THE FIBRINOLYTIC ACTIVITY OF HEMOLYTIC STREPTOCOCCI

WILLIAM S. TILLETT

Department of Bacteriology, New York University College of Medicine

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The fibrinolytic activity of hemolytic streptococci is a term used to designate the capacity of broth cultures of *Streptococcus hemolyticus* of the *beta* type to transform the solid clot of normal human blood into a liquid state. The rapid dissolution of human fibrin by hemolytic streptococci is dependent upon the presence in cultures of an extracellular enzymic substance which is excreted by the living organisms. Reports in the literature evidence the fact that the phenomenon has special characteristics of bacteriological and immunological interest.

The fact that the reaction involves a special kind of bacterial product acting upon a special kind of tissue substrate illustrates the particular qualities of streptococcal fibrinolysis. The process

seems to differ from the catabolic action of proteolytic enzymes which reduce complex protein material to split products of relatively simple chemical composition. Furthermore, a possible correlation with bacterial toxins, which are excreted extracellularly, remains uncertain in the present state of knowledge. However, the phenomenon appears to belong to the types of reaction which include enzymes and toxins, and, for this reason, warrants consideration from a biological standpoint and also as a possible agency in the mechanism of infections due to hemolytic streptococci.

It is the purpose of this article to review and to attempt to evaluate, when possible, the published reports concerning fibrinolytic action of streptococci and other bacteria. The editors of *Bacteriological Reviews* have urged their authors "to distinguish between the essential detail and the isolated, vanishing particular." The fulfillment of these conditions is rendered particularly difficult in the present report because of the fact that the investigations have developed only in recent years. From the published articles of numerous investigators, it is possible to define more clearly some phases of the reaction. However, lines of study suggested by certain factors of streptococcal fibrinolysis have yielded results which have served to broaden the scope of inquiry. Since these findings in many instances constitute new data, it is apparent that final conclusions cannot be drawn for the present. All the reports, which this author has encountered, have been included for the purpose of bringing the subject matter up to date, even though the diversity of some individual researches and the fragmentary reports of others renders difficult a critical assay of some of the results.

The terminology used in reference to some phases of the reaction is to some extent unsatisfactory. Certain phrases are perhaps awkward or confusing. However, at the present time, it would appear to be premature to offer a glossary of fixed expressions. In the current state of knowledge it seems desirable to refer to the phenomenon in terms which emphasize certain special conditions, not for the purpose of imposing exact and restricting definitions, but rather to identify the reaction by specifying the particularly striking features.

I. MATERIALS AND METHODS OF EXPERIMENTAL PROCEDURES

The fibrinolytic reaction of cultures of hemolytic streptococci is readily demonstrable without any unusual precision of technique. The occurrence of the phenomenon has been commonly encountered in tests with large numbers of strains of hemolytic streptococci. However, laboratory conditions which may affect the results, have been noted by several observers. Some of the conditioning factors assume importance only in certain types of technical procedure which will be referred to under appropriate headings. Other experimental details, however, require more careful general consideration because they need to be taken into account in interpreting results. They also indicate some of the biological factors which influence the production of fibrinolysin by the bacterial cells. From the standpoint of critical analysis it is, therefore, advantageous to review, first, data concerning experimental procedures.

One of the interesting aspects of the reaction concerns the source of the materials used in obtaining the lysis of fibrin by cultures. For example, the characteristically rapid and complete liquefaction of fibrin is usually most strikingly demonstrable when, on the one hand, the bacterial constituent of the test consists of cultures of hemolytic streptococci derived from *man*, and on the other hand, the fibrin, which serves as substrate, is also obtained from *man*. Furthermore, negative or inconclusive results are most frequently obtained when cultures isolated from animal sources are tested against human fibrin, or when cultures derived from human infections are tested against animal fibrin. As will be shown later, the findings just mentioned are not absolute, but are dependent upon quantitative as well as qualitative factors.

The experimental conditions under which the clot-dissolving property of cultures is most satisfactorily obtained consist in mixing the cultures with plasma or fibrinogen before inducing clot formation. By this procedure the organisms and their products are disseminated within the body of the clot as it forms, thus affording maximum surface contact between the active bacterial agent and the fibrin substrate.

It has been noted by several observers (Tillett and Garner (65), Hadfield, Magee, and Perry (19), Madison (31), Dack, Woolpert and Hoyne, (4), Schmidt (57), and others) that the time required for dissolution of normal human fibrin by active cultures varies from a few minutes with some strains to a partial effect exerted by other strains during twenty-four hours' incubation. 0.5 cc. of broth culture plus 0.2 cc. of a 1 to 5 dilution of plasma have been frequently used in tests and have given satisfactory results. Some investigators have, for convenience, employed approximate fractions or multiples of the ratio given above. The evidence is clear that the differences in speed and completeness of fibrin dissolution exerted by strains is dependent upon quantitative differences in the amount of fibrinolysin excreted by the cultures. Consequently, it is obvious that the demonstration of the occurrence of lytic action by strains, as well as the degree of potency, may be conditioned by the quantities of reagents selected for use.

The importance of the quantitative factor is also indicated by the report of Madison (33) concerning results obtained following the concentrations of fibrinolysin contained in cultures of several strains. (The methods of concentration will be described later.) Madison found that among 123 strains only 17 per cent were considered to be actively fibrinolytic when the tests were made with 0.5 cc. of broth culture. However, the percentage of demonstrably positive strains was raised to 35 per cent when cultural material which had been concentrated approximately twentyfold was employed.

The quantitative production of fibrinolytic substances by strains is also related to the cultural conditions under which the tests are made. Madison and Taranik (39) compared the curve of bacterial growth with the production of fibrinolysin, and found that "test tube proliferation" of the bacterial cells paralleled the rate of production of the lytic enzyme. Using quantitative titration, they were able to demonsrate lytic activity with cultures after a few hours' incubation and they also noted that the production of fibrinolysin was maximum when the phase of growth was nearest the peak, which was reached after approximately 12 to 14 hours. After this point in multiplication had

been reached, the production of fibrinolysin was markedly retarded, although some of the cultures retained maximum activity after twenty-four hours' incubation. The experiments did not indicate with certainty whether or not enzyme production requires cell division.

Without making quantitative titrations, Tillett and Garner (65) noted gradual deterioration of lytic activity in cultures which were kept in the incubator for several days. Decrease in potency was delayed, but not entirely arrested, by ice-box temperatures. Schmidt (57) found cultures five to six days old to be active, but he did not report measurements of activity.

It may be seen from the findings just cited that fibrinolysis by active cultures may be demonstrated within wide ranges with respect to age of cultures. However, it is also brought out that greatest activity is determined both by the abundance of growth of the strains and by the time in the phase of growth at which tests are made. With bacterial strains of high fibrinolytic activity the qualitative demonstration of lysis requires no special attention to cultural details. However, 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.

There is, also, suggestive evidence that additional elements in culture media, in the nature of accessory substances, may promote or retard the yield of fibrinolysin by streptococci. There are no published reports dealing with this point. However, the author has noted that when selected strains were cultivated simultaneously in samples of culture media containing different ingredients, the lytic potency of individual strains varied, even though the amounts of growth seemed to be comparable in the different kinds of media. When one considers by analogy the effect of culture media on the production by other bacteria of products such as toxins, it seems likely that the specific stimulation or impairment of the elaboration of fibrinolysin may be subject to conditions of the same order.

Whether variations in the potency of cultures depends upon

differences in the number of individual cells of the culture population, which excrete fibrinolysin, or is referable to the amount of the enzyme produced equally, in any single culture, by all the cells, has not been studied. The problem is common to the broader question concerning bacterial adaptation and selection, which, with respect to enzymes, has been discussed by Yudkin (84), who has considered the types of substances responsible for the increase in enzyme content of microorganisms.

According to the classification of bacterial enzymes employed by Karström (25), the fibrinolysin seems to belong to the group designated as "constitutive" enzymes, which do not require the presence of substrate for the production of the enzyme, as opposed to "adaptive" enzymes which are formed only in the presence of the specific substrate. No study has been made of the effect of the introduction of fibrin into cultures on the yield of fibrinolysin by the bacterial cells. *In vivo,* the possibility that the fibrin of inflammatory exudate might promote the production of fibrinolysin by the infecting organism is suggested by the occurrence of highly potent fibrinolytic strains in widespread infections.

Even though information concerning the effect of environment in the production of fibrinolysin is limited, it has been the common observation of many investigators that whereas many strains during artificial cultivation retain, as a constant property, the fibrinolytic potency exhibited in the initial tests, other strains have not maintained a uniform degree of lytic activity after repeated transplantations. For example, Hadfield, Magee, and Perry (19) observed that, after ten to thirty subcultures, some of their strains were decreasing in lytic potency. Of eleven active strains, they noted that six retained the same degree of activity during the period of study.

Observations also indicate that strains, with which the rate of reaction has slowed down, may be restored again to highly active ones both *in vitro* and *in vivo.* The factors which influence the yield of fibrinolysin by individual strains appear to be, in part, inherent in the bacterial cells and also to be related to the environments in which the organisms are kept viable. This

subject will be considered again further on. It is mentioned at this point to illustrate the fact that constancy in the yield of fibrinolysin has not been found to be a fixed attribute of all strains of hemolytic streptococci during periods of artificial cultivation.

The cultural factors which afford the most favorable basis upon which to make observations require: (*a*) Abundant growth. (*b*) Use of culture at time of maximum growth. (*c*) Use of culture media favorable for yield of fibrinolysin (possible influence of factors accessory to nutrition is suggested). It is important, also, to differentiate, in single tests on individual cultures, between strains that may, through prolonged laboratory cultivation or environmental circumstances, have become weakened in fibrinolysin production and other strains that are actually devoid of the property.

In the usual performance of the test, the plasma from the blood of *normal* human beings is regularly employed. However, the plasma-clots of different, apparently normal individuals may vary in susceptibility to lysis. For example, Tillett, Edwards, and Garner (66) noted that among thirty normal adults the plasma-clots from the blood of thirteen were liquefied within fifteen minutes, whereas the fibrin from eight others required from one to four hours before lysis was complete, even when a highly potent strain of hemolytic streptococcus was used in the tests. The dissolution time for the remaining nine normal persons ranged between fifteen minutes and one hour.

In addition to the clot available in whole oxalated plasma, fibrinogen and thrombin chemically isolated from blood have also served as a source of fibrin (65). The fibrin formed by combining fibrinogen and thrombin in the presence of active cultures has been found to liquefy at a greater speed and with smaller amounts of culture than does the clot of whole plasma. The probable explanations of the difference in speed of reaction between the substrates of whole plasma-clots and of fibrinogen-thrombin clots will be discussed in relation to immunological studies. From the standpoint of experimental procedure, the greater sensitivity of the fibrinogen-thrombin material has been

found to be useful in certain studies. However, it should be noted that additional complications may be introduced with the fibrinogen-thrombin technique. Investigators studying problems of blood coagulation have observed that when the fibrin, formed by relatively pure fibrinogen and thrombin, is allowed to stand for several hours, spontaneous lysis may occur with some preparations. Whether or not the spontaneous autolytic process may be catalyzed by the streptococcal fibrinolytic enzyme has not been studied. No information is available by which the end products of the autolytic and fibrinolytic actions may be compared. However, the possible effect of spontaneous lysis in fibrinolytic tests involving several hours' incubation may condition the evaluation of results obtained with fibrinogen-thrombin preparations, if the time required for lysis extends to several hours.

