JCI The Journal of Clinical Investigation

THE EFFECT IN PATIENTS OF STREPTOCOCCAL FIBRINOLYSIN (STREPTOKINASE) AND STREPTOCOCCAL DESOXYRIBONUCLEASE ON FIBRINOUS, PURULENT, AND SANGUINOUS PLEURAL EXUDATIONS

William S. Tillett, Sol Sherry

J Clin Invest. 1949;28(1):173-190. https://doi.org/10.1172/JCI102046.

Research Article

THE EFFECT IN PATIENTS OF STREPTOCOCCAL FIBRINOLYSIN (STREPTOKINASE) AND STREPTOCOCCAL DESOXYRIBONUCLEASE ON FIBRINOUS, PURULENT, AND SANGUINOUS PLEURAL EXUDATIONS[1]

By WILLIAM S. TILLETT and SOL SHERRY

(From the Department of Medicine, New York University College of Medicine, and the Third Medical Division of Bellevue Hospital, New York City)

(Received for publication August 6, 1948)

The results described in this article were obtained by the injection of concentrated and partially purified preparations derived from broth cultures of hemolytic streptococci into the pleural cavity of selected patients who were suffering from different types of diseases that gave rise to pleural exudations. The possibility has been explored of utilizing two of the defined properties elaborated by hemolytic streptococci that have the unique capacity of causing rapid lysis of the solid elements (fibrin and nucleoprotein) that are significant parts of exudates. In a companion article, Christensen (1) has described additional steps in the purification of the materials employed and has given quantitative methods of measurement of various increments concerned in the fibrinolytic system, including trypsin inhibitor and specific antibodies, which have been employed in this study.

In the study of patients it was first necessary to: (*a*) develop the investigation within nontoxic but effective ranges of doses of the material employed, and (*b*) determine whether or not the enzymatic activities of the streptococcal products were effectively operative when introduced directly into the site of the disease in the patients. This article is essentially limited to findings that are pertinent to the two aspects of the study mentioned above.

A brief description of the two streptococcal products that have been the subject of the investigation is as follows:

I. *Streptococcal fibrinolysin* (2) (*Strepokinase*)

This has been found to be elaborated characteristically by strains of hemolytic streptococci (Group A) causing acute infections in patients. It is also produced by some strains of streptococcal groups C and G (3). The product is abundantly excreted into the culture medium in which the organisms are grown and is readily obtainable free from the bacterial cells in sterile filtrates.

The fibrinolytic action, in tests conducted under optimal laboratory conditions, is unusually rapid in action on the fibrin coagulum of normal human blood, requiring only a few minutes when whole plasma is employed as a source of fibrin, and an even shorter time when preparations of fibrinogen and thrombin of human origin are used. The ultimate proteolytic nature of the fibrinolysis has been evident in the liberation of breakdown products of protein digestion (4).

Another interesting feature of the phenomenon is the high degree of activity of the streptococcal lytic system in the presence of normal human blood as contrasted with its inaction or delayed effect when tests are conducted with the blood of various animal species with the exception of monkeys (3). Although studies of factors involved in streptococcal fibrinolysis have indicated a basis for the differences in the behavior of blood from different animals, nevertheless the existence of the difference has rendered impossible most experimental studies, *in vivo,* on animals and has limited them to observations conducted in patients, such as those initially described in this article.

The mechanism of the reaction has been a subject of study by various investigators.

In an earlier report (2) it was noted that fibrin formed from mixtures of either human fibrinogen and rabbit thrombin, or of rabbit fibrinogen and human thrombin were susceptible to streptococcal lysis, whereas the fibrin derived from rabbit fibrinogen plus rabbit thrombin was unaffected. Milstone (5) demonstrated that an increment of human serum present in the euglobulin fraction,

[1] This study was supported by a grant from the National Institute of Health, United States Public Health Service.

was necessary for the reaction to occur and that if preparations of human fibrinogen and thrombin were highly purified no lysis would occur when the streptococcal product was added. Furthermore, if this human globulin fraction was added to fibrin made up of rabbit components, lysis occurred. He referred to the euglobulin constituent of human blood as "lysing factor" and suggested that streptococcal fibrinolysis was mediated by the same system as that involved in other examples of fibrinolysis.

Christensen (4), and Christensen and MacLeod (6) have presented results concerning the mechanism, and have concluded that the streptococcal product to which they have given the name streptokinase acts as a catalyst or kinase in causing the transformation of a zymogen of normal human blood, designated by them plasminogen, into a proteolytic enzyme, designated plasmin.

Kaplan (7) has made extensive studies on several aspects of the mechanism of the streptococcal fibrinolysin-serum protease system. These studies have in part dealt with the dissimilarity of serum protease and trypsin and also with the inhibition of streptococcal fibrinolysis by antiprotease. The findings are predicated upon the kinase nature of the action of the streptococcal product and the identity of the protease of serum as the lysing principle. Ratnoff (8), studying a proteolytic enzyme in human plasma, gave special attention to the fibrinolytic system activated by the streptococcal product and obtained data indicating that the activation of plasma proteolytic enzyme by streptococcal fibrinolysin behaved as if it involved a stoichometric reaction. This finding implies that the streptococcal product may take an active part in the lytic system rather than exert only a catalytic effect.

Without developing the discussion of the mechanism, it is sufficient to state that the objective demonstration of changes in constituents of pleural exudates that have been effected by the streptococcal fibrin-dissolving system, acting within the pleural cavity of patients, constitutes definitive results around which this report centers. The conditions, unavoidably inherent in the several factors that may be simultaneously operative in association with the processes of disease within patients, makes it impossible at the present time to analyze and identify the mechanism of fibrinolysis that may be responsible for the end results that we have observed.

Nomenclature. The fibrinolytic principle derived from hemolytic streptococci is referred to in this article as *streptokinase.* The kinase type of action of the streptococcal product has been consistently observed by several investigators. However, whether or not its complete activity is solely that of kinase remains to be determined.

The term *fibrin-lysing system* has been employed as an objectively descriptive expression which avoids the different recommendations of various investigators (6, 9–11). The *activatable fibrin-lysing system* may be closely identified with the plasminogen of Christensen and MacLeod (6), and the *active fibrin-lysing system* with their term plasmin.

II. *Streptococcal desoxyribonuclease*

In recent articles the occurrence of desoxyribonucleoprotein in significant amounts in purulent empyematous fluids was reported (12) and its depolymerization by a desoxyribosenuclease found to be present in the concentrated streptococcal filtrates was also described (13).

The solid sediments of the samples of pus were found to contain as much as 30% to 70% nucleoprotein. Since the physical characteristics of desoxyribose nucleoprotein are fibrous and gelatinous, the similarity of its appearance to that of fibrin became a matter of importance in identifying the dual enzymatic systems with which we were dealing; namely, fibrin substrate in the fibrinolytic system, and nucleoprotein substrate acted upon by nuclease. In each instance rapid liquefaction of the respective solid substrate occurred.

Each of the substrates mentioned has been identified in varying amounts in different types of pleural exudation. For example, empyemal pus of bacterial origin is high in its content of nucleoprotein, which is presumably derived from the leucocytes, while the constituent most conspicuously present in hemothorax is fibrin. Fibrinous pleural exudates such as may be encountered in association with pneumonia, pulmonary neoplasm, and other diseases, or the effusions of tuberculous pleurisy have been studied chiefly for the fibrinolytic effect, although from

observations on preparations of the exudates stained by the Feulgen method (12), it is evident that varying amounts of nucleoprotein are present.

Streptococcal concentrates [2]

The preparations employed in this study have been prepared by Christensen according to the method of partially purifying the concentrated filtrates of hemolytic streptococci previously described and further elaborated in the accompanying article (1, 14). A strain of hemolytic streptococcus (H46A) belonging to Lancefield Group C has been employed. This strain is potent in its production of both streptokinase and desoxyribose nuclease (13) and most of the lots of concentrated filtrate used in this study have possessed both properties. In addition it seemed reasonable to assume that since the strain (a group C) was not of patient origin its products might presumably be least likely to contain other noxious, but as yet unidentified, increments associated with acute infections in man and would, therefore, be more desirable for preparing material to be used in patients than would a Group A fibrinolytic strain. All the partially purified and concentrated preparations, with one exception, that were introduced into patients, contained both streptokinase and nuclease. In an additional preparation used in a single patient with hemothorax, the content of nuclease was reduced substantially by Christensen (1) but the streptokinase activity remained unimpaired.

The dosage of streptokinase has been based upon a *unit,* developed and described in the accompanying article by Christensen (1). The dosages per patient are given in the individual protocols. In general they ranged from 20,000 to 400,000 units. The concentration per cubic centimeter ranged from 2,000 to 40,000 units. The total dose was usually contained in 10 cc. of normal saline solution.

The desoxyribonuclease content of the various preparations of streptococcal concentrates has not yet been elaborated so extensively as that of streptokinase. Christensen (1) has suggested a unit basis which is at present being employed. In general the concentrates contained a ratio of approximately 6,000 units of nuclease to 15,000 units of streptokinase. The nuclease was of special significance in the cases of empyema, and the dosage is given in the description of the results obtained in that group.

