

THE ACTION OF STREPTOCOCCAL DESOXYRIBOSE NUCLEASE (STREPTODORNASE),¹ *IN VITRO* AND ON PURULENT PLEURAL EXUDATIONS OF PATIENTS²

By SOL SHERRY, ALAN JOHNSON, AND WILLIAM S. TILLET

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

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In previous reports it has been demonstrated that desoxyribose nucleoprotein is a significant constituent of the solid sediment of purulent exudates (1), and that hemolytic streptococci elaborate, during their growth in broth cultures, a desoxyribose nuclease (streptodornase) in considerable quantities (2). The latter finding was also described independently and concomitantly by McCarty (3).

In a more recent report (4) results have been described which were obtained when partially purified streptococcal concentrates, containing both streptokinase and streptodornase were injected into the pleural cavity of patients suffering from various types of diseases causing fibrinous, purulent, and sanguinous exudations.

The concentrates employed³ were prepared according to methods developed and described by Christensen (5). Since they contained both the fibrinolytic principle, streptokinase, and the pus-liquefying enzyme, streptodornase, it was possible to demonstrate that two enzymatic systems were operative when introduced locally within the areas of disease. Fibrin and fibrinogen were predominant in cases of uninfected loculated hemothorax; desoxyribose nucleoprotein was most conspicuous in cases with thick coarse purulent exudates; in other cases both substrates were present in varying amounts depending on the nature of the underlying disease.

With respect to the streptodornase activity, the earlier reports (1, 4) described the depolymerization of desoxyribose nucleoprotein and desoxyribose nucleic acid, and also demonstrated de-

creases in the amount of the solid sediment of purulent exudates in association with marked drops in the viscosity of the same specimens following intrapleural injections of the streptococcal concentrates.

It is the purpose of this article to describe in detail the biochemical changes induced by the action of streptodornase in association with the characteristic physical changes that have attended depolymerization, and, in addition, the histological and cytological changes that have been made evident by the use of microscopic preparations serially stained by the Feulgen method (6).

The data to be presented were derived from:

1. Determinations of the action of streptodornase on purified desoxyribose nucleic acid derived from calf thymus.

2. Observations of the action on purulent exudates produced, *in vivo*, following the intrapleural injection of streptococcal concentrates containing streptodornase; the effects, *in vivo*, have also been paralleled by comparable experiments conducted *in vitro*, by incubating streptodornase with specimens of purulent exudation derived from patients.

MATERIALS AND METHODS

Streptodornase. This enzyme was contained in the partially purified streptococcal concentrates that have been used throughout these studies. The concentrates also contained streptokinase in measurable quantities. However, the effects to be described in this article are limited to those caused by the nuclease. A unit of streptodornase has been arbitrarily defined as the amount that will cause a drop of one viscosity unit in 10 minutes at 37° C in an enzyme substrate mixture consisting of 2.5 cc. of desoxyribose nucleic acid and 0.1 cc. enzyme solution. The substrate consisted of 0.15–0.2% solution of thymus desoxyribose nucleic acid in M/40 barbital buffer and 0.003 M Mg⁺⁺ at pH 7.4. The substrate had an initial viscosity relative to water of 3–5 units.

The amounts of the enzyme employed will be given in individual protocols.

¹ The word "Streptodornase" has been employed in this article as an abbreviation of streptococcal desoxyribose nuclease.

² This study was supported by a grant from the National Institutes of Health, United States Public Health Service.

³ A considerable amount of the preparations was supplied by Lederle and Co.

Desoxyribose nucleic acid. This was prepared from calf thymus by the method of Mirsky and Pollister (7). A 0.15–0.20% solution in M/40 barbital buffer was heated at 56° C for two hours, to destroy any residual depolymerase activity, before its use as a substrate.

Viscosity. Viscosity measurements were done in a constant temperature bath at $37 \pm 0.2^\circ$ C. For purified desoxyribose nucleic acid solutions or thin exudates, Ostwald viscosimeters with a flow time of 10–15 seconds at 37° C were employed. For thick exudates an LV Model Brookfield viscometer was employed. Results are expressed in units of viscosity $\times H_2O$.

pH. pH was determined by glass electrode using a Beckmann pH meter.

Sediment. The per cent sediment was determined by filling Wintrobe tubes with exudate and spinning at 2500 RPM for one hour.

Phosphorus. Total acid soluble phosphorus was determined by a modification of the method of Fiske and SubbaRow (8) on digested 8% trichloroacetic acid filtrates. Inorganic phosphorus was determined by a modification of the method of Fiske and SubbaRow (8) on 8% trichloroacetic acid filtrates. Organic acid soluble phosphorus was obtained by subtracting the inorganic P from the total acid soluble P.

Acid soluble nitrogen. Determined on 8% trichloroacetic acid filtrates by micro Kjeldahl and nesslerization.