A final technical consideration concerns the influence of spontaneous retraction of the clot on the reading of the results of fibrinolytic tests. When active fibrinolysis occurs under the usual favorable conditions, the process is characteristic and the end point of the reaction is clearly defined. The quantities of materials employed in the usual test are such that, when coagulation occurs, the tube may be inverted without disturbing the position of the clot at the bottom. However, when the tubes are allowed to stand for a period of time, retraction of the clot may occur regardless of the presence or absence of bacterial cultures. The factors which determine the retractility of blood clots appear to be unknown. Consequently, neither the speed nor the degree of retraction is controlled in fibrinolytic tests. When the clot remains attached to the inner wall of the tube, it seems to occupy most of the space up to the top of the fluid level and it is saturated with liquid. Under these conditions, the reading of negative fibrinolysis is definite. However, when the clot is released from the sides of the tube, it settles to the bottom and may progressively shrink in size depending upon the degree to which the fluid contained within the interstices of the clot is squeezed out. An appearance comparable to the latter

incident may occur in fibrinolytic tests. In the experience of the writer, it may be difficult to differentiate weakly acting strains which have induced partial lysis after prolonged incubation from nonfibrinolytic strains in the tests of which a considerable degree of spontaneous retraction has occurred without lysis. Consequently some degree of reservation is indicated in the exact classification of cultures when the results are not clearly defined. It seems likely that a correct interpretation of some of the doubtful tests requires a method more accurate than the visual estimation of lysis on the basis of the size or shape of the ball of fibrin.

In performing the tests, the greatest number of observations have been made by incubating the preparations in the water bath at 37°C. Reports have indicated that incubation at higher temperatures may be preferable. Hadfield, Magee, and Perry (19) allowed the tubes to stand at 37°C. until coagulation had occurred. Following clot formation, 52°C. was used. They believed that lysis was hastened at the higher temperature. In some of his experiments, Schmidt (57) considered that more satisfactory results were obtained at 45 than at 37°C. Sherman and Niven (59) have advocated incubation at 53°C., after coagulation has occurred at room temperature. They pointed out that this procedure eliminated the growth of the organisms during the test period so that the result of the test was dependent upon the amount of preformed fibrinolysin. They noted that the duration of the tests could be shortened, since, if no lysis occurred in four hours, the result was not altered by prolonged incubation. Garner and Tillett (15) found that the reaction proceeded at a slower rate at room temperature than at 37°C., and that lysis was even more retarded at ice box temperature.

In summarizing the data concerning the materials and methods, emphasis has been placed upon factors which may affect the results obtained in fibrinolytic tests. Inasmuch as the phenomenon is readily demonstrable, the possible importance of some of the conditions may appear to have been unduly stressed. However, a review of some of the technical details

indicates the possible significance of experimental procedures in interpreting the results to be reported, in some of which discrepancies may be referable to materials and methods.

II. THE TYPES AND KINDS OF BACTERIA, PARTICULARLY STREPTOCOCCI, WHICH POSSESS FIBRINOLYTIC ACTIVITY

The first positive tests of fibrinolytic activity were obtained with strains of hemolytic streptococci derived from patients suffering from acute illnesses. Additional information concerning the fibrin-dissolving action among many strains of streptococci has accumulated from the published reports of several investigators. The incidence of the fibrinolytic property has been considered in relation to certain individual and group characteristics of the organisms and also to other biological reactions of streptococci. Other species of bacteria have also been tested for the presence of lytic properties. Although in most instances the results have been negative,certain interesting findings have been reported.

a. *Streptococcus hemolyticus* of the *beta* type

The first series of articles to be summarized under this heading deal with the results of fibrinolytic tests performed with hemolytic streptococci which were described by the authors as being associated with infections of varied clinical manifestations and degrees of severity. The details of the association have not been given in every instance nor is the correlation between the culture tested in the laboratory and the etiological status clearly established with many of the strains. However, the findings illustrate the occurrence of fibrinolytic properties among human pathogenic strains.

Tillett and Garner (65) tested the fibrinolytic activity of twenty-eight strains from different conditions including septicemia, acute tonsillitis, scarlet fever, erysipelas, empyema, and cellulitis. All of the strains were actively fibrinolytic. *Result:* 28 strains; 28 positive.

Hadfield, Magee, and Perry (19) reported the results obtained with twenty-nine strains that were derived from cases of moderate and severe scarlet fever, fatal septicemias, puerperal sepsis, peritonitis, tonsil-

litis, and rheumatic fever. The strains all caused lysis with varying degrees of potency and completeness during the test period. Five of them were highly active, six were somewhat less active, and eighteen produced slow or partial lysis. The highly active ones were from the severe cases. *Result:* 29 strains; 29 positive, with 11 highly active and 18 weakly lytic.

Madison (31) recorded the results obtained with thirty-two strains from "internal human tissues" and 123 from "superficial human tissues". The first group consisted of strains from cases of pneumonia, septicemia, empyema, and meningitis. Thirty of these were fibrinolytic. Of the 123 strains in the second group, which came from erysipelas, furunculosis, fistula, sore throat, sinusitis, and acute gastritis, only twenty-one were fibrinolytic. Five strains from erysipelas were highly potent. *Result:* 1st group, 32 strains; 30 positive (94 per cent). 2nd group, 123 strains; 21 positive (17 per cent).

Morales-Otero and Pomales-Lebron (43 to 45) in separate communications cite their results with thirty-three, fifteen, and forty-eight strains, respectively. The first and third groups were derived from a variety of disease sources. Of these eighty-one strains, seventy-nine exhibited the capacity to dissolve fibrin. (One of the negative strains came from a patient convalescent from scarlet fever; the other from a case of lymphangitis.) The group of fifteen strains were obtained from cases of recurrent tropical lymphangitis. Two of the strains were described by the authors as effecting incomplete hemolysis and appear not to have been of the beta type. The remaining thirteen strains were fibrinolytic. *Result:* 94 strains; 92 positive (98 per cent).

Hare and Colebrook (20), in one of their articles concerning infections due to hemolytic streptococci in parturient women, described the biological characteristics of a large number of strains. Of fifty-six derived from cases of puerperal infection, fifty-five were actively fibrinolytic. From eleven of the cases which had low-grade fever during puerperium, the strains in three instances were fibrinolytic. In some of these mild cases the authors considered that the fever was of uncertain origin. *Result:* 56 strains from puerperal fever; 55 positive (98 per cent). 11 strains from mild febrile puerperium; 3 positive (27 per cent).

Dack, Woolpert, and Hoyne observed the lytic action of 303 strains from scarlet fever. Twenty-five of them were derived from infected mastoids, and were all fibrinolytic. Of the remaining 278 strains, only twenty-eight caused lysis of fibrin. *Result:* 25 strains from scarlet

fever complicated by mastoiditis; 25 positive. 278 strains from scarlet fever; 28 positive.

Fraser and Madison (12) tested sixty strains from scarlet fever and found them all to be fibrinolytic. The highest potency was most frequent in the strains from severe cases. *Result:* 60 strains from scarlet fever; 60 positive.

Tillett (67) reported the results obtained with 157 strains. Of these, 140 were grouped, according to the source, into those from septicemia, acute suppurative diseases, (such as meningitis, peritonitis, empyema, mastoiditis, etc.), erysipelas, acute tonsillitis with and without rheumatic fever or nephritis, and a single additional group including chronic disorders and normal carriers. In these observations, the tests were made with the first subculture of the organisms after isolation from the patient. Of the 140 strains of definite etiological significance, 139 were fibrinolytic. An additional group of seventeen human pathogenic strains, obtained from other laboratories, were found to be actively lytic. *Result:* 157 strains from various disease sources; 154 positive (98 per cent).

Kodama (26) studied the biological properties of a large number of strains. Of 130 strains recently isolated from human infections and from the throats of normal people, 128 were fibrinolytic. *Result:* 130 strains; 128 positive (98 per cent).

Stewart (62) observed the lytic activity of 211 strains which produced soluble hemolysin. Of these, 146 were from surgical sources including puerperal infection, forty-five were from scarlet fever, and twenty from removed tonsils. One hundred and eighty-six of the total were classified as fibrinolytic. Of the twenty-five negative strains, sixteen were from surgical sources, seven from scarlet fever, and two from removed tonsils. The negative strains were tested on the first subculture. *Result:* 211 strains from various sources; 186 positive (88 per cent).

Evans (11) in a report on the properties of *Streptococcus pyogenes* cited the fibrinolytic properties of thirty-three strains. Thirty-two were fibrinolytic. In a subsequent article on *Streptococcus scarlatinae,* thirteen strains were tested. The average potency of the strains was not great, and four were found to be negative. Evans designated as *Streptococcus scarlatinae* strains which exhibited certain selective sugar fermentations, the most important of which was inability to ferment salicin. *Result:* 33 strains of *Streptococcus pyogenes;* 32 positive (97 per cent). 13 strains of *Streptococcus scarlatinae;* 9 positive (69 per cent).

Tunnicliff (71) studied, among several groups of streptococci, the occurrence of lysis by nineteen which were of the hemolytic type. They were isolated from scarlet fever, erysipelas, septic sore throat, endocarditis, and septicemia. All were actively fibrinolytic. *Result:* 19 strains; 19 positive.

Summarizing the findings just given, the figures are: Total number of strains, 1299, of which 899 (69 per cent) were actively fibrinolytic.

In the greatest number of the reports, however, the incidence of fibrinolytic activity by the pathogenic strains was greater than 90 per cent. Madison (31) in the tests with strains described as obtained from superficial human tissues, and Dack, Woolpert, and Hoyne (4) in their scarlet fever strains reported the lowest incidence (17 and 16 per cent respectively) of lytic properties. It may be noted that Evans (11) also considered *Streptococcus scarlatinae* to be less actively fibrinolytic than the *Strep. pyogenes* group. All of the reports with respect to *Strep. scarlatinae* strains have, however, not been consistent. Dack and co-authors considered the latter strains from severe cases to be highly potent.

The findings have demonstrated that strains from suppurative and invasive types of infection are, with few exceptions, not only regularly possessed of fibrinolytic properties, but are also usually the most potent in causing lysis of fibrin. For example, cultures derived from cases of septicemia, peritonitis, meningitis, or infections of the throat (acute tonsillitis, scarlet fever) where the organisms have invaded beyond the local pharyngeal tissues, constitute the strains which elaborate fibrinolysin in considerable quantity. The findings with cultures from minor infections and perhaps with some *Strep. scarlatinae* strains, indicate either the absence of lytic properties or that the production of fibrinolysin is characteristically impaired during laboratory cultivation. The suggestion, implied in these results, of a possible association between lytic activity and pathogenicity will be discussed later.

The occurrence of fibrinolytic properties in strains derived from normal persons has been studied less extensively. In twenty-five strains isolated from the throats of patients with various chronic disorders and from normal persons, Tillett (67) found the

incidence of weakly lytic strains to be greater than that of highly active ones. More detailed findings with cultures from normal throats will be given in association with the studies of relations to the Lancefield groups.