Toxicity of streptococcal concentrates. Among the initial studies (2) concerned with the characterization of the streptococcal fibrinolytic phenomenon, preliminary observations (unpublished) were made with respect to the skin reactivity of active filtrates derived from strains of fibrinolytic streptococci. Following intracutaneous injections, areas of local erythema developed that simulated the type of a positive reaction elicited by the erythrogenic scarlatinal toxin. However, by comparative tests in which both scarlatinal toxin and fibrinolytic filtrates were injected into Dick positive and Dick negative individuals, no correlation was detected. From the limited observations, it seemed reasonable to assume that the primary toxicity of the fibrinolytic filtrate was due either to the fibrinolytic principle itself or to some contaminating product other than the established erythrogenic toxin of hemolytic streptococcal origin.

When the recent concentrates prepared by Christensen (1) in the process of purifying the streptococcal fibrinolysin were being considered for introduction into patients, intradermal tests were performed. Although the concentration of streptokinase was increased many hundred-fold over the preparations of earlier use, the degree and frequency of erythematous reactions were found not to be any greater than those previously encountered nor to be related to the concentration.

Samples of the concentrates were then injected intramuscularly. No reactions occurred that were detectable by the development of local redness, tenderness or swelling, or as a generalized pyrogenic effect. When increasingly large concentrations failed to produce untoward effects intramuscularly, small amounts were introduced intrapleurally into patients with empyema, inflammatory pleural effusions, or hydrothorax.

Without describing the details of the progressive observations following intrapleural injections it may be stated that as the procedures of purification and concentration conducted by Christensen developed, the evidences of toxicity have diminished in relationship to amount injected. The studies have, therefore, up to the present time indicated that the primary toxicity of the fibrinolytic principle or the accompanying desoxyribonuclease is not great and that the transient pyrogenic reaction may be due particularly to contaminating substances still present in even the purest preparations now available.

The manifestations of toxicity when they occurred have consisted of a pyrogenic reaction beginning *approximately* six to eight hours after the injection, reaching its peak of a rise of one to four degrees at 24 hours after injection and decreasing gradually over the next 24 to 72 hours to the pre-injection level which in some instances was a normal temperature and in others was elevated prior to the injection due to the underlying disease. General malaise accompanied the febrile period. Nausea and gastro-intestinal discomfort were noted in some patients. Local pain, although never severe, was present in some instances, and not in others.

An outpouring of polymorphonuclear leucocytes occurred which was demonstrable 24 hours after the injection and decreased to the pre-injection level within the next two to ten days. The increase in the leucocyte counts ranged from 1,000 to 25,000 cells per cubic centimeter.

The general pyrogenic reaction together with individual symptoms as mentioned, and the local outpouring of cellular constituents did not follow any uniform or correlated pattern, since all of the manifestations were not present to the same degree and some of them were entirely absent. In several instances in which two injec-

[2] Lederle and Co. have cooperated by growing large volumes of culture and supplying the filtrate in its initial concentrated form.

tions were given, the first may have caused a reaction while the second elicited no toxic response, or the first was silent and the second evoked some reaction. Furthermore, the occurrence of reaction was not clearly related to dosage of concentrate.

In this study a total of 34 injections was given to 23 patients. Utilizing a rise of 1° F in fever as an indication of a reaction—and irrespective of whether or not the patient was febrile before the injection—on 17 (50%) occasions a pyrogenic response was noted.

The degree of reaction appeared to be most directly related to the nature of the patient's disease. In instances where considerable thickening of the pleura was present or other circumstances that may have impaired the absorbing surface, minimum or no toxic reactions occurred. In other cases where the contrary situation of a thin pleura or presumably a relatively freely acting absorbing surface was present, the reactions as described were more consistently noted.

Other factors that may participate in the reaction in a manner as yet undetermined are the breakdown products derived on the one hand from the fibrinogen and fibrin system and, on the other hand, from the nucleoprotein system through the action of nuclease. No specific information on this phase of the subject is available except the fact that the toxic reaction, whatever its exact cause may be, was a transient one of the classical brief pyrogenic type without any residual effects. No indication of chronic alterations of an untoward nature have been evident. Because of the implications of hemolytic streptococci in relation to rheumatic fever and acute hemorrhagic nephritis, detailed examinations for their possible presence have been frequently made with uniformly negative findings.

It has been of special importance to make preliminary tests with each new lot of purified concentrates made available for use in patients by injecting a small amount intrapleurally and noting the degree of reaction. When the reaction appeared to be relatively greater than would be expected from the small dose, the lot was not further employed in the study of patients. This precautionary procedure of selecting individual preparations for intrapleural use has been regularly adhered to, and, as a result, no contraindications for the development of the study have been encountered.

PROCEDURE

Observations on Patients. In the study of patients numerous quantitative determinations have been made on samples of exudate obtained prior to the injection of streptococcal concentrates and at stated intervals thereafter, usually one hour, 24, 48, 72 hours, etc., according to the nature of the case and the findings.

The tests and procedures employed have included the following: fibrinogen, N.P.N., total protein, formal titration, amount of sediment, viscosity, pH, volume of pleural exudate, free streptokinase, activatable fibrin-lysing system, active fibrin-lysing system, trypsin inhibitor, and specific anti-fibrinolytic antibody (anti-streptokinase).

Cytological studies, including total and differential counts, determination of motility and viability of cells and the appearance of Feulgen-stained preparations for the purpose of identifying intra- and extracellular desoxyribose nucleoprotein have also been made. These results will be referred to briefly in this article and be reported in detail in a subsequent communication.

The technical procedures in each instance have been as follows:

Streptokinase titre, activatable fibrin-lysing system (equivalent to plasminogen), trypsin inhibitor, and anti-streptokinase titre were determined on chest fluid according to the methods described by Christensen (1).

Active fibrin-lysing system (equivalent to plasmin) was determined as follows: To 0.1 cc. samples of chest fluid, brought to neutral pH, were added 0.5 cc. of 0.25% Bovine Fibrinogen (Armour) in 0.01 M saline phosphate buffer (pH 7.4), and 0.5 cc. of 0.25% Human Plasma Fraction III (Cohn) in saline phosphate buffer. One tenth ml. of 1/3 dilution of Lederle Hemostatic Globulin (or 1/500 dilution of Parke Davis Bovine thrombin) in saline phosphate buffer was then rapidly added. The tubes were incubated in a water bath at 37°. One tenth cc. of saline instead of chest fluid was used for controls. The time of complete lysis of the fibrin clot was noted. Since the lysis of fibrin in the absence of extra increments of streptokinase was relatively slow and, therefore, could not be expressed in the same unitage scale, the results were expressed as $0 - ++++$ as follows:

lysis up to one hour ++++
lysis one to six hours +++
lysis six to 24 hours ++
lysis 24 to 48 hours +
lysis 48 to 72 hours but no lysis in controls ±
No lysis 0

Estimations of the degree of activity of the fibrinlysing system with respect to time have proved important since the period required for lysis, *in vitro,* reflected to some degree the activity of the process, *in vivo,* which was significant with respect to changes in the exudates that continued for several days after the initial injection.

Amount of sediment: Wintrobe hematocrit tubes were filled to the mark with a heparinized sample of chest fluid and centrifuged at 2,500 rpm for one hour. The results are expressed as per cent.

pH was determined on separately collected heparinized samples by glass electrode after chest fluid was allowed to equilibrate with room temperature. A previously determined temperature correction for the instrument used was applied, so that the results are expressed as pH at 37° C.

Viscosity: The viscosity of thin fluids was determined on heparinized samples in an Ostwald viscosimeter at 37° C. The viscosity of thick purulent fluids was determined with an LV model Brookfield electric viscometer at 37° C.

Total protein and N.P.N. were done on the supernatant of centrifuged oxalated specimens of chest fluid. The total protein was determined by Kjeldahl digestion and nesslerization. The N.P.N. was determined by digestion and nesslerization of a trichloracetic acid filtrate. The results are expressed as milligrams per cent for N.P.N., and grams per cent for total protein.

Fibrinogen was determined on the supernatant of centrifuged oxalated specimens. Five-cc. samples were diluted to 30 cc. with normal saline, to which were added 1 cc. of 2.5% $CaCl_2$ solution and 1 cc. of a 1/100 dilution in normal saline of Parke, Davis Bovine Thrombin. The tubes were then placed in the refrigerator overnight. The clots were removed with a stirring rod, washed with distilled water, and the nitrogen determined by digestion and nesslerization. The results are expressed as milligrams per cent.

Formol titration: Ten-cc. samples of heparinized fluid were brought to pH 7.0 by addition of either 0.1 N NaOH or 0.1 N HCl. Five cc. of 40% formaldehyde were added, and the mixture titrated with standard 0.1 N NaOH by glass electrode to pH 8.6. The volume of alkali necessary to bring 5 cc. of 40% formaldehyde to pH 8.6 was subtracted from the titration. The results are expressed as cubic centimeters of 0.1 N NaOH per 100 cc. of chest fluid.

Volume of pleural fluid: Since information concerning the volume of pleural effusions was desirable, the following method was developed. The fluid volume was determined in pleural transudates and non-purulent exudates by injecting known quantities of T-1824 and sampling the pleural fluid from 15 to 60 minutes thereafter. This method is not applicable to purulent empyemas where a significant portion of the dye is bound to the sediment of the fluids and probably to the shaggy pleural surface, but quantitative determinations on non-purulent pleural effusions have yielded satisfactory results.

