibrinolysin following infection and the type of group A streptococcus causing the disease (8, 20). Further studies established a relationship between the *in vitro* fibrinolytic capacity of the infecting organisms and antifibrinolysin formation (8, 20). The present investigations confirmed these findings. Of the 26 *proved* streptococcal infections caused by organisms producing little or no fibrinolysin when tested in the routine manner, 6 or 23 per cent of the patients exhibited an increase in fibrinolysin antibodies during

convalescence. In contrast, 73 per cent of 30 patients infected with organisms producing large amounts of fibrinolysin exhibited a specific antibody response.

Thus, there was a distinct correlation between the antifibrinolysin response in patients and the fibrinolytic capacity of the infecting strains. The six cases in which a response was detected in the absence of appreciable fibrinolysin production of the strain warrant special comment. It seems probable that these strains actually produced considerable fibrinolysin *in vivo* which was not detected in the standardized *in vitro* test used in this study. As mentioned previously, the culture medium used as routine was not ideal. In exploratory studies of this problem, certain strains of type 3 organisms which failed to produce fibrinolysin in the routine test were retested after growth in beef heart infusion broth buffered with Mote's modification of the Hodge and Swift buffer (21). Under these more ideal but less standardizable conditions, most of these organisms were found to produce limited amounts of fibrinolysin.

It seems likely, therefore, that many of the variations which have been observed in the fibrinolysin production of beta hemolytic streptococci in the past may be related to variations and irregularities in the tests used and in the media on which the organisms are grown. The studies reported in the present paper in which a more quantitative and more reproducible test was employed failed to confirm many of the relations which have been reported previously. There is still need, however, for further standardization and quantitation of the methods of determining fibrinolysin.

SUMMARY

A method for the measurement of fibrinolysin production by beta hemolytic streptococci is described. The test was shown to be highly accurate in that repeated determinations showed only small variations. A study of 766 strains of beta hemolytic streptococci isolated from normal soldiers and patients with respiratory disease showed that fibrinolysin was produced by Lancefield groups A, C, and G, and, in addition, by a few strains of groups B and F.

Group A streptococci produced more fibrinolysin on the average than the other groups. The median titers were 117 for group A, 61 for group C, and 20 for group G streptococci. In a study of 388 typed group A streptococci from different subjects the fibrinolytic capacity of an organism was shown to be related to the serological type. The importance of this observation in relation to the rôle of streptococcal fibrinolysis in infections is discussed.

Finally, it was demonstrated that strains of streptococci which produced large amounts of fibrinolysin were capable of stimulating antifibrinolysin formation in patients whereas strains that produced small amounts only occasionally caused antibody formation.

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