From the standpoint of the classification of hemolytic streptococci on the basis of biological, biochemical and serological reactions, the admirable and detailed review of Sherman (58) includes data concerning fibrinolytic activity of strains in relation to other findings. It would be repetitious to record here the reports which he has analyzed. Consequently, the reader is referred to Dr. Sherman's article for comprehensive data.

The fundamental observations of Lancefield (28) concerning the serological classification of hemolytic streptococci has had such wide and important application in the orientation of this species of organisms that it is of paramount importance in the study of strains. Sherman has brought together various findings concerning streptococci under the Lancefield groupings. Consequently the results given here will be limited to the fibrinolytic activity of strains with respect to the Lancefield classification.

b. Relation to Lancefield serological classification

Group A hemolytic streptococci have come to be recognized as the group characteristically responsible for acute infections in man. The fibrinolytic activity of strains identified serologically as belonging to Group A has been described in several articles with uniform results. Hare (21) reported on sixty-three strains from the nose and throat of normal persons, and found sixty-two possessed fibrinolytic properties. Kodama tested 160 Group A strains from cases of infections, from normal persons, and from stock cultures; and 157 were fibrinolytic. Davis and Guzdar found each of twenty-eight Group A strains from normal throats to possess lytic properties. Sherman and Niven reported four out of five strains which dissolved fibrin. They cite one strain, originally isolated from a case of epidemic sore throat which belonged to Group A but was nonfibrinolytic when tested. Hare and Maxted isolated ten Group A strains from the stools of patients with scarlet fever; each culture was active against fibrin.

Seegal, Heller, and Jablonowitz recovered from monkeys nineteen Group A strains and found them fibrinolytic.

Of the 285 strains identified as belonging to Group A by the investigators who tested them against fibrin, 280 (98 per cent) possessed fibrinolytic activity. Madison (32) suggested "a possible genetic linkage between these two specific bacterial characters." From observations upon 189 strains, he reported that the titre of fibrinolysin and the titre of Group A carbohydrate as determined by the ring test were closely correlated. However, as will be described in the reports which follow, strains other than Group A have been found to be fibrinolytic.

Groups B, D, E, F, and H. Without extending the details, strains belonging to these groups have been found to be negative by Hare, Hare and Maxted, Kodama, Sherman and Niven, and Seegal, Heller, and Jablonowitz. The reports include forty-one strains of Group B, fifty-four of Group D, a small number of Group E, eighteen of Group F and ten of Group H. Sherman and Niven have recorded some of their results as ±, indicating that possibly a slowly acting lysis may have occurred with some of the strains. The source of all of the strains was the throat, stools, or vagina of normal persons, or milk.

Group C. Among fifty-seven strains derived by several investigators (21, 26, 6, 45, 59) from throat, or vagina of normal persons or throat of monkeys (56), fifty-four were found to be fibrinolytic. Of eleven strains isolated from milk (59), none was fibrinolytic. In the biochemical tests of the strains isolated from normal persons, trehalose was fermented but not sorbitol. In the milk strains, Sherman and Niven observed that trehalose was unaffected, but sorbitol was fermented. Sherman has suggested, therefore, on the basis of this difference the terms "Human Group C" and "Animal Pyogenes Group C." It is interesting to note that many of the strains belonging to the "Human Group C" are fibrinolytic, but that the "Animal Group C" are negative. The strains belonging to Group C have only rarely (21, 58) been reported, up to the present time, as occurring in infections in man. They constitute, therefore, a group of fibrinolytic streptococci, which have not been considered significant in human infections,

although Hare refers to two strains originally isolated from cases of erysipelas.

Reich has described the transformation of a strain of hemolytic streptococcus, Group A, which by prolonged and repeated passage through rabbits lost the original serological classification and gave positive precipitin reactions first with Group C antiserum and then with Group E antiserum. Coincidentally the fibrinolytic activity was also lost. When, however, the strain was cultivated in repeated subcultures in broth, the original Group A reaction returned and fibrinolysis was again demonstrable. Gay and Clark (17), on the contrary, reported that a human strain "H", which had been passed through rabbits for nineteen years, belonged, at present, to Group A, and was capable of liquefying fibrin. Data with regard to the loss by a strain of fibrinolytic activity coincident with change in serological type are limited to the report of Reich. It is apparent that confirmation by the use of a large number of strains is necessary before the suggestive finding is established.

Group G. Of seventy-nine Group G strains, derived like the Group C strains from the throat, vagina, or stools of normal persons, seventy were fibrinolytic (21, 22, 26, 6, 45, 56).

From the data with respect to serological classification, strains belonging to Groups A, C, and G have been found to be fibrinolytic. Strains belonging to Groups B, D, E, F, and H have proved to be negative. Reports of a considerable number of other strains which are nonfibrinolytic will be reported in connection with animal strains. In these latter strains, however, the serological classification was not made.

Using the Lancefield classification for the identification of human strains both from patients and normal persons, the combined tests of serology and fibrinolysis demonstrate a correlation in 98 per cent of the tests with Group A strains. In combination with the observations made with strains derived from active infection but not classified serologically, the similarity of the two groups of findings is apparent.

As an arbitrary test for the separation of human pathogenic strains from innocuous ones, the determination of fibrinolysis

is a helpful procedure but is not necessarily conclusive in every instance. The large proportion of nonfibrinolytic strains among the serological groups has been found in Groups B, D, E, and F, which on the basis of previous experience with immunological and biochemical tests have been classified as nonpathogenic for man. Fry has reported three fatal cases of infection due to Group B hemolytic streptococci. The strains were without fibrinolytic activity. Fry described the special characteristics of the pathological anatomy which differed from the usual changes observed in fatal cases of hemolytic streptococcus infection, and discussed the possible significance of the disease picture from the standpoint of the qualities of the infecting organism.

In addition to the non-fibrinolytic Group B strains which caused infection in Fry's cases, limitations on the evaluation of negative strains as non-pathogenic are also exemplified by a few other exceptional strains which have possessed definite etiological significance in active infection and which have been tested under advantageous laboratory conditions but did not exhibit lytic properties.

From the standpoint of the interpretation of positive fibrinolytic tests as indicative of pathogenicity, restrictions in the significance of the results are based on reports that strains belonging to Groups C and G are only occasionally significant in human infections but are frequently fibrinolytic. It is interesting to note, however, that the fibrinolytic strains of Groups C and G have usually been derived from human sources. Sherman and Niven are of the opinion that some strains of various hemolytic species may induce the slow lysis of fibrin.

In contrast to the dissolving action of human pathogenic strains, Tillett and Garner reported that hemolytic streptococci from animal sources were usually incapable of liquefying the fibrin of human blood. These findings have been extended in several reports, although serological classification was not regularly reported. Since the factors pertaining to animal strains concern the source of the fibrin substrate as well as the origin and biological characteristics of the cultures, a consideration of this interesting phase of the subject is given in Section IV. The present

section continues with results obtained with other kinds of streptococci and other species of bacteria commonly associated with man.

c. *Streptococcus viridans*

Of this variety of streptococci, Tillett and Garner tested six strains and found each to be nonfibrinolytic. Madison (33) reported thirty-three strains as negative in fibrin-dissolving tests. The same author, even after using methods of concentrating fibrinolytic material, was unable to obtain lysis with green streptococci. Stewart recorded that thirty-three strains belonging either to the *Strep. viridans* or *Strep. anhemolyticus* type, were not active in the liquefaction of clot. Schmidt obtained no lysis with green streptococci. Laca and Porzecanski found strains of *Streptococcus viridans*, *Streptococcus fecalis*, and *Enterococcus* to be nonfibrinolytic. Tunnicliff (71) stated that strains of *Streptococcus viridans* were nonfibrinolytic but that some of them inhibited clot formation.

Neter and Witebsky (48) subsequently presented a series of reports on the fibrinolytic and anticoagulating action of several species of bacteria. Although the immediate purpose of this review is not concerned with the so-called anticoagulating action of organisms, the findings which are related to fibrinolysis warrant consideration. Neter and Witebsky reported that, when the bacteria were cultivated in 2 per cent glucose broth, some strains of the following species were fibrinolytic; *Streptococcus hemolyticus*, *Streptococcus viridans*, *Enterococcus*, *Pneumococcus*, *B. coli*, *B. lactis aerogenes*, *B. friedländeri*, *B. pyocyaneus*, and *B. proteus*. They concluded that "fibrinolysin production is not limited to hemolytic streptococci alone, if, for instance, the sugar content of the culture media is increased." If this reviewer understands the article correctly, tests for fibrinolytic activity were considered positive if clot formation failed to occur when $CaCl_2$ was added to the mixtures of plasma and culture. Witebsky and Neter (81) also described what they considered to be the properties of two different fibrinolysins produced by streptococci. One of these had the following characteristics: It developed when the organisms were grown in 2 per cent glucose broth; it inhibited

clot formation; it was effective in both human and animal plasma; it acted only in an undiluted state; it was thermostable; and it was not neutralized by antistreptococcus sera. The other fibrinolysin was produced in 0.05 per cent glucose broth; it acted only upon human fibrin-clot; it was effective in high dilutions; it was thermostable, and was neutralized by antistreptococcus sera.

Witebsky and Neter stated that, when cultures of *Streptococcus viridans*, *Enterococcus*, or *Pneumococcus* were cultivated in 2 per cent glucose broth, fibrinolysin developed like that present in cultures of hemolytic streptococci also grown in 2 per cent glucose broth.

The inhibiting effect on clot formation exerted by cultures of streptococci had previously been noted by Dennis and Berberian and by Tunnicliff. In the latter studies, the culture medium of choice was, respectively, 2 per cent dextrose broth (9) and 1 per cent meat extract broth with 1 per cent dextrose (71).

Dart reported a confirmation of the findings of Neter and Witebsky with respect to fibrinolysin and anticoagulant (second fibrinolysin) if hemolytic streptococci were cultivated in 0.4 per cent dextrose broth. The fibrinolysin was obtained from cultures by precipitation with alcohol according to the method described by Garner and Tillett; the anticoagulant factor was recovered from the supernatant fluid by evaporation; it resisted heating at 100°C for 30 minutes.

Dennis and Adham in a further study of the anticlotting factor of dextrose-broth cultures of streptococci described it as being soluble in 75 per cent alcohol, absolute alcohol, and ether; it was dialyzable; it gave a strongly positive Kelling's test for lactic acid. They concluded that the anticoagulant was primarily lactic acid. The anticlotting constituent seldom occurred with cultures grown in media having less than 0.4 per cent dextrose; and the authors considered the anticlotting action to be more closely correlated with the total acid content of the cultures than with pH.