RESULTS

Twenty-three patients have been studied following the injection of amounts of streptococcal concentrates sufficiently large to bring about definitive and measurable biochemical and biophysical changes in the area of exudation at the site of the injection. Although the general plan of study has been the same in all instances the presentation of the results has been arranged according to a grouping of the cases based on the types of exudates which were present in the pleural cavities of the patients. The changes that have been effected by the concentrates have been found to bear a relationship to the character of the exudates.

The three major groups were: acute fibrinous pleurisy, bacterial empyema, and hemothorax.[8]

Group I. Acute fibrinous pleurisy, 13 patients.

Five cases of tuberculous pleurisy with effusion.
Six cases of primary or metastatic malignant pulmonary neoplasm with pleural effusion.
Two cases of congestive heart failure with pleural effusion.

Figure 1 contains data derived from serial determinations of fibrinogen and nonprotein nitrogen made on seven patients before and after the introduction of streptococcal concentrate. The dosages, given in the figure, ranged from 20,000 to 200,000 units of streptokinase, and approximately 20 to 200 units/cc. chest fluid. Three of the patients received a second injection.

The fibrinogen represents the chief parent protein (plus an undetermined amount of solid fibrin) that was acted upon by the fibrin-lysing system, and the N.P.N. represents the end-product of the local proteolysis.

The findings in each of the seven patients demonstrate the rapidity of the beginning decrease in measurable fibrinogen and increase in N.P.N., the samples taken as early as one hour after the injection revealing changes in the values of each. In patient W. H., for example, the immediate drop in fibrinogen was from 41 to 20 mgm.%, and in patient J. G., from 108 to 26 mgm.%. The concomitant rise in N.P.N. in patient W. H. was from 45 to 52 mgm.%, and in patient J. G., from 31 to 41 mgm.%.

In five of the seven patients of the charted group, a further drop in fibrinogen was noted in the specimen obtained 24 hours after the injection, and in each of the seven the N.P.N. either continued to rise or maintained a higher level than that of the pre-injection figures.

Three of the patients received a second injection of streptococcal concentrate; patient C. L. received 100,000 units of streptokinase four days later, patient E. H. received 40,000 units of streptokinase three days later, and patient J. G. received 200,000 units of streptokinase three days later. In each case there was a further abrupt

[8] The authors wish to acknowledge the cooperation and many helpful suggestions which they have received from the Attending and Resident Medical and Surgical Staffs of the Chest Service of Bellevue Hospital, Dr. J. Burns Amberson, Director.

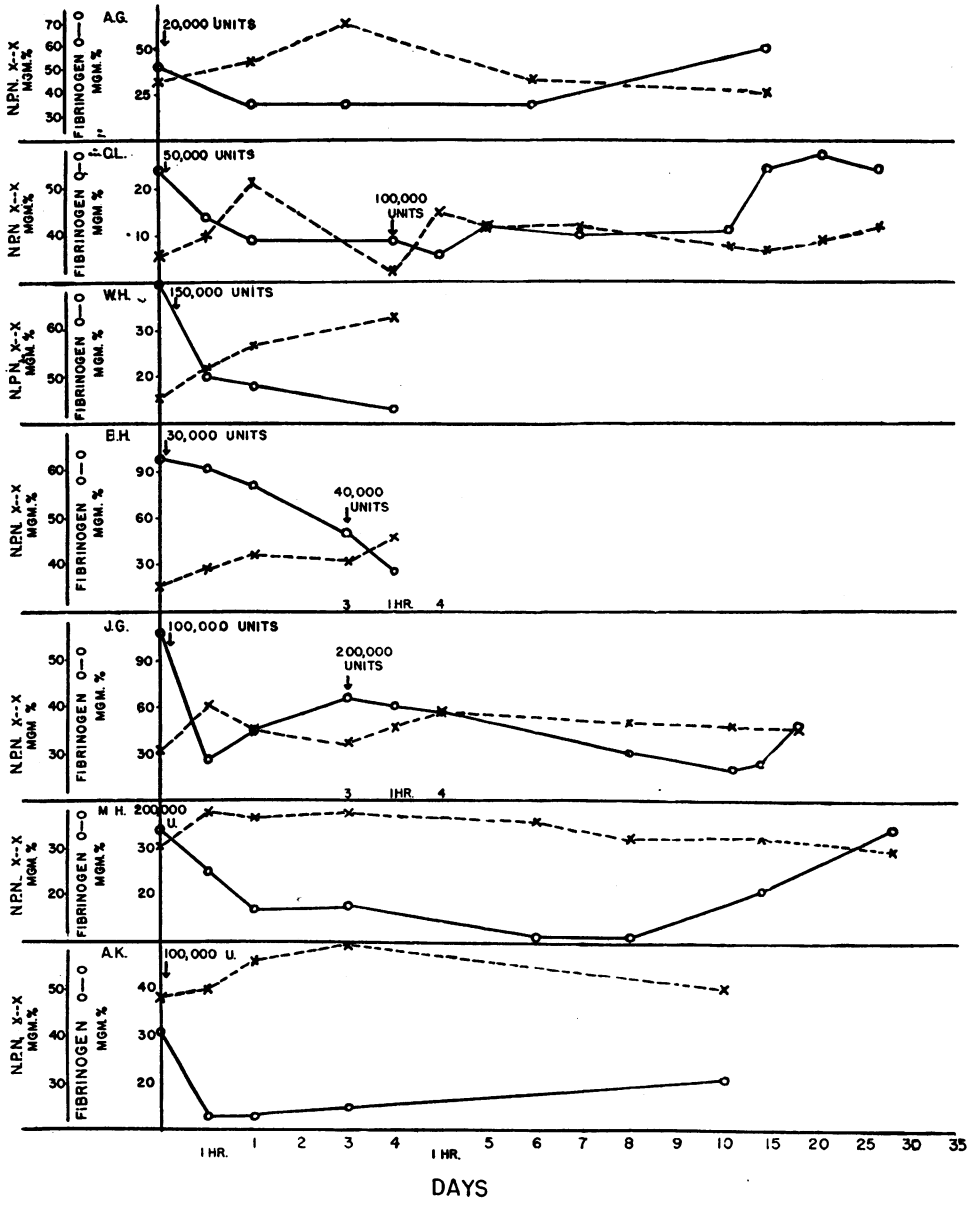


FIG. 1. THE EFFECT OF INTRAPLEURALLY INJECTED STREPTOKINASE ON THE FIBRINOGEN AND N.P.N. CONCENTRATIONS OF THE CHEST FLUID OF SEVEN PATIENTS WITH FIBRINOUS PLEURAL EFFUSIONS

The crosses represent N.P.N. (mgm.%), and the circles, fibrinogen (mgm.%).

decrease in fibrinogen and rise in N.P.N. following the second injection.

In five cases in which the fibrinogen content was followed for long periods of time by examining samples of pleural fluid obtained at repeated intervals, the levels of fibrinogen were found to return to the control value only after ten days to one month. Why the fibrinogen remained low for this period of time is not readily apparent. Continuing lytic action during this period was not evident by the measurements of the active fibrinlysing system employed. Furthermore, the fibrinogen levels remained low despite the appearance of antistreptokinase, and was independent of the reappearance of additional increments of the activatable fibrin-lysing system through further exudation of serum into the pleural area. It may represent the time necessary for the exudation of

additional fibrinogen to occur into the area of pleurisy, or slow proteolysis may have continued but at a rate not determinable by our present methods.

There were in the patients several uncontrollable factors that altered the serial findings as they extended over several days. For example, when the patient's disease was in an acute active state, there was a continuing increase in the effusion together with the inflammatory constituents. When the disease was stationary or subsiding, the contents of the pleural cavity were not substantially altered during the period of observation. In addition, the variable degree of local irritation caused either by the injection material itself or the breakdown products of proteolysis contributed