Ammonia. Determined by method of Van Slyke and Cullen (9).

Uric acid. Determined on 1% tungstic acid filtrates by the method of Folin (10).

Absorption at 2600 Å. Determined on trichloroacetic acid filtrates in a Beckmann Spectrophotometer.

Feulgen stain. An adaptation of Rafalko (11) and Stowell's (12) modification of the method originally described by Feulgen (6) has been regularly employed.

Solutions

1. *Leuco-fuchsin reagent.* 0.5 Gm. basic fuchsin suspended in 100 cc. boiling distilled water. Shake. Cool to 50° C. Filter. Decolorize by slowly bubbling SO_2 through the solution for one to two hours. Store in dark for 24 hours. Add 0.25 Gm. activated charcoal (preferably norit). Shake for one minute and filter rapidly through coarse filter paper. Store in icebox in dark bottle.
2. *1N HCl solution.*
3. *Sulfurous acid solution.* Bubble SO_2 through 1 liter of distilled water for three to four hours in the cold. Store in icebox in dark bottle.
4. *Fast Green F.C.F. solution*—0.02% alcoholic solution.

Technique*

1. Thick and thin smears of exudate are made and air dried.

* All procedures are carried out at room temperature unless otherwise specified.

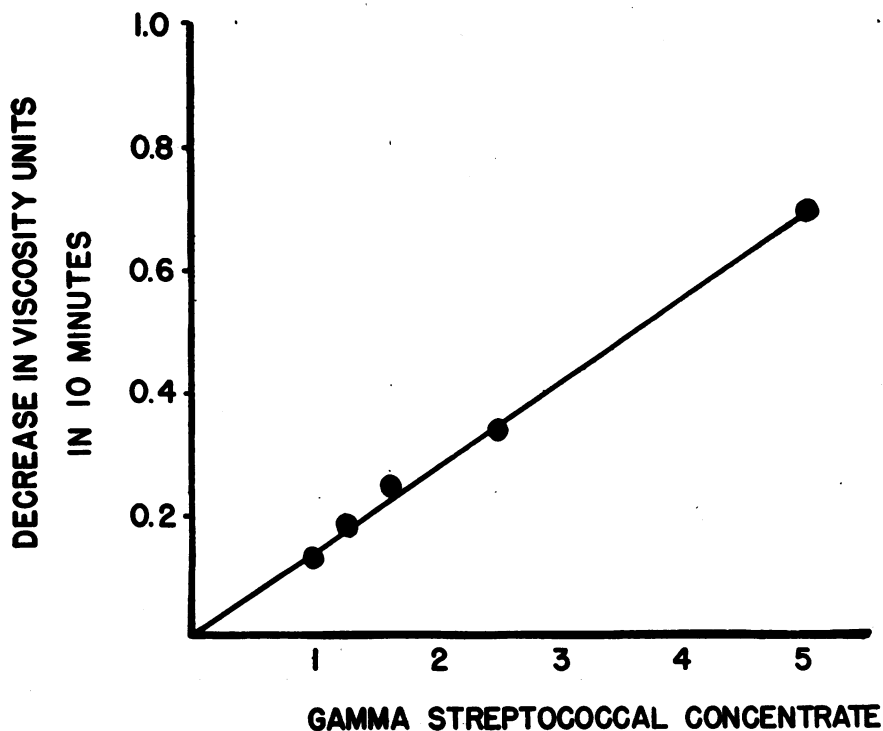


FIG. 1. THE EFFECT OF VARYING AMOUNTS OF STREPTODORNASE CONCENTRATE ON THE DEPOLYMERIZATION OF PURIFIED DESOXYRIBOSE NUCLEIC ACID

2. Immerse in 1N HCl for one minute.
3. Immerse in 1N HCl for 15 minutes at 56° C.
4. Rinse in distilled water.
5. Rinse in sulfurous acid solution.
6. Immerse in leuco-fuchsin solution for two hours.
7. Rinse in three successive baths of sulfurous acid solution for one minute each.
8. Immerse in tap water for five minutes and then rinse with distilled water.
9. Immerse in Fast Green solution for 20-30 seconds.
10. Rinse in distilled water, and air dry.

RESULTS

1. The action of streptodornase on purified desoxyribose nucleic acid (calf thymus).

In Figure 1 the depolymerase activity of 1 to 5 gamma of a streptodornase concentrate in 0.1 cc. was tested with 2.5 cc. of a 0.15% solution of desoxyribose nucleic acid in M/40 barbital buffer containing 0.003 M of Mg^{++} at pH 7.4. The tests were conducted for periods of ten minutes in a water bath at 37° C. From the data it may be noted that a linear relationship between concentration of enzyme and depolymerase activity was demonstrable.

In Figure 2 the effect of temperature on the depolymerase activity of the streptococcal concentrates is illustrated.