Tillett (69) studied the anticoagulating effect and the fibrinolytic activity of strains of *Streptococcus hemolyticus*, *Streptococcus viridans*, and *Pneumococcus*. The cultures were cultivated in 0.05, 1.0, and 2 per cent dextrose broth. With respect to the anti-

clotting effect, he found that when the ultimate pH of the 1.0 or 2.0 per cent dextrose-broth cultures was below 5.0, coagulation of plasma was inhibited; when the pH of the cultures was above 5.0, clotting occurred in all the tests but fibrinolysis was effected only with strains of *Streptococcus hemolyticus*. With uninoculated sterile broth of varying hydrogen ion concentrations, the effects on the coagulation of plasma paralleled the findings obtained with cultures of the same pH. Furthermore, when the high degree of acidity (pH 4.4 to 4.9) produced in dextrose broth cultures was altered by the addition of NaOH to pH 6.0 to 7.0, coagulation occurred. When cultures in 0.05 per cent dextrose broth (pH 6.5 to 7.0) were acidified to below pH 5.0, coagulation was inhibited. In studies on the physiology of blood coagulation, the lower limit of pH at which fibrin is formed is placed at 5.6 to 6.0. It is also interesting to note that the anticoagulative action of organic acids, including lactic acid, has been described (80). Tunnicliff and Hammond (72) in continuing a study of the anticlotting action of *Streptococcus viridans* found that the smooth form, which prevented coagulation, lowered the pH of 1 per cent dextrose broth to 4.4–4.8; cultures of rough forms, however, which did not inhibit coagulation, reached a pH of 5.2–6.0.

From a consideration of the data concerning the anticlotting action of various bacterial species, it seems probable that the effect depends to a considerable degree on the action on oxalated plasma of the products of the hydrolysis of sugar by the organism, or on pH, or on both of these factors. Furthermore, from an analysis of the findings with respect to organisms other than hemolytic streptococci, it appears that the action designated as fibrinolysis by *Streptococcus viridans*, pneumococci, and other bacterial species, is not due to a lytic agent comparable to the fibrin-dissolving substance of hemolytic streptococci.

d. Other streptococci; Dissociants

Pseudo-hemolytic streptococci. This term has been frequently employed by English investigators in designating strains which differ from other hemolytic streptococci on the basis of negative tests for "soluble hemolysin." Hare and Colebrook describe the

results of fibrinolytic tests with thirty-four such strains. None of them caused lysis of fibrin. Twenty-seven of the strains were from pregnant women who had afebrile puerperium. Seven came from puerperal cases with mild fever. Stewart (62) found that twenty-seven strains of the pseudo-hemolytic variety were nonfibrinolytic.

Streptococcus anhemolyticus. Only a few strains of this type have been tested. Tillett and Garner obtained negative results with two strains; and Stewart described anhemolytic strains as being negative in fibrinolytic tests.

Double-zoned hemolytic streptococci. Brown has described strains having this characteristic appearance when cultivated in blood agar. Strains of this type have been derived from both human and animal sources. The author has tested some of them, obtained through the courtesy of Dr. Brown, and found them to be nonfibrinolytic.

Dissociants of streptococci. Mellon and Cooper (42) described the action of various dissociants which they obtained from individual strains of hemolytic streptococci. Some of the dissociated forms were described as nonhemolytic diphtheroids. The variants, which caused only partial lysis in 24 hours, were definitely less active in liquefying fibrin than the original cultures. The authors also state that diphtheroids with acid-fast granules considered to be in the tubercle bacillus cycle were indistinguishable in their fibrinolytic activity from diphtheroids dissociated from streptococci. Subsequent reference will be made, in relation to virulence, to the findings of Tunnicliff (68), who noted the loss of lytic activity by certain strains associated with the change from cultures producing smooth colonies to those producing rough, irregular colonies, and also to the results obtained by Dawson and his coworkers (7), who, with M, S, and R variants of the same strain, observed no difference in fibrinolysis, each of the cultures being active.

e. Staphylococcus and other bacterial species

The effect of staphylococci on the coagulation of blood and the dissolution of fibrin has received the attention of many investigators. It is not within the scope of this review to consider these

properties of staphylococci, because the slowly liquefying action of staphylococci on fibrin, although constituting an example of bacterial fibrinolysis, differs in many respects from the rapid fibrin-dissolving effect of hemolytic streptococci. Madison (35) has described the immunological differences between the products of the two bacterial species.

Concerning other bacterial species, the available reports are limited. Tillett and Garner tested several members of the colon-typhoid group and also *Hemophilus influenzae* and found them to be nonfibrinolytic. Schmidt tested a heterogeneous group of organisms and obtained uniformly negative results. Madison (40), however, obtained interesting results with *B. pestis*. Sixteen strains were tested for fibrinolytic activity. One of them was of human origin (20 years old), and the others were derived from field mice and ground squirrels. For the fibrin clot, he used fibrinogen and thrombin obtained from the plasma of man, guinea pig, rabbit, rat, and other animal species. Using methods of titration which he described, Madison found that the cultures of *B. pestis* induced lysis of the fibrin-clots from the blood of several of the animal species, including man. The potency of lytic action was, however, greatest against the coagulum of rat's blood.

Fisher (11a) in studying the fibrinolytic properties of staphylococci noted that certain contaminating bacterial species dissolved plasma-clot slowly in one to six days. The strains consisted of *B. subtilis* (5 strains), and single cultures of *B. proteus*, *B. pyocaneus*, diphtheroids, and *B. alkaligenes*. Owing to the fact that an incubation period of several days was necessary before dissolution occurred, the possibility that the liquefaction might be dependent upon proteolytic digestion warrants consideration. No studies dealing with this point have been made.

Weiss (80a) made observations with two strains of *Bacterium melaninogenicum*. The cultural material was concentrated through alcoholic precipitation, and the tests were made with human fibrinogen-thrombin preparations. A 1 to 4 dilution of the concentrate of one strain caused lysis in forty minutes, while original concentrations of the other strain caused partial lysis (designated 2+).

Neter and Witebsky (48) found that *Pneumococcus* behaved like *Streptococcus viridans* in fibrinolytic studies with dextrose-broth cultures. Tillett and Garner, Schmidt, Lippard and Johnson, and others could demonstrate no fibrinolysis with pneumococci.

III. CORRELATION OF FIBRINOLYTIC ACTIVITY WITH OTHER BIOLOGICAL PROPERTIES OF HEMOLYTIC STREPTOCOCCI

a. Relation to proteolysis

Laca and Porzecanski studied the proteolytic, fibrinolytic, and hemolytic activity of ninety-six strains of streptococci. They found all of these properties commonly present in many of the pathogenic strains. However, in certain strains, fibrinolysis was present but proteolysis was absent; while in others the proteolytic effect was marked, but fibrin dissolution did not occur. Garner and Tillett by determinations of amino nitrogen contrasted the action on fibrin of fibrinolysin and streptococcal peptase.

b. Relation to the production of hemolysin and of toxin

With respect to the qualitative differences of hemolysins of streptococci, since the relationship is contained in the reports listed under the kinds of streptococci classified on the basis of their action on blood agar, the results need not be restated. Among strains of hemolytic streptococci of the *beta* type, accurate comparative measurements of hemolysin and fibrinolysin have not been made. However, on the basis of the size of the zone of hemolysis created by colonies in blood agar, Hadfield and associates, Schmidt, and others have stated that no strict relationship exists between potency of strains in the production of hemolysin and of fibrinolysin.

Fraser and Madison using scarlatinal strains attempted to correlate fibrinolytic activity, toxin production, and severity of scarlet fever. They found a 63 per cent correlation between the titre of toxin produced by the strains and severity of disease graded according to degree of fever, duration, and complications. On the same basis they reported an 80 per cent correlation between the titre of fibrinolysin and severity of illness. They stated that their results agreed with the conclusions of Dack and

his associates that a high fibrinolytic titre is significant in relation to the complications of scarlet fever.

Morales-Otero and Pomales-Lebron (44) compared fibrinolytic activity with toxigenicity as determined by intracutaneous tests in the shaved skin of white goats. Of fifteen strains, thirteen were both toxigenic and fibrinolytic.

c. Relation to virulence

The types of illnesses resulting from hemolytic streptococcus infections are characteristically diverse. The manifestations of the diseases range from clinical entities, the etiology of which may be diagnosed or suspected without laboratory aid, to other disorders which have characteristics common to many pyogenic infections. The mechanisms of hemolytic streptococcus infections appear to involve properties which are integral parts of the bacterial cell body, such as capsule formation, and perhaps others, and also substances which are elaborated and excreted by the organisms. That hemolytic streptococci produce different kinds of noxious agents is evidenced by many reports and is particularly well illustrated by the erythrogenic toxin and the hemolysin. These substances possess different properties and have been studied as separate entities, although elaborated by the same types of organism. Furthermore, with the possible exception of the studies of Mudd and his associates (3), the occurrence of the excretory products in strains has not, up to the present time, been found to parallel any individual constituent of the bacterial cell structure. Concerning the production of fibrinolysin by streptococci in relation to structural characteristics of the organisms, a few observations have been made. Hadfield, Magee, and Perry observed with two strains, which produced matt colonies (virulent) at the time of high fibrinolytic activity, that the subsequent change to cultures producing glossy colonies (avirulent) was attended with marked reduction in the production of fibrinolysin. They found the average virulence for mice of their strains most potent in the production of fibrinolysin was higher than that of the least active. Tunnicliff (71) reported that strongest lytic action was associated with virulent strains

which possessed capsules and produced smooth colonies. She found that the production of fibrinolysin was lost when cultures were altered by dissociation so that granular colonies with irregular edges were formed. She reported further that reversion of strains to the type which formed smooth colonies, was accompanied by the restoration of active fibrinolysis. Schmidt noted the loss of lytic activity with some strains after repeated subculture, and that virulence for mice was also lost. When, however, by mouse passage, virulence was restored, lytic action also increased.

Morales-Otero and Pomales-Lebron (44) cited their experience with strains which were virulent for mice at a time when the organisms were fibrinolytic. Two years later, the same strains had lost mouse virulence but had retained fibrinolytic and toxigenic powers. Dawson, Hobby, and Olmstead in describing the results of their extensive studies on M, S, and R variants of hemolytic streptococci briefly record, without giving details, that no significant differences were observed in the fibrinolytic capacity of the three variants of the same strain.

These findings indicate that although the production of fibrinolysin by hemolytic streptococci may frequently accompany the presence of experimental indices of pathogenicity (colonial structure and virulence for mice), the relationship is not an inseparable one. In the author's experience, strains of hemolytic streptococci of highest fibrinolytic potency may not be virulent for mice. Furthermore, as will be subsequently discussed, human strains of hemolytic streptococci are not regularly capable of causing dissolution of the fibrin of mouse's blood. Since the presumptive evidence of the rôle of fibrinolysin in virulence is derived from the capacity of the invading organism to dissolve the fibrin of the infected animal, it follows that invasion in the absence of fibrin susceptibility is referable to other conditions. In experimental infections, the mechanism of virulence often centers around factors which involve susceptibility or resistance to phagocytosis. These same factors, in all probability, play an important and often decisive rôle in human infections. However, in infections in man due to hemolytic streptococci, supple-

mentary factors may influence the pathogenesis of the diseases. For example, the erythrogenic toxin, which seems to be of limited significance in infections of laboratory animals, induces toxic manifestations in man. Whether or not the fibrinolytic properties of human pathogenic strains may also be a contributing factor to some of the characteristic elements of hemolytic streptococcus infections, has not been determined but may be surmised from suggestive indirect evidence.