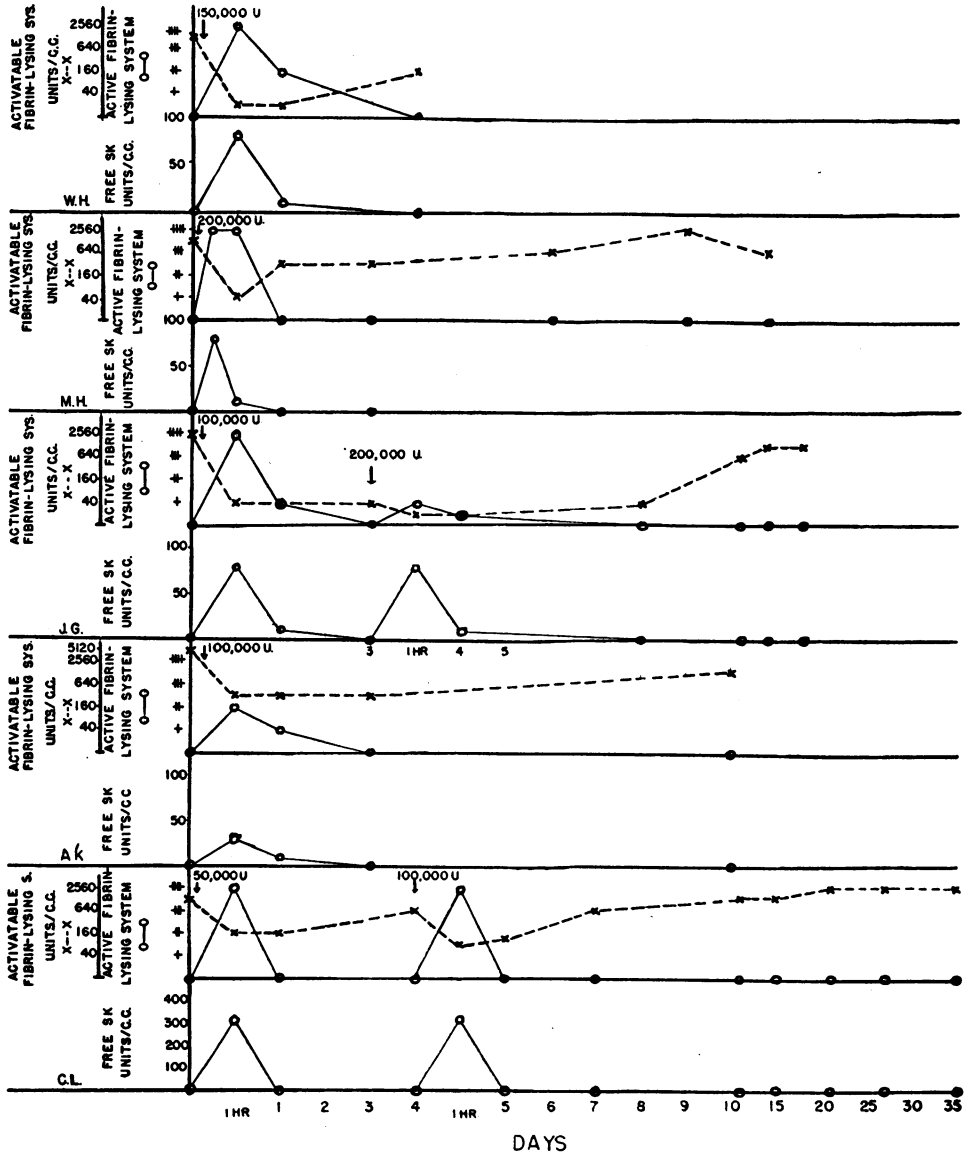


FIG. 2. THE EFFECT OF INTRAPLEURALLY INJECTED STREPTOKINASE ON THE FREE STREPTOKINASE, ACTIVATABLE AND ACTIVE FIBRIN-LYSING SYSTEMS OF THE CHEST FLUID OF FIVE PATIENTS WITH FIBRINOUS EXUDATES

In the graphs for each patient, in the top sections the crosses represent the activatable fibrin-lysing system (units/cc.) and the circles the active fibrin-lysing system; in the lower sections, the circles represent free streptokinase (units/cc.).

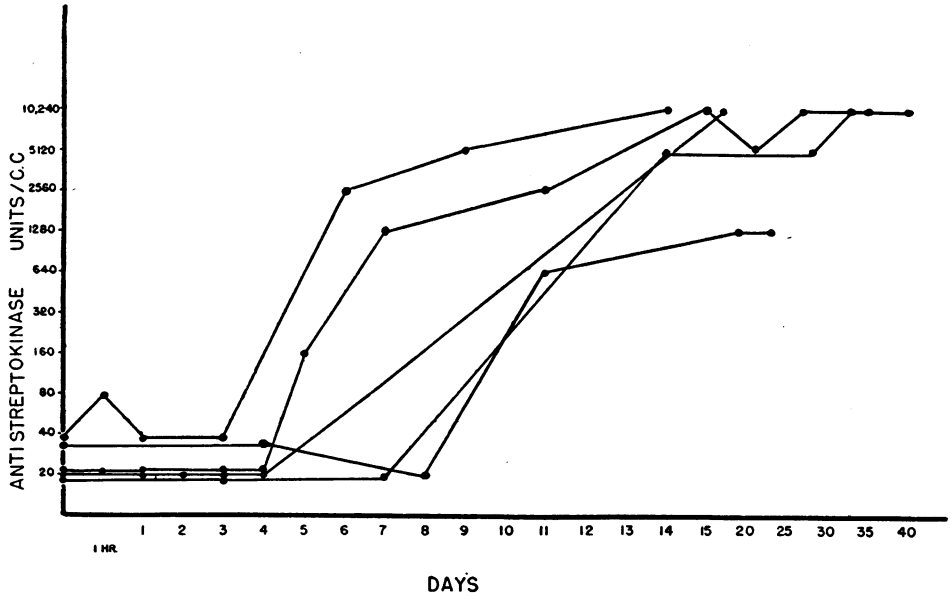


FIG. 3. THE ANTISTREPTOKINASE TITRE IN PLEURAL EFFUSIONS FOLLOWING INTRAPLEURAL ADMINISTRATION OF STREPTOKINASE IN FIVE PATIENTS WITH FIBRINOUS PLEURAL EXUDATES
Streptokinase was injected at zero time.

an additional increment of exudate. Furthermore, the rate of absorption of the newly formed non-protein nitrogen proceeded at variable rates. Consequently, the figures obtained by analysis of the exudate were only relative with respect to total proteolysis.

In spite, however, of the variables outlined above, the intrapleural degradation of fibrinogen in significant amounts following each injection of the streptococcal concentrates has uniformly occurred in each of the patients in this series, as well as in those of the other groups. The extent to which solid preformed fibrin underwent lysis was not measurable but its breakdown undoubtedly contributed to the N.P.N. that was liberated.

In some of the patients, estimations of blood N.P.N. were done but no significant rise above normal levels was noted, the rate of absorption from the pleural area being insufficient to raise the content of the general circulation.

The data contained in Figure 2 concern three elements involved in the streptococcal fibrin-lysing system, namely: free streptokinase, and both the activatable and the active fibrin-lysing system. They are recorded for five patients of the acute fibrinous pleurisy group. Similar findings were obtained from most of the patients studied of all the groups.

Prior to injection the amount of activatable fibrin-lysing system present in the pleural exudate was approximately the same as that found in normal serum but within one hour after the injection of streptokinase striking changes occurred in the elements of the fibrin-lysing system of the pleural exudate. When streptokinase was introduced, the activatable elements of the lytic system were replaced by active increments which were demonstrable immediately following injection but progressively disappeared during the next 24 to 48 hours. This implies that once the system assumes the active form it is subsequently destroyed, or disappears through combination with substrate. Its replenishment must await, through further exudation from the general circulation into the local area, the addition of new components of the activatable system which were rapidly depleted following the introduction of streptokinase. The rate at which the replenishment occurred has depended upon factors that control the reformation of exudate. In the series of cases used in this study, it has been demonstrable within a few days. These findings indi-

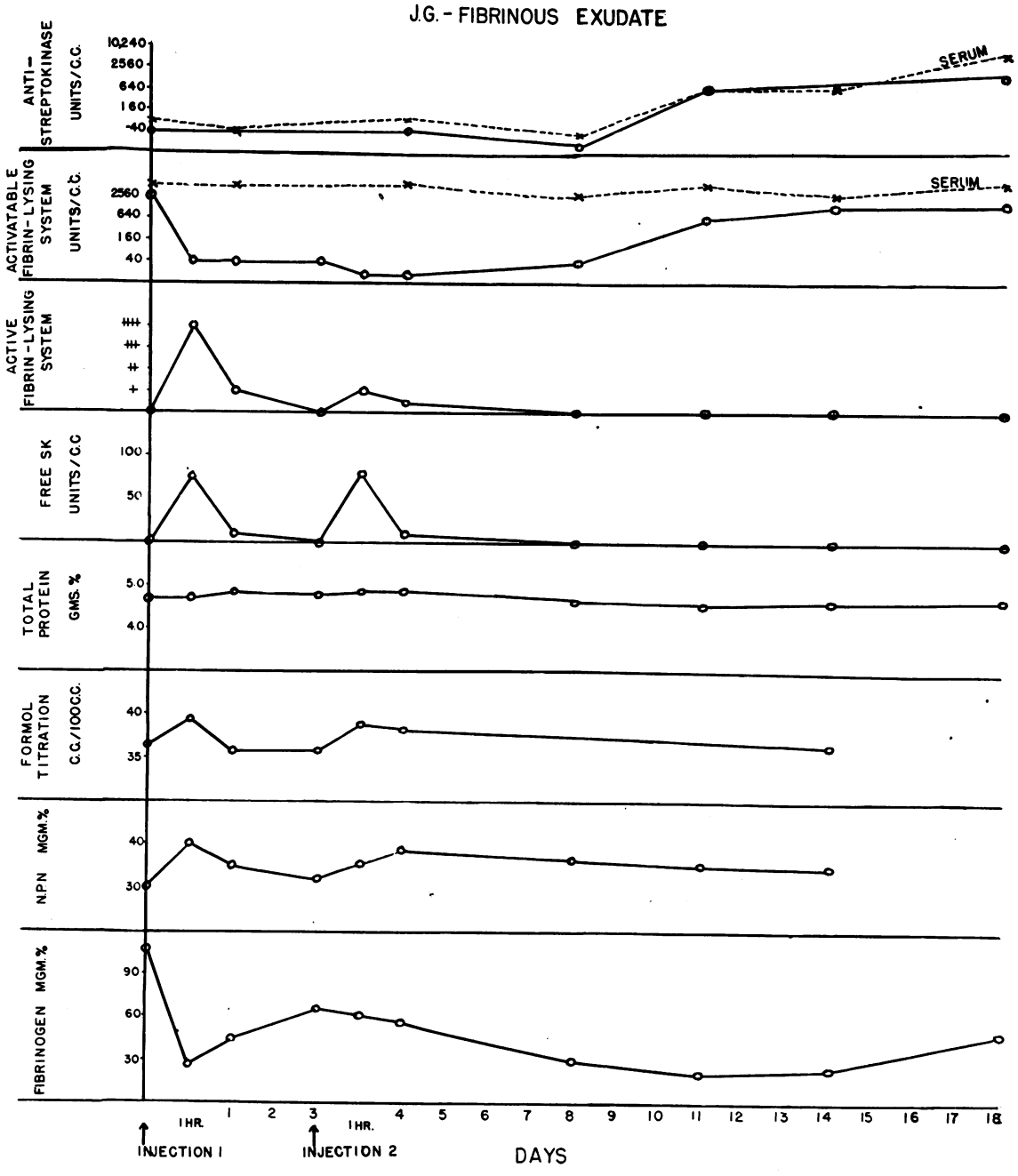


FIG. 4. THE EFFECT OF TWO INTRAPLEURAL INJECTIONS OF STREPTOCOCCAL CONCENTRATE IN A CASE OF TUBERCULOUS FIBRINOUS EXUDATE ON THE FIBRINOGEN, N.P.N., FORMOL TITRATION, TOTAL PROTEIN, FREE STREPTOKINASE, ACTIVE AND ACTIVATABLE FIBRIN-LYSING SYSTEMS, AND THE ANTISTREPTOKINASE TITRE

100,000 units of streptokinase were given in injection 1, and 200,000 units in injection 2.

cate the self-limiting nature of the activity following a single injection of streptokinase.

In our observations trypsin inhibitor has not changed after streptokinase injections and the results indicate that this inhibitor plays little part under these circumstances in the inactivation of the system.

An excess of free streptokinase was found to be present one hour after the injection but was not demonstrable 24 to 48 hours later. The extent

to which it may be dissipated by combining directly with substrate, or by inactivation by some inhibitor, or by neutralization with specific antibody is not at present determined. Also suggested by the data in Figure 2 is the finding of active fibrin-lysing elements *only* in the presence of free streptokinase. This observation may have a bearing on the mechanism of the phenomenon as it occurs in exudates and will be given further study. However, once the activatable system has been depleted, new increments of free streptokinase alone produce very little new active fibrin-lysing elements.

The development of specific antifibrinolytic antibodies following acute streptococcal infections has been previously demonstrated (15, 16). That the injection intrapleurally of streptococcal concentrates containing streptokinase also evokes the specific antistreptokinase response has been mentioned by Christensen (1), and was observed in connection with the earlier injections which were made primarily to estimate degrees of toxicity.

In Figure 3 evidence is presented from five cases of the development of antistreptokinase antibodies that were demonstrable in pleural exudates and followed intrapleural injections of streptococcal concentrates.

The rise in titre in the exudates occurred between the sixth and 11th days and was noted simultaneously in the serum.

The exact significance of the presence of specific antibody in nullifying the action of the fibrin-lysing system in local areas is not at present clearly established. Subsequent reference will be made to this point in connection with patient A. M. of Figures 7 and 8.

Figure 4 is presented for the purpose of demonstrating the total findings with respect to their correlation obtained serially in a single patient. In addition to the data described in Figure 1 (fibrinogen and N.P.N.) and Figure 2 (activatable fibrin-lysing system, active fibrin-lysing system, and free streptokinase) and Figure 3 (specific antistreptokinase), measurements of total protein and formol titratable substances are included.

The patient, J. G., had acute fibrinous pleurisy of tuberculous origin which had been active for ten weeks when first studied. He received two injections of streptococcal concentrate, the first containing 100,000 units and the second, three days later, containing 200,000 units of streptokinase.

The individual findings are comparable to those presented in Figures 1 to 3 and they demonstrate the rising and falling changes previously described. The fibrinogen drops and the N.P.N. rises; the activatable fibrin-lysing system drops as it is rapidly transformed into the active fibrin-lysing system which attacks the fibrinogen and the local deposit of fibrin; antistreptokinase being evident in increased titre in the exudate on the 11th day.

The *total proteins* have exhibited no quantitative changes. They have been determined in grams rather than milligrams as has been the case with fibrinogen. This is in agreement with Christensen's findings (unpublished) of little or no *in vitro* digestion of serum albumins or globulins.

The formol titrations which have risen coincident with the rise in N.P.N. after each injection were of the same magnitude and probably represent further evidence of the intrapleural breakdown of fibrinogen and fibrin.

The findings in the case of patient J. G. of Figure 4 are similar to those obtained in each of the other cases of Group I. In addition to the results noted in Figures 1 to 4, the following observations have been noted in this group of patients. The pH of the chest fluids fell significantly (0.2 to 0.3 units) in 24 hours after the injection of the streptococcal concentrate, slowly returning to the control values, and has been considered as part of the phenomenon of increased proteolysis. Small but significant falls in viscosity were noted, which are consistent with the breakdown of the long eccentric fibrinogen molecules and depolymerization of any small amounts of desoxyribosenucleic acid that may be present. Twenty-four to 48 hours after injection, the sediment increased from 0.5% to 2.0%, then slowly decreased over the next two to ten days to the control levels. This appeared to be directly related to the increased cellular content occurring in the pleural fluids in association with the local irritation. As noted earlier, no significant changes in the amount of trypsin inhibitor were found in the samples of pleural fluid. Finally, the values for activatable fibrin-lysing system, trypsin inhibitor and antistreptokinase were found to agree

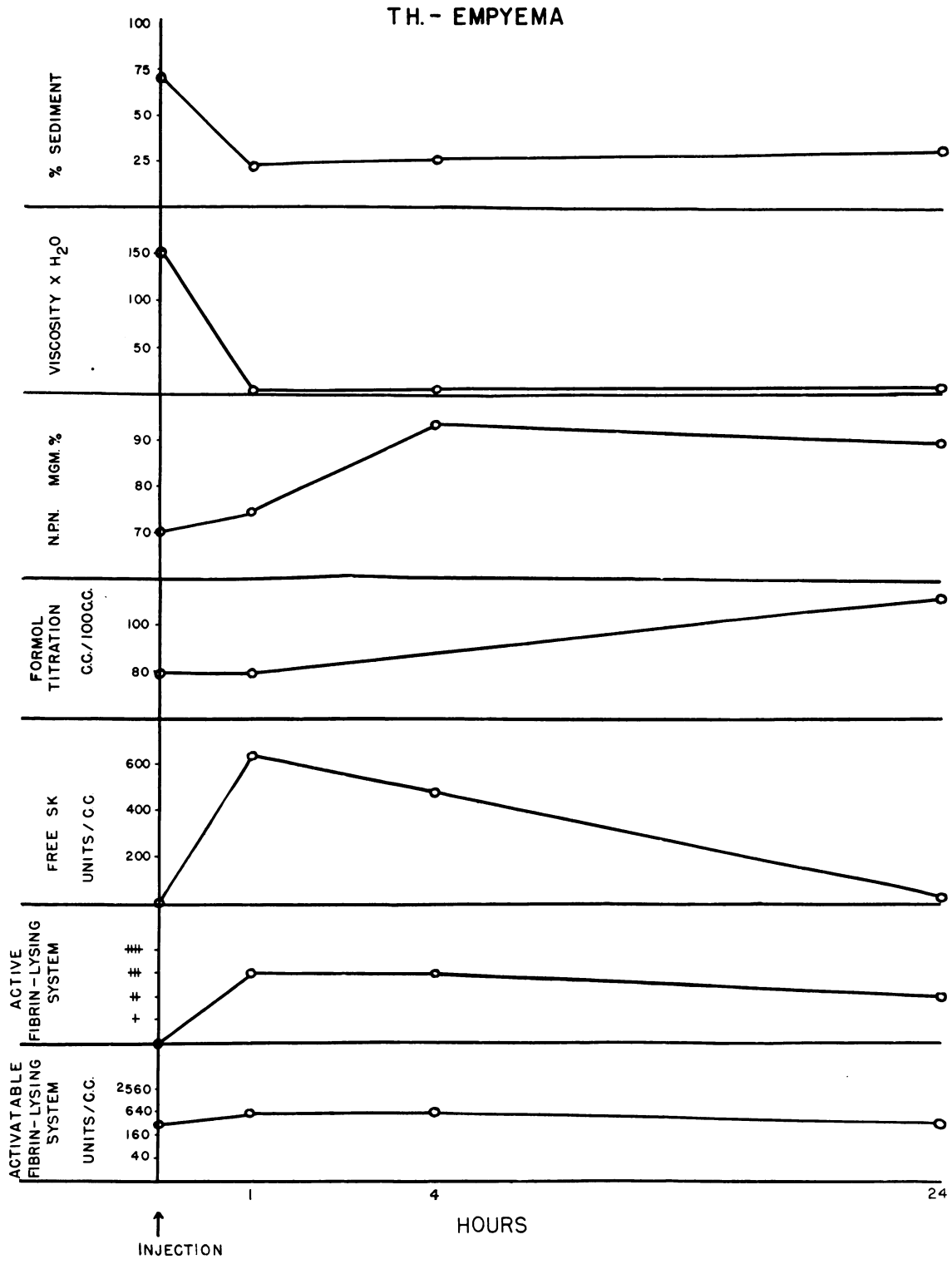


FIG. 5. THE EFFECT OF STREPTOCOCCAL CONCENTRATE (CONTAINING APPROXIMATELY 160,000 UNITS OF DESOXYRIBONUCLEASE AND 400,000 UNITS OF STREPTOKINASE INJECTED INTRAPLEURALLY), IN A CASE OF MIXED TUBERCULOUS EMPYEMA ON THE ACTIVATABLE AND ACTIVE FIBRIN-LYSING SYSTEMS, FREE STREPTOKINASE, FORMOL TITRATION, N.P.N., VISCOSITY, AND SEDIMENT OF THE CHEST FLUID

The striking change in the character of the fluid is seen in the accompanying photo (Figure 6).

closely for both chest fluid and serum prior to the injection of streptococcal concentrates. The decrease noted in the activatable fibrin-lysing system after streptokinase injections occurred only locally since no changes in this value occurred in the serum at any time. Antibody changes, however, as previously described, were evident simultaneously both locally and in the general circulation.