Neter (50) found the fibrinolysin present in the spinal fluids of four out of five patients with meningitis due to hemolytic streptococci. With samples of the spinal fluids of cases of meningitis due to other organisms, he obtained negative results, except in one case of pneumococcus meningitis. He also reported the occurrence of lytic activity in peritoneal and pleural exudates from hemolytic streptococcus infections, and with the pericardial and peritoneal fluids from *Staphylococcus aureus* infections. He examined also the peritoneal exudate of mice infected with hemolytic streptococci and pneumococci. In the infections with streptococci, the peritoneal washings induced lysis of human fibrin, but the material from mice infected with pneumococci was negative.

In connection with the production *in vivo* of fibrinolysin, it may also be mentioned that the thinness of the fluid so characteristic of the exudate obtained early in cases of infections of the serous cavities due to hemolytic streptococci, particularly empyema, appears to be due to the lytic action of the infecting organisms on the fibrinous exudate. Goodpasture in describing the pathological changes occurring in bronchopneumonia due to hemolytic streptococci of 1917–18 refers to cases in which "microscopically the alveoli are filled with polymorphonuclear leukocytes and usually enormous numbers of streptococci, with little or no fibrin." In MacCallum's account of pneumonia during the World War, reference was not infrequently made to areas in which fibrin was scarce or absent. The density of fibrin deposits in many of the lesions was also commented upon. The present writer has examined material from two cases of empyema. Fibrinolysin was demonstrable in the thin pleural fluid obtained early in the disease. However, as the exudate became thick with

fibrin, antifibrinolytic properties were demonstrable in the blood of the patients. It seems not unlikely that the pathogenesis of some aspects of hemolytic streptococcus infections may be explained on the basis of the fibrinolytic potency of the organism in relation to the antifibrinolytic properties of the host.

IV. HEMOLYTIC STREPTOCOCCI FROM ANIMAL SOURCES, WITH PARTICULAR REFERENCE TO ACTION ON FIBRIN OF BLOOD FROM DIFFERENT ANIMAL SPECIES

Tillett and Garner reported that, although cultures of hemolytic streptococci derived from patients caused lysis of normal human fibrin-clot, normal rabbit fibrin-clot was resistant to dissolution when tested under comparable conditions. Observations concerning differences in fibrinolytic activity referable to animal sources of fibrin have yielded interesting results.

Van Deventer and Reich (73) tested three human strains and two animal strains (P 454 and K 158 E of Lancefield) against the plasma-clot of the following animals: rabbit, guinea pig, rat, domestic fowl, horse, cow, goat, sheep, dog, and cat. All tests were negative. The three human strains were lytic for human fibrin. They were also tested against the plasma-clot of rhesus monkeys. Two of the strains caused lysis of monkey fibrin but at a slower rate than the effect on human fibrin. One of them was equally active against human and monkey fibrin.

Madison (34) tested twelve strains of hemolytic streptococci derived from horses suffering from strangles against samples of fibrin derived from horse, man, hog, cow, and rabbit. Fibrinogen-thrombin preparations were employed because of their greater susceptibility to lytic action. The equine strains caused dissolution of horse fibrin but did not liquefy the fibrins derived from the other animal species, including man. Two human strains of hemolytic streptococci were weakly lytic against *horse* fibrin. In addition, Madison found that three strains of hemolytic streptococci obtained from hogs (septicemia) were highly active against *hog* fibrin. The same strains were weakly active against *human* fibrin, but negative against the fibrin of the other animal species.

Planet also compared the action of *human* and *equine* strains of

hemolytic streptococci against the plasma-fibrin of *human* and *equine* sources. The single *human* strain of hemolytic streptococcus, which he employed, caused dissolution of the fibrin from five different *human* plasmas, but was inactive against the fibrin of twenty-two different *horse* plasmas. One of his *equine* strains caused lysis of all of the samples of fibrin from *horses* but was negative against *human* fibrin-clot. With other *equine* strains of hemolytic streptococci, varying degrees of lytic activity for *equine* fibrin were noted but no alteration of human fibrin occurred. Some of the equine strains fermented lactose and some did not. No relationship was noted between the fermenting activity and fibrinolytic capacity.

Smith, Hankinson, and Mudge tested twenty-two strains of hemolytic streptococci derived from *cow's milk* against the plasma-clot of *bovine* blood. Nine of the strains caused varying degrees of lysis of *bovine* fibrin. Of these, two were from normal cows, five were from cows with mastitis in a quarter other than that which supplied the infected milk, and two were from cows with chronic mastitis. The results were not conclusive, but suggested the possibility that strains lytic for bovine fibrin might be significant in mastitis.

Pilot, Buck, and Davis (53) examined one hundred strains of hemolytic streptococci obtained from the tonsils of cows; and ninety-two gave negative fibrinolytic tests with human fibrin. Among forty-three strains derived from the tonsils of hogs, thirty-nine were negative. No report was made of tests made with fibrin of the cow or hog. In a subsequent article twenty-two canine strains were reported as negative for human fibrin (54).

Seegal, Heller, and Jablonowitz in a study of hemolytic streptococci derived from *monkeys*, tested the fibrinolytic activity of the cultures against fibrin from *man* and from *monkeys*. Nineteen Group A strains caused lysis of *human* fibrin within 3½ hours, and also dissolved *monkey* fibrin but at a slower rate, ranging from 6 hours with 3 strains to a negative result with 2 others. With four Group C and five Group G strains, *human* fibrin was liquefied regularly within 3¼ hours, and lysis of *monkey* fibrin occurred with the same prolonged rate of activity obtained with

the Group A strains. The lysis of *human* fibrin was uniformly more efficient than that of *monkey* fibrin.

Yen, in studying the problem of the resistance of animal fibrins to dissolution, observed the influence of quantitative factors in the reaction. Using hemolytic streptococci from patients, he concentrated the fibrinolysin from filtrates of cultures by alcoholic precipitation. In order to have a more sensitive substrate, he employed fibrinogen-thrombin preparations isolated from the plasma of man, rabbit, and guinea pig. He found that human fibrin was dissolved in 3 to 5 minutes, that rabbit fibrin was liquefied in 30 to 180 minutes, and that guinea pig fibrin failed to liquefy. He concluded that rabbit fibrin-clot was not absolutely resistant to lysis by human strains of hemolytic streptococci, if sufficiently high concentrations of fibrinolysin were tested.

Schmidt also emphasized the importance of the quantitative factor in determining the results obtained with materials from different animal species. Although he found exceptions with some strains, he confirmed the findings of others with respect to the homologous source of materials, *provided* the usual test dose of culture was used and the fibrin was contained in the clot of whole plasma. When, however, large amounts of fibrinolysin were employed and added to fibrinogen-thrombin preparations, the principle of species specificity was not regularly maintained.

Concerning the sensitivity of the fibrin substrate to dissolution by streptococci, an additional complicating factor is introduced when the fibrinogen constituent of the clot and the thrombin component are each derived from a different animal species. Tillett and Garner reported that when fibrinogen from rabbit's blood was coagulated in the presence of cultures of hemolytic streptococci by thrombin from human blood, dissolution occurred; also, when fibrinogen of human blood was clotted by thrombin of rabbit's blood, liquefaction took place. When, however, both constituents of the coagulum were derived from the rabbit, the results were either negative or slow dissolution occurred after many hours. In the above experiments, the determining factor in the occurrence of active fibrinolysis was the

presence of at least one human element in the fibrinogen-thrombin complex.

Madison (36) used materials which he designated as "hybrid fibrins." He derived fibrinogen and thrombin from eleven different animal species, including man. Using a human strain of hemolytic streptococcus, he found that dissolution occurred in every instance when the fibrinogen component of the fibrin was of human derivation regardless of the source of the thrombin. When the human component was thrombin and the fibrinogens were from various animals, dissolution occurred, but proceeded at a slower rate than the control of human fibrin. If neither constituent was of human origin, the results were negative. Comparable but somewhat less striking homologous species relationships were found to exist when an equine strain active against horse fibrin, and a porcine strain active against hog fibrin, were tested with hybrid fibrins. In these latter experiments, however, there were some irregularities not explicable on the basis of the individual animal source of the materials.

The subject of hybrid fibrins is obviously a somewhat confused one. Schmidt emphasized the importance of the quantitative proportions between fibrinolysin and fibrin substrate. He found that small doses of a highly active human strain acted only upon fibrins when one element was of human origin. When the amount of fibrinolysin was increased, however, some of the hybrid fibrins were dissolved. Schmidt extended the studies by considering whether or not strains, which are highly pathogenic for a given species, would dissolve the fibrin of the species provided thrombin of the homologous animal was employed. His results were not harmonious. They conformed to a homologous species relationship between virulence and source of fibrin with some strains, but the correlation was not demonstrable with others. For example, he described an equine strain, virulent for mice, which liquefied mouse fibrin formed with horse thrombin, but was inactive against mouse fibrin formed with human thrombin.

It is obviously impossible in the present state of knowledge to interpret clearly the results obtained with the manifold hybrid fibrins. It seems probable that the results are dependent upon

quantitative factors in some instances, and upon qualitative differences of materials in others. However, even when the differences are quantitative, homologous fibrin has been found to be more susceptible than heterologous material. Viewed as a chemical reaction involving a system consisting of enzyme (fibrinolysin) and substrate (fibrin), variations in sensitivity are dependent upon the sources of materials, but the degree of specificity necessary to elicit the dissolving effect is not established. Furthermore, since the materials used are not chemically pure, accessory factors, which may influence enzyme systems such as the fibrinolytic process, merit consideration. Additional information on this complex subject seems to require chemical procedures which are more technically exact than the methods employed at present.

In spite of the limitations on the interpretation of the results just discussed, the apparent predilection of strains of hemolytic streptococci for the fibrin of a species homologous to that in which the organisms may survive, and in some instances invade, contains implications of biological interest which invite additional study.

V. CHARACTERIZATION OF FIBRINOLYSIN AND NATURE OF THE REACTION

The fibrinolysin has been found to be freely excreted by the living, growing bacterial cells. Consequently, it has been possible to obtain active fibrinolytic material, free from the microorganisms, by filtration. Garner and Tillett found that the fibrinolytic principle could be partially purified by (a) precipitation of culture filtrate with 3 volumes of 95 per cent alcohol, and, (b) adsorption on polyaluminum hydroxide B of Willstätter followed by elution with M/10 sodium phosphate buffer, pH 7.3. Concentration was accomplished by dissolution of the precipitates in small quantities of solvent, but was best obtained by vacuum dialysis (15). Concentration by alcoholic precipitation has also been reported by Madison (33), Yen, and Schmidt.

It should be noted that when high degrees of concentration are attempted, preparations may be encountered, which inhibit

coagulation. The explanation of the anticoagulative effect is not clear. It seems possible that it may be referable to some other constituent of the filtrate which is also concentrated together with the fibrinolysin. For example, peptone is known to contain anticoagulating material, which might be responsible for the effect. It seems also possible that inhibition of the clotting process might be dependent upon the physico-chemical action of highly concentrated proteins or other organic materials.

Garner and Tillett found that active culture filtrates were relatively heat stable, in some instances resisting heat of 100°C. for 60 minutes. Dennis and Berberian (9) reported that fibrinolytic activity was markedly weakened by boiling for one-half hour. In contrast to the heat stability of culture filtrates, Garner and Tillett observed the activity of material obtained by alcoholic precipitation was destroyed at 57°C. for one hour. However, when the fibrinolytic agent was purified by adsorption and elution, the resultant material was again heat-stable as in the case of the culture filtrate. The sensitivity of the material obtained by alcoholic precipitation suggests that the procedure separated the active principle from other substances which afforded protection from the deleterious effects of heat. Although an explanation of the differences in the effect of heat is not clear, the thermal properties suggest that the fibrinolysins of different preparations may exhibit variations in sensitivity to other inactivating substances, such as chemicals or antisera.