Group II. Cases of bacterial empyema, Eight patients.

Four cases of mixed infections associated with tuberculous empyema and bronchopleural fistula.

One case of empyema due to Friedlander's bacillus.

One case of pneumococcal (Type XVII) empyema.

One case of post-pneumonic empyema (sterile).

One case of empyema due to anaerobic streptococcus.

Interesting differences were observed between the constituents of purulent empyemal fluid and those of acute fibrinous pleurisy. For example, fibrinogen has not been found to be present in the purulent material aspirated before the injection of the concentrates. The supernatant portion of centrifuged specimens failed to produce coagulum when thrombin was added. It seems probable that the formation of fibrin had previously occurred to a considerable degree at the site of infection and depleted the exudate of its fibrinogen.

In the empyemal group, the presence of desoxyribose nucleoprotein as a significant portion of the granular sediment was regularly noted, and, as previously reported (12), constituted from 30% to 70% of the total solids. In addition, although not recorded in detail in this article, preparations stained by the Feulgen method have revealed the presence of nucleoprotein occurring extracellularly as fibrous reticulum, granules, and amorphous plaques.

In Figure 5 comprehensive data are presented concerning one of the patients with empyema. The patient, T. H., had chronic pulmonary tuberculosis, with a left bronchopleural fistula, draining thoracotomy, and a mixed empyema. After aspirating a sample of pus, streptococcal concentrate containing approximately 160,000 units of desoxyribonuclease and 400,000 units of streptokinase in 10 cc. of saline were injected. The rapid and extensive liquefaction of the nucleoprotein by the desoxyribonuclease was demonstrated by the marked fall in viscosity from 150 × water to 3 × water and decrease in measurable sediment from 70% to 23% in one hour. These quantitative changes persisted over the 24 hours of sampling. Subsequent drainage of the empyema was temporarily facilitated following the liquefying changes. However, the lysing procedure was not continued and consequently the clinical value was not extensively tested.

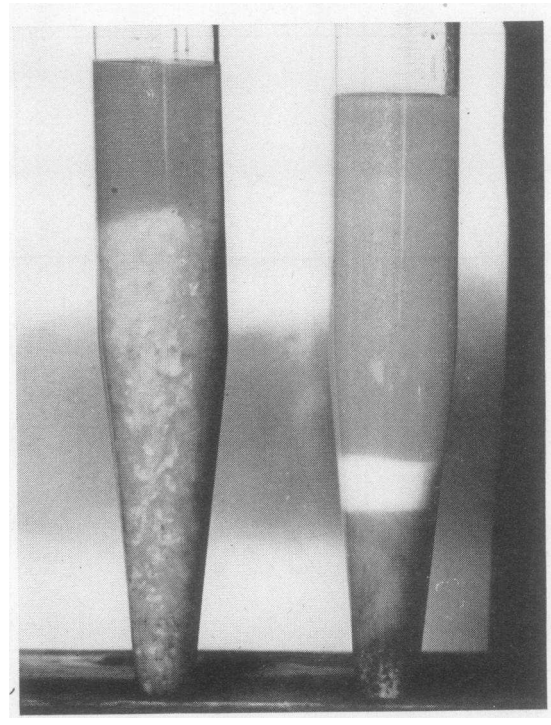


FIG. 6. RAPID LYSIS BY STREPTOCOCCAL CONCENTRATE OF PURULENT PLEURAL EXUDATE WITHIN PLEURAL CAVITY OF PATIENT

Patient *T. H.* Chr. Pulm. Tbc, lt. bronchopleural fistula, pyopneumothorax and thoracotomy. Streptococcal concentrate containing desoxyribonuclease and streptokinase injected intrapleurally 12/4/47.

Specimen at left: Taken immediately prior to injection. Ten cc. centrifuged for one hour. Sediment 70%—thick, pink, viscid, and containing large cheesy shreds. Viscosity of whole fluid 150 × water.

Specimen at right: Removed from chest one hour after injection. Ten cc. centrifuged for one hour. Sediment 23%—separates readily into three layers. From bottom up: small viscid layer; large pink layer of mixed red and white blood cells; and white layer composed of white cells and granules. Viscosity of whole fluid 4 × water.

Definite increases in N.P.N. and formol titratable material were also observed. One hour following the injection the free streptokinase titre was 640 units/cc., and subsequently fell to 10 units/cc. in 24 hours. As in the cases previously described, the active fibrin-lysing system appeared but to a less degree than in the fibrinous pleuritic group.

Even though the above data indicate some degree of activity of the fibrinolytic system, the findings in this patient demonstrate the significant action of the streptococcal desoxyribonuclease. The changes in the physical character of the empyemal pus are strikingly illustrated in the photographs shown in Figure 6. The amount of lysis of sediment that occurred at the site of infection within the patient's thorax is evident and it was to the greatest degree due to the depolymerization of the nucleoprotein of the pus by the nuclease contained in the concentrate that was introduced.

Similar findings were obtained in six of the eight cases of empyema. One of them is worthy of further mention. The patient, T. P., entered the hospital with acute lobar pneumonia of four days' duration. He was treated with penicillin but his course and X-ray were suggestive of empyema. Aspiration of the chest performed on the tenth day of hospitalization yielded only a few cubic centimeters of thick green fluid which was sterile on culture. Introduction of air intrapleurally revealed the presence of several loculations within the empyemal cavity. Ten days later, 50 cc. of thick greenish pleural fluid was withdrawn and streptococcal concentrate containing approximately 40,000 units of desoxyribose nuclease and 100,000 units of streptokinase in 10 cc. of saline was introduced into the site of aspiration. Examination of specimens of fluid obtained by subsequent repeated aspirations revealed that the viscosity of the pus fell from 38 × water to 14 × water in one hour, and to 5× water in 24 hours. The sediment decreased from 35% to 14% in one hour, and to 10% in 24 hours. The N.P.N. rose from 58 mgm.% to 73 mgm.% in one hour, and to 84 mgm.% in 24 hours. Twenty-four hours after the injection 465 cc. of thin greenish-grey fluid was removed with ease. An X-ray of the chest at this time revealed that the latest aspiration had effected almost complete drainage of the empyemal cavity and that the previous evidence of loculation had disappeared. Following this tap, the temperature fell rapidly to normal, the leucocytosis disappeared within a few days, and the patient made an excellent clinical recovery.

In one case of mixed empyema and in the one due to Friedlander's bacillus infection, the pHs of the fluids were found to be very acidic (5.50, and 5.21) apparently due to extensive proteolysis and digestion (N.P.N.'s 184, and 400) that had occurred spontaneously as a result of the diseases themselves. The injected streptococcal concentrate had no effect due to the low pH since studies, *in vitro,* have shown that both streptokinase and desoxyribonuclease are rapidly inactivated at this pH.