The fibrinolysin conforms in many of its characteristics to a protein. The partially purified materials give positive tests for protein, and fibrinolytic activity is destroyed by digestion with trypsin or papain (15).

Using fibrinogen-thrombin preparations, Garner and Tillett found that the fibrinolysin was not bound to the reaction products, since the active material was recovered approximately quantitatively after dissolution of fibrin was complete.

In characterizing the fibrinolysin, therefore, on the basis of the data available at present, the active agent may be considered to be enzymic in nature for the following reasons: 1. It is of biological origin. 2. Catalytic property is indicated by the fact

that active material is recoverable, approximately quantitatively, after the reaction is completed. 3. Destruction by heat (high temperatures for broth filtrate; low temperature for material isolated by alcoholic precipitation). 4. Tests for protein are positive.

The fibrinolysin differs, however, from proteolytic enzymes in that preparations of the former exert no hydrolytic action on casein, gelatin, or peptone. Furthermore, it also differs from the so-called streptococcal peptase, which is obtained by rupturing the bacterial cells and which acts upon casein but is especially vigorous against peptone (15).

Fibrinogen is the only substrate besides fibrin which has so far been found to be susceptible to fibrinolysin. Demonstration of the action on *human* fibrinogen was made in experiments (15) in which fibrinogen, incubated for short periods with fibrinolytic cultures, was incapable of forming fibrin following the subsequent addition of thrombin. *Rabbit* fibrinogen, however, in parallel experiments, retained the capacity to form fibrin even after preliminary incubation of eighteen hours with fibrinolysin.

One of the interesting features of the fibrinolytic phenomenon concerns the nature of the end products of the reaction. Following dissolution of fibrin and during subsequent incubation, determinations have been made of increases in amino N (Garner and Tillett), and also of non-protein N and of the evolution of ammonia (Garner). It was found that, during the experimental period, there is a small and gradual increase in the amino N content of the solution. The results contrast, however, in degree very markedly with the observed effect of trypsin on fibrinogen, where the sharp increase in amino N, characteristic of proteolytic fermentation, occurred. Whether or not the action of fibrinolysin is accompanied by proteolytic hydrolysis, is not clear. The end products appear to be protein but to have somewhat different properties from fibrinogen with respect to thermal precipitation point and the precipitating concentration of salts. Garner did not detect the evolution of ammonia during the experimental period.

From these experiments it seems likely that the chemical deg-

radation of the highly complex molecules of fibrin is not great, even though the physical change of solid fibrin into a solution is striking.

In referring to the observations of Garner and Tillett, Jablonowitz calls attention to the fact that globulin present in the impure preparations of fibrinogen may have accounted for the properties of the end products of the reaction rather than a change in the characteristics of fibrinogen to globulin through the action of fibrinolysin. Jablonowitz studied the alterations in the immunological specificity of fibrinogen following the action of fibrinolysin derived from a strain of hemolytic streptococcus of human origin. For purposes of obtaining highly purified material, he prepared fibrinogen by methods of repeated precipitation. This material, when tested against the antiglobulin serum described by Kendall, gave only a very faint reaction. Consequently it was used in the immunization of rabbits. The sera of the immunized rabbits was tested against two preparations: (a) sterile broth + fibrinogen, (b) fibrinolysin + fibrinogen. The two mixtures (a and b) were incubated for 24 hours at 37°C. before being used in precipitation tests with antifibrinogen serum. After the precipitation tests had been incubated, the precipitates were centrifuged, washed, and analyzed for total N. The total N in the precipitate produced with fibrinolysin + fibrinogen (b) was less (0.075 mgm.) than that obtained from the sterile broth + fibrinogen mixture (0.31 mgm.). Jablonowitz concluded therefore that fibrinogen was altered immunologically by the action of fibrinolysin. In other experiments to determine the rate of alteration, he found that there was an initial lag period of approximately 15 minutes followed by a rapid change which seemed to be complete in about an hour.

Garner (16) reported that the end product was not differentiated from fibrinogen by serological reactions. The findings, on which that observation was based, were obtained by Garner and Tillett (unpublished) in determining the precipitative titre by the usual technique. Using progressive dilutions of precipitinogen, the differences in the end points of the tests with fibrinogen and dissolved fibrin were not sufficiently great to indicate differences in the precipitinogenic preparations.

Doudoroff investigated the effect on fibrinolytic filtrate of cultures of various bacterial species. After mixing 48 hour cultures with the filtrate, he subsequently killed with chloroform the organisms which had been added and tested the mixture for fibrinolytic action. He found that the fibrinolysin was most regularly inactivated by bacteria which were capable of liquefying gelatin. The inactivating effect of the cultures was usually destroyed by heating at 60°C. for 30 minutes.

Madison and Snow (41) tested the antifibrinolytic effect of several antiseptics which they employed in sub-bacteriostatic doses in cultures. The results were not striking. They also added antiseptics to fibrinolytic tests and concluded that tincture of iodine impaired lytic action more definitely than other drugs.

Huntington cultivated strains of hemolytic streptococci in 0.05 per cent glucose-broth with and without 20 mg. per cent of sulfanilamide, and was unable to observe any deleterious effect upon the production of fibrinolysin by the drug.

VI. IMMUNOLOGICAL STUDIES

In immunological studies, oxalated plasma from the blood of normal individuals and patients has been most regularly employed. By this procedure, the measure of antifibrinolytic resistance is made with the fibrin of the patient's blood in the presence of whatever antifibrinolytic properties may be concomitantly contained in the additional constituents of the same sample of plasma. Serum has also been employed as in other immunological reactions. However, owing to special conditions of the tests, which will be referred to later, the serological method has not been regularly adopted.

Although 0.2 cc. of plasma has been usually employed, inquiry has been made into the possible significance of differences in the amount of fibrinogen contained in blood in different diseases. Hadfield and associates investigated this point and found that the content of fibrin in plasma did not appreciably affect the dissolution time, even when as much as 1400 mgm. per 100 cc. of blood was present. Van Deventer (75) concentrated fibrinogen fourfold and found that the speed of dissolution was slowed but did not result in complete refractoriness. From these find-

ings, it seems unlikely that, under the condition of usual tests, significant variations in the dissolution time are referable to the quantities of fibrin in the blood.

The value of using strains of hemolytic streptococci of highly potent fibrinolytic activity in antifibrinolytic tests has been advocated by investigators of the subject. In order to emphasize the difference between the results obtained with normal susceptible fibrin and patients' resistant plasma-clot, Tillett, Edwards, and Garner (66) employed the whole broth culture of a strain of maximum potency. By this procedure the greatest amount of fibrinolysin was contained in the test material, including such additional amounts as the living organisms might produce during the period of incubation.

Hadfield and his co-workers considered the use of a powerfully lytic strain important in differentiating between the rate of dissolution of normal fibrin and of that from patients. Stuart-Harris (63), using data derived from titration experiments, illustrated graphically the characteristic curve of the relationship between concentration of lytic agent and time required for fibrinolysis. On the basis of the ratios obtained, he concluded that the use of weakly active strains or high dilutions of potent strains so prolonged the dissolution time with normal fibrin that the assay of the degree of resistance in patients' fibrin was masked. Furthermore, differences between samples of fibrin, which were minor when potent material was used, were unduly emphasized when weakly acting preparations were employed. Other observers have employed three to five strains in each test and used the average results.

Limited consideration has been given to the possibility that the fibrinolysins of different disease-producing strains may be immunologically distinct. Tillett, Edwards, and Garner tested the blood of a few patients with the homologous strain derived from each patient but were unable to detect any difference in antifibrinolytic resistance. Van Deventer (74) tested forty strains against the fibrin of three normal persons and two resistant patients. He concluded that there was only one type of fibrinolysin among the strains. Yü and Zia described their findings with

plasma from a patient convalescent from scarlet fever which was shown to be resistant to a strain from a case of puerperal sepsis, but susceptible when tested with some of the scarlet fever strains. They did not clearly indicate whether all of the test strains possessed the same degree of fibrinolytic potency. At the present time, among human strains no definite evidence of immunological differences of the fibrinolysins has been obtained, although an exhaustive study of the subject has not been made.

Determinations of the presence or absence of resistance have been made by contrasting the brief length of time required to liquefy normal fibrin with either the absence of any dissolving effect on patients' fibrin or the prolonged period necessary to effect liquefaction. The three variables in fibrinolytic tests are: quantity of fibrin; quantity of fibrinolysin; time required for dissolution. Fibrinolytic "units" have been suggested by some observers. Madison and Taranik (39) proposed that the highest serial dilution of broth culture causing complete liquefaction of the fibrinogen-thrombin clot by the end of two hours incubation be assumed to contain one fibrinolytic unit. From the dilution, the number of lytic units per cubic centimeter of broth culture was calculated. Van Deventer (76) referred to a unit of fibrinolysin as three times the amount necessary to dissolve, within two hours, the fibrin of fibrinogen-thrombin preparations. Standards, however, have not been used extensively enough in studies of antifibrinolysin to be evaluated. A sufficient amount of information is not yet available concerning methods of quantitative measurement and the mechanism of the reaction to make improved procedures practicable. Consequently, estimations of resistance based on the factor of time has been most widely used. From the standpoint of exact quantitative measurements, the limit of experimental error is in all probability relatively broad. For this reason, rates of dissolution which might serve as sharp dividing line between normal and abnormal results have not been advocated. In the absence of arbitrary standards, most observers have, with minor variations, employed the following scheme for estimating degrees of resistance, when the amount of culture and plasma were kept constant: Dissolution

in less than one hour indicates susceptibility; dissolution in one to three hours indicates doubtful to weak resistance; dissolution requiring three hours or longer up to twenty-four hours indicates "definite" or "marked" or "partial" resistance; no dissolution during the twenty-four hour period of the test indicates "maximum" resistance. When several tests are set up with constant quantities of the same samples of plasma and culture, the dissolution time is constant within a narrow range of variation. Consequently, when the difference in time of liquefaction of two separate specimens of blood is a matter of several hours, the delayed rate assumes significance.

Concerning the susceptibility of the fibrin from normal persons, a sufficient amount of information has accumulated to indicate the average findings among healthy adults. Among thirty normal individuals, Tillett, Edwards, and Garner (66) found the dissolution time to be 8 to 15 minutes in thirteen instances, 15 to 60 minutes in eight tests, and from one to four hours with nine specimens. Morales-Otero and Pomales-Lebron (46) found that the time required for dissolution varied in tests with normal fibrin from 30 minutes to two and a half hours. Myers, Keefer, and Holmes reported that the average time for lysis with samples of blood from fourteen adults was one hour, the minimum time being 14 minutes and the maximum five hours. Waaler (78) stated that of tests made with specimens of blood from thirty-nine normal persons, thirty-four were classed as susceptible, and five as partially resistant. In a second article by Waaler (79) the blood of fifty of fifty-five normals were found to be susceptible and five partially resistant. Hadfield and associates stated that in tests with specimens of blood from twenty-eight adults none was totally resistant. Stuart-Harris (64) found the fibrin from 98.6 per cent of seventy-two persons to be susceptible. From his average results, and using a factor of standard deviation, he placed the limit of time for normal tests at 51 minutes.