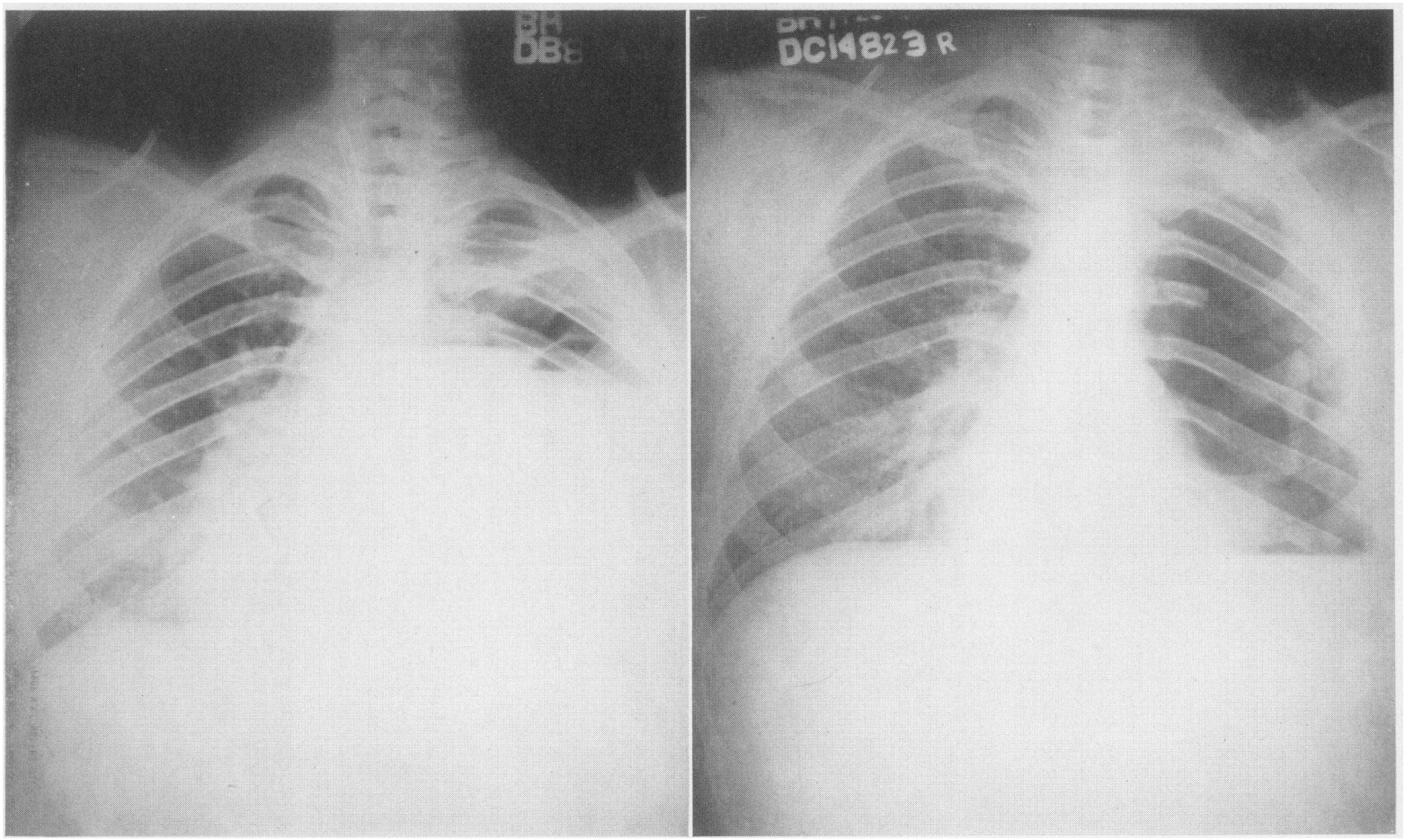
In cytological studies of the sediment of exudates, both of the acute fibrinous and the purulent groups, one feature of special interest has been the consistent breaking up of clumps of white blood cells. This change has been evident in the contrast between the cells of the pre-injection specimen and those of the sample obtained one hour after the introduction of the streptococcal concentrate. Whether or not the clumping was due to the fact that the cells were made adherent by a coating of fibrin or fibrous nucleoprotein is not yet established. However, since the concentrates contained both streptokinase and nuclease a final explanation of the cellular effect awaits further study. As mentioned previously a detailed report of the cytological studies will be the subject of an early communication.

From the results obtained in the observations on the empyemal cases there is ample evidence that nucleoprotein depolymerization and probably fibrinolysis occurred, the former by the extensive decrease in the nucleoprotein sediment and fall in viscosity, the latter by changes in the fibrinlysing system and changes in the N.P.N. and formol titration.

Group III. Cases of hemothorax, two patients. In each, the intrathoracic blood coagulum developed following pneumonectomy.

Figures 7 and 8 illustrate changes in a case of loculated hemothorax that were brought about by the streptococcal fibrin-lysing system.

400,000 units of streptokinase were introduced into a single pocket containing 71 cc. of



BEFORE STREPTOKINASE (Note multiple fluid levels)

AFTER STREPTOKINASE (Note all loculations due to fibrinous bands have been eradicated)

FIG. 7. EFFECT OF STREPTOCOCCAL FIBRINOLYSIN (STREPTOKINASE) IN A CASE OF LOCULATED HEMOTHORAX

Patient A. M. 20 yrs. old. Left pneumonectomy for cystic bronchiectasis. Postoperative course complicated by persistent fever and loculated hemothorax preventing aspiration of contents and subsequent thoracoplasty. 400,000 units streptokinase were introduced intrapleurally into small pocket. In 24 hours, chemical methods (chest fluid volume determinations) indicated conversion of small pocket into a single large cavity. In next two days 1,300 cc. of sanguinous fluid were removed readily from one site, and X-ray revealed that pockets were gone. Thoracoplasty followed shortly with good results.

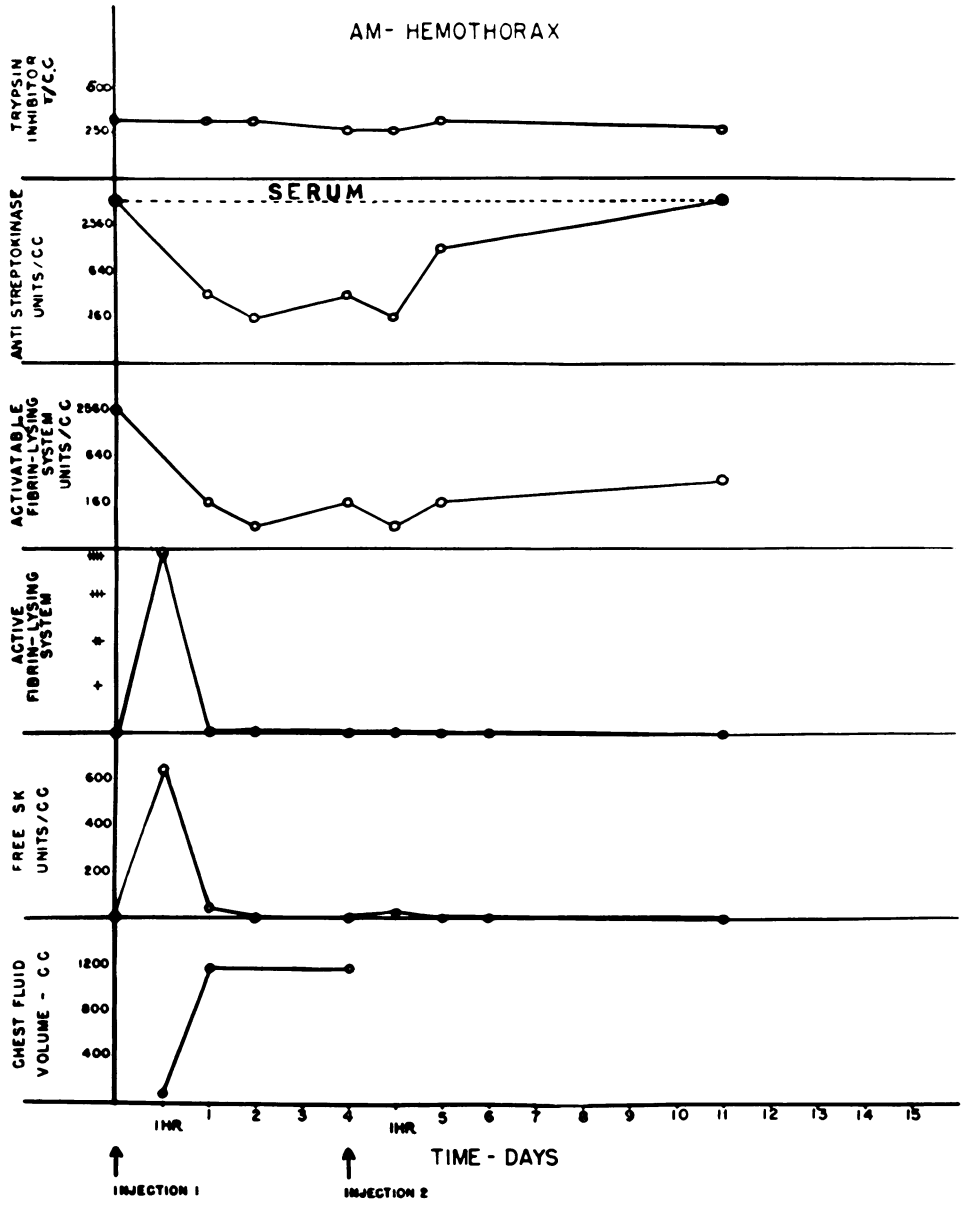


FIG. 8. THE EFFECT OF TWO INTRAPLEURAL INJECTIONS OF STREPTOCOCCAL CONCENTRATE IN A CASE OF POSTOPERATIVE HEMOTHORAX ON THE CHEST FLUID VOLUME, FREE STREPTOKINASE, ACTIVE AND ACTIVATABLE FIBRINLYSING SYSTEMS, ANTISTREPTOKINASE, AND TRYPSIN INHIBITOR OF THE CHEST FLUID

400,000 units of streptokinase were given in injection 1, and 200,000 units in injection 2.

The striking clinical change is described in the text, and seen in the accompanying X-rays (Figure 7).

fluid. The concentration in this isolated area was, therefore, 5,600 units/cc. before resolution of the loculating bands took place.

The legend under Figure 7 gives the essential details of the findings in this patient both before and after the introduction of streptococcal concentrate and illustrates objectively the fibrinolytic effect. In this instance the postoperative coagulum prevented drainage of the intrathoracic contents. However, after dissolution of the fibrinous bands and a considerable amount of the associated blood clot it was possible during the ensuing 48 hours to drain by thoracentesis a large proportion of the hemothorax which had become liquefied.

The multilocular condition had been transformed into a unilocular state. It seems likely that the fibrinolytic system was chiefly operative.

It is of special interest to note that this patient had a high titre of antifibrinolysin (antistreptokinase) at the beginning of the study. Although not proved by cultures it seems likely that he had previously had one or more infections with hemolytic streptococci since the height of antibody titre is consonant with that previously found in patients convalescent from known infection with hemolytic streptococci.