From these results it may be seen that lysis of the fibrin clot of the blood of the great majority of normal individuals occurs in less than one hour, and commonly requires a considerably shorter time. Although each of the investigators has employed individ-

ual strains selected for the purpose but not standardized on the basis of any arbitrarily adopted unit of accurate measurement of of fibrinolytic potency, the results are in general agreement.

On the basis of these findings, it may be estimated that the blood of approximately 85 to 90 per cent of normal healthy individuals may be arbitrarily classified as susceptible on the basis of tests in which the dissolution time is less than one hour.

In tests made with the blood of normal children, the data for age groups ranging from three to fifteen years of age are consonant with the findings in adults. Owing, however, to the frequency of upper respiratory infections in children during the winter months, it has been suggested that varying degrees of resistance may occur more frequently than in adults.

Among the acute diseases, directly referable to infection with hemolytic streptococci, immunological studies of the following conditions have been reported: Acute tonsillitis, with and without extension to mastoid, middle ear, or sinuses; scarlet fever, with and without complications; erysipelas; suppurative infections such as empyema, peritonitis, and abscesses in different locations; septicemia arising from different sources. The data to be given were obtained by consolidating all of the findings presented by various authors. Although the averages are not entirely in accord with the individual findings of each report, the differences are not sufficiently great to warrant a separate account of each.

In immunological studies it has been found that the development of antifibrinolytic properties may be demonstrable at variable times during the course of the disease up to as late as the third or fourth week in convalescence. The summarizing data which follow are in many instances derived from repeated examinations of the blood during acute illness and convalescence. However, in some of the cases, only one or two tests were made. The conclusions, therefore, are to some extent based on partially complete results which limit final conclusions.

Acute tonsillitis. Tillett, Edwards, and Garner (66), Myers, Keefer, and Holmes, Stuart-Harris (63, 64), and Tillett (68) have reported results obtained in forty-eight cases. In thirty-two of

the patients (67 per cent) an antifibrinolytic response was noted during convalescence. The time in the course of the disease at which the specific resistance developed varied from the first week to as late as the fifth week. In uncomplicated cases, the period of lag between the cessation of active disease and the detection of antifibrinolytic properties in the blood usually ranged from two to four weeks.

The degree of antifibrinolytic response was also found to vary in individual cases. The severity and extent of the infection were not infrequently found to be important factors not only in evoking the development of high antifibrinolytic response, but also in shortening the time of appearance of the specific immunity.

Scarlet fever. Tillett and associates, in eight cases, Dack and associates in forty-seven cases, Stuart-Harris, in fifty-eight cases, and Waaler in fifty-seven cases found the blood of 86 (50 per cent) out of 170 cases to possess antifibrinolytic properties, observed in most instances during convalescence. As in the patients with acute tonsillitis, the results in scarlet fever indicated that the development of antifibrinolysis becomes demonstrable usually within two to five weeks after the cessation of active disease. Dack and his associates noted that in the first test with some of the patients, the dissolution time was prolonged. Waaler found antifibrinolytic properties more frequently in cases complicated by otitis media and nephritis than in cases with adenitis or arthritis. Stuart-Harris obtained antifibrinolysis most frequently in cases complicated by arthritis, carditis, and nephritis.

In view of the fact that scarlet fever in recent years has been relatively mild, it seems reasonable to presume that the somewhat less frequent occurrence of antifibrinolytic immunity in patients with scarlet fever (50 per cent) than in those with acute tonsillitis (67 per cent) may be ascribed to differences in the severity of the infections. Cases of scarlet fever are usually hospitalized regardless of the degree of illness, whereas only relatively severe cases of acute follicular tonsillitis seek admission to hospitals, and become available for study. The reports,

referred to earlier, that scarlatinal strains of hemolytic streptococci possess less fibrinolytic potency than other strains, also suggests limitations in the antigenicity of the fibrinolysin.

Erysipelas. Combining the results obtained in different laboratories (66, 47, 63), resistance to fibrinolysis developed in 37 (80 per cent) of forty-six patients. Tillett, Edwards, and Garner noted that the development of resistance coincided in some cases with the cessation of the spread of the lesion. However, in other instances, the same authors observed a delay of one to three weeks in demonstrable antifibrinolysis. In ten of their cases, Myers, Keefer, and Holmes noted a high degree of resistance which was present during the period of active disease and persisted after recovery. In general, the antifibrinolytic response appeared in erysipelas at an earlier time during the course of the disease than in the uncomplicated cases of either acute tonsillitis or scarlet fever.

Suppurative infections with and without septicemia. This group includes cases of unusual severity. In some of the patients the occurrence of septicemia was reported. The mortality rate was high. In six fatal cases with septicemia, Tillett (68) found that none developed antifibrinolytic immunity. The patients died between the 6th and 25th day of disease. It is apparent that the survival period may not have been long enough to permit the appearance of the immune response. However, the limited data suggest that the occurrence of antifibrinolysis is less frequent in overwhelming infections than in local processes. Dack and his associates reported among the patients with scarlet fever, one fatal case in which the blood contained a high degree of antifibrinolytic resistance. Myers, Keefer, and Holmes also described a case which ended fatally with maximum resistance to lysis present in the blood. Of six cases with septicemia, which recovered, (4 reported by Tillett, and 2 by Stuart-Harris), three developed antifibrinolytic properties; the fibrin-clots of the other three remained susceptible even after the infection was overcome.

The findings with a miscellaneous group of infections, including cellulitis, empyema, mastoiditis, peritonsillar abscess, etc. may be collected from the several articles dealing with anti-

fibrinolytic immunity. Of twenty-two such cases, sixteen (73 per cent) developed the specific immune response.

A summary of the results just given is as follows:

	Number of cases	*Resistance present in per cent*
Acute tonsillitis	48	67
Scarlet fever	170	50
Erysipelas	46	80
Miscellaneous	22	73
Septicemia with recovery	6	50
Fatal cases	8	25
Normal individuals	165*	10–15

* Approximate.

In a large number of the observations just summarized, the changes in the reaction of fibrin from susceptibility to resistance were demonstrated during the course of the diseases. The findings in serial tests with samples of blood from patients, who recovered, indicate that the fibrinolytic substance is frequently antigenic under the conditions of naturally occurring infections, and that the antifibrinolytic response is a specific immune reaction. However, additional observations suggest that insusceptibility to lysis may occur under conditions which are not referable to specific antibody response. The interpretation of single tests, carried out with plasma obtained during phases of active disease and convalescence will be subsequently discussed.

Furcolow and Fousek (14) performed eighty-four tests on seventy patients. In twenty-two instances no antifibrinolytic resistance was present. None of the latter had proven hemolytic streptococcus disease, although six were suspected. In twenty-five tests, the dissolution time ranged from one to three hours, which was interpreted as a suggestive but doubtful indication of resistance. Twenty-two of the patients either had proven hemolytic streptococcus infections or had been contacts. Antifibrinolytic resistance was marked in thirty-seven tests. Thirty-six of the cases had proven hemolytic streptococcus infections.

Rheumatic fever. Hadfield, Magee, and Perry made tests with the blood of forty-four children with rheumatic disease. The patients were divided into a group of twenty-one who had had recent active disease, and a second group of twenty-three quiescent cases. The first group was further subdivided into nine cases with sedimentation rate (red blood cells) above 20. Among them, five exhibited either maximum or partial resistance. Of twelve cases having had recent attacks but with a sedimentation rate below 20, five had maximum or partial resistance. The blood from each of the quiescent cases was susceptible to lysis.

Myers, Keefer, and Holmes made observations on thirty-four cases of rheumatic fever, twenty-nine of which either gave a history of a recent attack of acute respiratory infection or carried hemolytic streptococci in their throats at the time of admission to the hospital. Of these twenty-nine cases, the blood in twenty-seven possessed maximal antifibrinolytic resistance. Of the five remaining patients who had active disease but who gave no evidence either by history or by throat culture of having had hemolytic streptococcus infection, four possessed either maximal or partial resistance. The average time required for lysis in all the tests was nineteen hours.

Stuart-Harris (64) among twenty-two convalescent cases, found partial resistance in seven (32 per cent), and in tests with the blood of forty-eight active cases, twenty-nine (60 per cent) partial or complete resistance was present.

Waaler (79) tested the blood of seven patients with rheumatic fever, all of whom had acute infections of the throat. Six possessed maximal or partial resistance. He stated that when manifestations of active disease subsided, the antifibrinolytic property of the blood decreased.

Tillett (68) examined the blood of eight patients with active rheumatic fever, all of whom had had preceding acute upper respiratory infections. Six possessed maximum resistance, and two partial resistance.

Lippard and Johnson made observations on the blood of five cases (8 to 15 years of age). The dissolution time varied from 3 hours to maximum resistance. The authors also found high

titre of streptolysin antibodies in the same specimens of blood. However, the parallelism of antistreptolysin and antifibrinolysin was not quantitative, since the specimens with the highest titre of antistreptolysin did not exhibit the greatest degree of antifibrinolytic resistance. Stuart-Harris (63) also brought out the fact that titre of antistreptolysin and antifibrinolysin were not concomitantly present to the same degree.

Summary of antifibrinolytic tests in patients with rheumatic fever

	Number of cases	*Resistance present in per cent*
Active disease	123	72
Quiescent disease	45	15

From these results it is interesting to note that the findings obtained with cases of active rheumatic fever demonstrate that the frequency of the development of antifibrinolytic resistance (72 per cent) is essentially the same as that obtained in cases of acute tonsillitis without the visceral manifestations of rheumatic disease (67 per cent). Whether or not the rheumatic process has in itself the capacity to evoke an antifibrinolytic response or or whether upper respiratory tract infections due to hemolytic streptococci occurring frequently in rheumatic subjects elicit resistance to fibrin dissolution cannot be assayed from the results so far available. It is not within the scope of this article to discuss the broader subject of the possible relationship of hemolytic streptococci to rheumatic fever. However, the observations in cases of acute upper respiratory diseases of hemolytic streptococcal origin and also in cases of active rheumatic fever appear to be sufficiently consonant to indicate that the frequency with which antifibrinolytic properties develop in these disorders is comparable.

Rheumatoid arthritis. Myers, Keefer, and Holmes tested the blood from eleven cases; two had maximal resistance, and another, following acute sinusitis developed maximal resistance. The average dissolution time for the group was six hours, which is somewhat higher than the average of one hour for the normal controls, but considerably less than the average of nineteen hours for the cases of rheumatic fever.

Stuart-Harris in sixty cases of rheumatoid arthritis found resistance in six. Among ten cases of other types of chronic arthritis, no resistance was noted.

Waaler observed nineteen cases and recorded two as having 3+ resistance, four with 2+ resistance, four graded as 1+; nine were susceptible. He considered many of the reactions to be weak but suggested that resistance might have been more frequently encountered if the tests had been performed with samples of blood obtained earlier in the course of the disease. Neither the history of respiratory infections nor the results of bacteriological studies were reported.

Gonoccocal arthritis. Of six cases studied by Myers and associates two had maximum resistance. The average dissolution time was five hours which is approximately the same as that obtained in cases of rheumatoid arthritis. Tillett and associates found in one case that normal suseptibility remained unchanged in tests repeatedly performed during sixty days of activity and convalescence. Stuart-Harris also noted susceptibility in one case of gonoccocal arthritis.

Still's disease. In five children, Waaler observed no antifibrinolytic resistance.

Summary of antifibrinolytic tests in patients with arthritis

	Number of cases	*Resistance present in per cent*
Rheumatoid arthritis	90	14
Gonococcal arthritis	8	25
Still's disease	5	0

The findings with the arthritic group are significant when contrasted with the results obtained in rheumatic fever. It is also interesting to note that the frequency of antifibrinolytic resistance was slightly greater than in normal individuals. A discussion of the possible significance of these results will be reserved until the findings in other diseases are described. However, from the standpoint of critical analysis, it would appear to be necessary to exclude the possibility of a relatively recent hemolytic streptococcus infection—whether causal or incidental—as the incitant of the antifibrinolytic response in order to

interpret the findings obtained in chronic disorders of uncertain etiology.

Acute Nephritis. In five patients, all of whom had previously suffered from acute tonsillitis due to hemolytic streptococci, four developed moderate to maximal resistance (68). Waaler (78) commented upon the frequency of antifibrinolysis in seven cases. In four cases, Myers and associates found the average dissolution time to be six hours, but did not comment upon the occurrence of antecedent respiratory infection. Similarly, Stuart-Harris found the fibrin-clot in two cases to be susceptible. No bacteriological details were given.

Recurrent tropical lymphangitis. Morales-Otero and Pomales-Lebron (46) in a study of the relationship of hemolytic streptococci to tropical lymphangitis tested for the presence of antifibrinolytic properties in the blood of fourteen patients suffering from this disease. They found maximum antifibrinolytic resistance in 7 instances, moderate resistance in 3. In the remaining 4 cases resistance was either absent or doubtful. They reported that the resistance was usually most marked early in the disease, gradually decreased during convalescence, and rapidly reappeared with a recurring attack of lymphangitis.

Bacterial endocarditis. Myers and associates studied two cases due to *Streptococcus viridans* from each of which the fibrin-clot exhibited maximum resistance. A third case, with infection due to an indifferent streptococcus, was found in repeated examinations to be without antifibrinolytic properties.

Waaler (79) tested the blood of four cases which were due to *Streptococcus viridans.* Three of the four possessed antifibrinolytic properties. A fifth case, due to a fecal streptococcus, gave tests rated as 2+ resistance.

Stuart-Harris reported observations in two cases which were due to *Streptococcus viridans.* One of the patients, who had previously suffered from rheumatic fever, developed partial resistance. The fibrin-clot of the other was susceptible; and at autopsy no signs of rheumatic fever were noted. In a third case of undetermined bacterial etiology, no antifibrinolytic resistance was present.

The high incidence of antifibrinolytic properties in the blood

of patients with endocarditis due to *Streptococcus viridans* is an interesting finding, the interpretation of which is not apparent. If the resistance to lysis is dependent upon the presence of specific immune properties, the antifibrinolytic response appears to be evoked either by green streptococci or in association with the underlying rheumatic disease. The possible influence of non-specific factors in antifibrinolysis will be presently considered. It is interesting to note in passing that both McEwen and Coburn have reported in personal communications that the antistreptolysin titre of the serum of patients with bacterial endocarditis is usually not increased.

Diseases not associated with hemolytic streptococci.—It is unnecessary to consider individually the large number of diseases which have been used for comparison with infections due to hemolytic streptococci. The control groups have consisted of diseases of diverse bacterial etiology, such as pneumonia, tuberculosis, typhoid fever, diphtheria, staphylococcal infections, etc. The findings in pneumonia will be considered separately. With respect to the other non-streptococcal diseases, the results have not indicated that any specific type of disorder is characterized by the presence of antifibrinolytic properties in the blood. However, it is of interest to note that the average degree of antifibrinolysis in the control group of patients is somewhat greater than that found in normal persons. For example, Myers, Keefer, and Holmes reported the average dissolution time of the two groups to be four and one-half hours and one hour, respectively. Stuart-Harris inquired into the past history of the non-streptococcal cases which possessed antifibrinolytic properties, and in several instances noted that a preceding attack of tonsillitis or rheumatic fever may have accounted for the resistance to lysis. However, even though occurrence of a concomitant or preceding hemolytic streptococcus infection may be responsible, in some instances, for the antifibrinolytic response of patients with non-streptococcal diseases, there is suggestive evidence that alteration in the blood associated with the acute active phase of infection may inactivate the fibrinolytic process. In this connection the findings in pneumonia are of interest.

Pneumonia. Waaler (78) reported six cases of pneumonia,

in which the fibrin-clot was resistant. In one of the cases the resistance persisted for two months. Of five cases of pneumococcus pneumonia in adults reported by Tillett, Edwards, and Garner, the fibrin-clot of four was found to be susceptible both during the phase of acute, active disease and also during several weeks of convalescence. In one patient, however, the blood obtained during active pneumonia exhibited maximum resistance, but within a few days after recovery there was a sudden and complete loss of resistance. The rapid disappearance of the antifibrinolytic properties in this patient, associated with critical recovery, contrasted markedly with the gradual reduction over weeks or months of the resistance in patients with proven hemolytic streptococcus infections. Stuart-Harris studied four cases of pneumococcus infection, two of which were pneumonia, one of mastoiditis, and one of pericarditis. The fibrin-clot, in each instance, was found to be susceptible. The tests apparently were performed during acute illness although no specific statements are made as to the time in the course of the illness at which the specimens of blood were obtained. Myers, Keefer, and Holmes included cases of pneumonia in their large group of non-streptococcal diseases. As previously mentioned, the average dissolution time of the whole control group was four and one-half hours. The results with the blood from patients with pneumonia were not separated from the others.

The conflicting results with pneumonia consist of the uniform finding by Waaler of high antifibrinolytic resistance, and the negative results of others, with the exception of the one case mentioned. Waaler concluded from his studies of patients with bacterial endocarditis and pneumonia that the occurrence of antifibrinolytic properties in the blood of patients was not decisive evidence of hemolytic streptococcus infections.

Interesting information is obtained from the studies of Lippard and Johnson concerning children with pneumonia. They noted that, in the youngest patients, maximum resistance was present early in the disease but abruptly disappeared three to thirteen days after onset. This finding was, however, not regularly obtained in all of the children with bronchopneumonia. Boisvert

reported that in the pediatric age group, the majority of patients with pneumococcus pneumonia possessed antifibrinolytic resistance during the period of active disease but rapidly lost it after recovery.

An interpretation of the data obtained in pneumonia is not apparent at the present time. The factor of age of the patient may be important. In addition, certain other possibilities warrant consideration. The results obtained by some of the investigators were characterized by the fact that insusceptibility to fibrinolysis did not gradually appear over periods of time after the beginning of the infection, as occurs in usual immunological responses. On the contrary, the high antifibrinolytic potency of early tests was followed by abrupt loss instead of gradual disappearance. In view of this particular course of events, the possibility suggests itself that the inactivating effect exerted on the fibrinolysin of hemolytic streptococci by the blood of some cases of pneumonia is not dependent upon immunologically specific antibody but to non-specific substances present in the blood during acute illness and rapidly lost during recovery.[1] On the basis that the fibrinolysin is an enzyme, it is interesting to speculate whether antienzymic effects comparable to the rise of antitrypsin which occurs during acute infection might account for the inactivation of the fibrinolytic enzyme. An additional example of the effect of blood from cases of acute illness on hemolytic streptococci is furnished by the report of Tillett (70) who found that the serum of patients with pneumonia and other types of infection is highly streptococcidal, but the property is rapidly lost following cessation of active disease.

On the basis of the present information, the interpretation of single tests may be summarized as follows:

During active acute infections of streptococcal or non-streptococcal origin. *In children,* antifibrinolytic properties are fre-

[1] In a recent personal communication Dr. P. L. Boisvert of the Department of Pediatrics of Yale University School of Medicine outlined studies of antifibrinolytic immunity which are in progress. It would be premature to comment in this article on his extensive but uncompleted data. However, the findings, up to the present time, differentiate, in the pediatric age groups, between the specific immunity and probable non-specific inactivation.

quently present (Lippard and Johnson; Boisvert). *In adults*, antifibrinolytic properties are frequently absent (66, 47, 63) but have been noted in pneumococcus pneumonia (78).

During convalescence, the development of antifibrinolytic properties, following non-streptococcal disease, has not been reported; following infections due to hemolytic streptococci, antifibrinolytic properties have appeared in approximately 60 to 80 per cent of the cases.

The fact that the dissolution time in 10 to 15 per cent of normal persons is prolonged may account for the findings in which "moderate resistance" remains unchanged during acute illness and recovery. Since "maximum resistance" has not been noted in normal healthy persons, its occurrence during convalescence is strong presumptive evidence of relatively recent infection due to a hemolytic streptococcus.

Tillett and Garner reported that the serum from convalescent patients, the fibrin-clot of whose blood was resistant to dissolution, conferred antifibrinolytic properties when added to normal plasma. Demonstration of the presence of antifibrinolysin in the serum suggested that specific resistance to fibrinolysis was not dependent upon properties of the fibrin substrate itself. Van Deventer (75) isolated fibrinogen from the blood of several individuals. Tests with the plasma-clot of these subjects indicated varying degrees of resistance. However, when fibrinogen-thrombin preparations were used, no differences in susceptibility were noted. The same author (75a, 76) tested twenty-eight commercial antistreptococcus sera by "passive transfer" to normal human fibrin. He added the fibrinolysin in arbitrarily designated units to dilutions of sera and incubated the mixtures for 3 hours before adding to the fibrin constituents of the test. Six of the sera were found to possess high titres of antifibrinolytic antibodies. He added potent antisera to rabbit and monkey blood, allowed them to clot, and was able to demonstrate the antifibrinolysin in the serum expressed from the clots. Van Deventer also attempted to immunize rabbits with fibrinolysin, using several cultural preparations as antigens. However, the sera of the animals, even after many injections, failed to exhibit

antifibrinolytic properties when tested with susceptible human fibrin. Schmidt (57) titrated samples of antistreptococcus horse sera, and according to the quantitative procedures, which he described, 0.0025 cc. of highly potent sera were capable of inhibiting fibrinolysis.

In some respects the use of serum in testing for antifibrinolytic resistance is more advantageous for quantitative titration than is plasma. However, factors which have not up to the present time been studied in detail may condition the serological results. Because of an insufficiency of experimental data it is unnecessary to discuss the problem in detail. However, mention may be made of the fact that the thrombin contained in sera may be sufficiently high to coagulate, either wholly or partially, the substrate without the addition of $CaCl_2$ to oxalated plasma, or of specially prepared thrombin to fibrinogen. Since both thrombin and antibodies are closely associated with the globulin fraction of blood, the possibility suggests itself that the thrombin of immune sera might carry antifibrinolysin into the forming fibrin, whereas the thrombin of normal sources results in the formation of susceptible fibrin. It is apparent that the standardization of serological procedures must await additional studies.

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