In spite of the presence of immune bodies, however, impairment of the action of the streptococcal product was not apparent. It is not possible at the present time to offer an explanation of these findings. However, the possibilities suggest themselves either that a sufficient amount of streptokinase was injected to bind with antibody and leave an excess, or that the active lysing system was put in operation before the antibody took effect. Further study of this phase of the problem is required. Also evident from the data, as in the results with the previous cases, is the fall in the activatable fibrin-lysing system coincident with the appearance of an active fibrin-lysing system, and its slow return toward the control value. Trypsin inhibitor did not change despite the activation and rapid inactivation of the fibrin-lysing system.

The course in the patient just described appears to represent an example of a beneficial therapeutic result effected by the action of the streptococcal fibrin-lysing system.

The second patient with postoperative hemothorax will be referred to only briefly since he is still under observation. Thirty-two days postoperatively the patient was transferred to our Service because it was impossible to obtain more than a few cubic centimeters of fluid from the postoperative side of the thorax. X-ray examination revealed a large shadow with fluid levels occupying approximately one-half of the thoracic cavity. From three aspirations at different sites a total of 3 cc. was obtained. Into each of the sites streptokinase was injected up to a total of 250,000 units. In the preparation of concentrate employed in this case almost all of the nuclease had been eliminated. Consequently only the fibrinolytic system was operative to any significant degree. Twenty-four hours later 600 cc. of thin brownish fluid were removed by thoracentesis from a single site without difficulty. Immediately after the aspiration, by fluoroscopy and X-ray, only a small amount of effusion remained. Although fluid reaccumulated over the next few days, successful aspirations were readily performed indicating a considerable reduction in the presence of loculation, and the creation of a favorable situation for further intercostal drainage.

DISCUSSION

A group of unique enzymatic activities have been utilized to produce changes in fibrinous, purulent and sanguinous exudations within the pleural cavities of patients. Thus through the mediation of substances elaborated by hemolytic streptococci, fibrin is caused to undergo lysis; fibrinogen is altered so that it no longer can assume the solid form of fibrin; and the coarse sediment of purulent exudate (primarily desoxyribose nucleoprotein) is degraded to a thin solution..

Once it had been well established by detailed preliminary observations that the methods of purification of the streptococcal products developed extensively by Christensen (1) yielded preparations that were progressively less toxic, although maintaining constant or increased potency, a demonstration of the occurrence and degree of action in patients of the active principles—streptokinase and desoxyribose nuclease—became a center of interest. For this purpose quantitative estimations were made of the results of the action of each enzymatic system on its respective substrate in the areas of disease. The rapidity of the beginning of action, and the duration of continuing action after a single, and in some instances after a second injection, were determined.

Definitive enzymatic changes of a significant degree were regularly obtained and were demonstrable within an hour after the injection and endured for several days before the effect subsided and disappeared. The phenomenon was a self-limiting one following each injection.

The clinical courses of the patients have been followed by frequent physical, laboratory, and X-ray examinations over extended periods. Following the injections a febrile response occurred

not infrequently and there was also evidence of local irritation. Both signs of reaction were, however, transient.

Since the periods, in which the action of the concentrates was operative, were limited to a few days, striking evidence of favorable alterations of a permanent nature was not expected nor definitely demonstrated except for the results obtained in cases of loculated hemothorax, one example of which is described in detail, and in some of the instances of empyema. In patients with hemothorax, it is obvious that consideration must be given to the broad principles and problems of thoracic surgery before the application of the procedure may be most advantageously made.

The findings, however, constitute a background against which the scope of the study is being broadened to include a consideration of the possible usefulness of the procedure when utilized under appropriate circumstances. The desirability of causing the liquefaction of fibrin or of preventing its formation, or of resolving purulent nucleoprotein containing sediments, would on theoretical grounds depend upon a variety of conditions associated with the pathogenesis and expected evolution of the disease processes to which this method involving lytic activities might be applied. Comparable principles would also be applicable to exudative diseases involving locations in the body other than the pleural area.

If the solid increments constitute part of an advantageous walling off process then the liquefaction of the wall might promote a spread of the infection in its acute phases before the patients' immunity was sufficient to restrain the dissemination of the infection. On the other hand, the same walling off may prevent the introduction of antibacterial reagents of an immunological nature, as well as antibiotic substances, into the field of disease. Under the latter circumstances elimination of the wall might be advantageous. Other sets of conditions concern factors that are involved in the ultimate formation of scar tissue, or of adhesions, and the permanent thickenings that are related to the final organization of infected areas.

The results presented in this article serve as a basis for extending the study along the lines that have been discussed.

SUMMARY

1. Twenty-three patients suffering from exudative pleurisies associated with different types of diseases have received intrapleural injections of partially purified concentrates containing streptococcal fibrinolysin (streptokinase) and desoxyribose nuclease.

2. Intrapleural fibrinolytic and proteolytic changes due to the activity of the fibrin-lysing system were demonstrable in samples of the exudates taken at repeated intervals after the injection. The effects were self-limiting after each injection.

Intrapleural depolymerization of the nucleoprotein of the solid sediment of the exudates was also demonstrable in a similar manner.

3. When toxic manifestations occurred following the injections, they were limited to transient febrile reactions with general malaise, and a local outpouring of leucocytes of a few days' duration.

4. The possibility is discussed of influencing favorably by the enzymatic systems the course of exudative types of diseases and it is being given additional study.

The authors wish to acknowledge the excellence of the technical assistance which they have received from Miss Harriet Heffernan, Mr. John Goeller, and Miss Doris Bitz.

BIBLIOGRAPHY

1. Christensen, L. R., Methods for measuring the activity of components of the streptococcal fibrinolytic system, and streptococcal desoxyribonuclease. J. Clin. Invest., 1949, **28**, 163.
2. Tillett, W. S., and Garner, R. L., The fibrinolytic activity of hemolytic streptococci. J. Exper. Med., 1933, **58**, 485.
Garner, R. L., and Tillett, W. S., Biochemical studies on the fibrinolytic activity of hemolytic streptococci. I. Isolation and characterization of fibrinolysin. II. Nature of reaction. J. Exper. Med., 1934, **60**, 239 and 255.
3. Review article for references up to 1938: See Tillett, W. S., The fibrinolytic activity of hemolytic streptococci. Bact. Rev., 1938, **2**, 161.
4. Christensen, L. R., Streptococcal fibrinolysis: a proteolytic reaction due to a serum enzyme activated by streptococcal fibrinolysin. J. Gen. Physiol., 1945, **28**, 363.
5. Milstone, H., A factor in normal human blood which participates in streptococcal fibrinolysis. J. Immunol., 1941, **42**, 109.

6. Christensen, L. R., and MacLeod, C. M., A proteolytic enzyme of serum: characterization, activation, and reaction with inhibitors. J. Gen. Physiol., 1945, **28**, 559.
7. Kaplan, M. H., Studies of streptococcal fibrinolysis. I. The dissimilarity of serum protease and trypsin as indicated by the separate specificities of their kinases, fibrinolysin and enterokinase. II. The inhibition of streptococcal fibrinolysis by antifibrinolysin and antiprotease. J. Clin. Invest., 1946, **25**, 331 and 337.
8. Ratnoff, O. D., Studies on a proteolytic enzyme in human plasma. I. The probable identity of the enzymes activated by chloroform and by filtrates of cultures of beta hemolytic streptococci. II. Some factors influencing the enzymes activated by chloroform and by streptococcal fibrinolysin. J. Exper. Med., 1948, **87**, 199 and 211.
9. Loomis, E. C., George, C., Jr., and Ryder, A., Fibrinolysin: nomenclature, unit, assay, preparation and properties. Arch. Biochem., 1947, **12**, 1.
10. Ferguson, J. H., Travis, B. L., and Gerheim, E. B., Fibrinogenolytic demonstration of activation and inhibition of tryptase in plasma protein fraction—I ("antihemophilic globulin"). Proc. Soc. Exper. Biol. & Med., 1947, **64**, 285.
11. Astrup, T., and Permin, P. M., Fibrinolysis in animal organism. Nature, 1947, **159**, 681.
Permin, P. M., Properties of the fibrinokinase-fibrinolysin system. Nature, 1947, **160**, 571.
12. Sherry, S., Tillett, W. S., and Christensen, L. R., Presence and significance of desoxyribose nucleoprotein in the purulent pleural exudates of patients. Proc. Soc. Exper. Biol. & Med., 1948, **68**, 179.
13. Tillett, W. S., Sherry, S., and Christensen, L. R., Streptococcal desoxyribose nuclease: significance in lysis of purulent exudates and production by strains of hemolytic streptococci. Proc. Soc. Exper. Biol. & Med., 1948, **68**, 184.
14. Christensen, L. R., Protamine purification of streptokinase and effect of pH and temperature on reversible inactivation. J. Gen. Physiol., 1947, **30**, 465.
15. Tillett, W. S., Edwards, L. B., and Garner, R. L., Fibinolytic activity of hemolytic streptococci. The development of resistance to fibrinolysis following acute hemolytic streptococcal infections. J. Clin. Invest., 1934, **13**, 47.
16. Tillett, W. S., The occurrence of antifibrinolytic properties in the blood of patients with acute hemolytic streptococcus infections. J. Clin. Invest., 1935, **14**, 276.