For the experiment on thermal effects, 0.1 cc. of a 1:1000 dilution of a 5% crude streptococcal concentrate, and containing approximately 1.4

units of streptodornase was added to 2.5 cc. of a 0.2% solution of desoxyribose nucleic acid in M/40 barbital buffer (pH 7.4) containing 0.003 M of Mg^{++} . Measurements of viscosity were made over periods of ten minutes following mixture of enzyme and substrate.

As may be noted from Figure 2 the rate of depolymerization progressively increased with rising changes in temperature reaching a maximum at 45° C. Above 45° C the rate of enzyme inactivation exceeded the increased velocity of reaction. The activity of the enzyme rapidly fell off as the thermal death point of 56° C was approached. Temperature inactivation of the enzyme appeared to begin above 30° C.

In Figure 3 the relation of pH to streptodornase activity is demonstrated.

To a 0.2% solution of desoxyribose nucleic acid in M/40 sodium barbital containing 0.003 M of Mg^{++} , 3% acetic acid was added in different amounts in order to obtain solutions of substrate varying in pH from 5.2 to 9.0. 0.1 cc. of a 1:1000 dilution of a 5% preparation of concentrate and containing 1.8 units of streptodornase was added. Measurements of viscosity were made over periods of ten minutes in a water bath at 37° C following the mixture of enzyme and substrate. The final pH was checked before and at the end of each experiment.

As may be noted from the data in Figure 3, activity was greatest between pH 7.0 and 8.5.

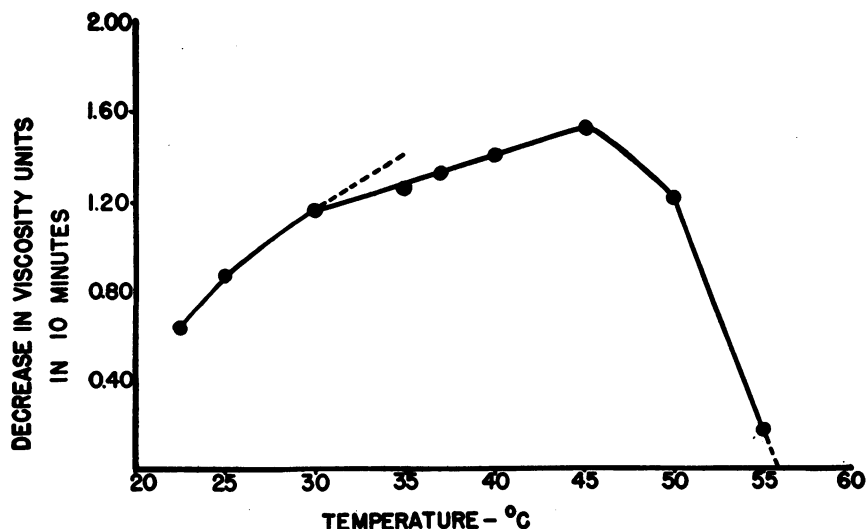


FIG. 2. THE EFFECT OF TEMPERATURE ON THE DEPOLYMERASE ACTIVITY OF STREPTODORNASE

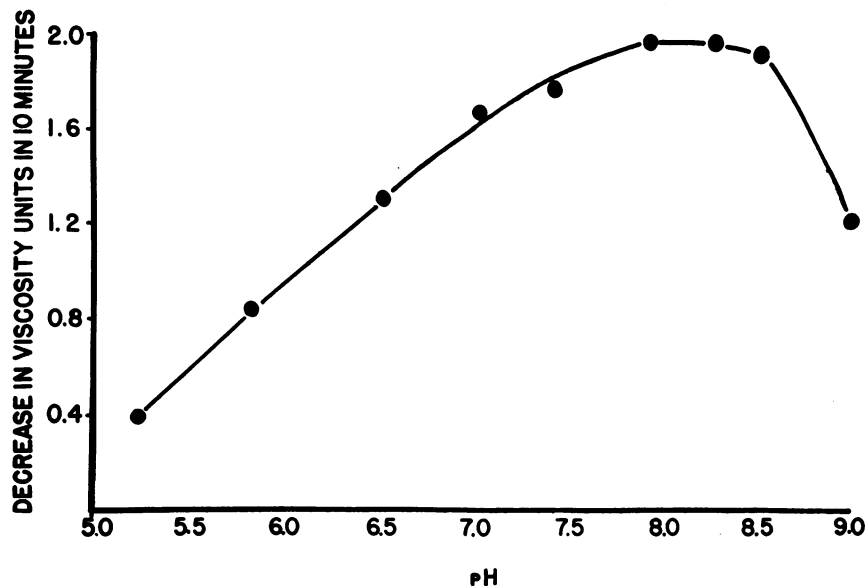


FIG. 3. THE EFFECT OF pH ON THE DEPOLYMERIZATION OF PURIFIED DESOXY-RIBOSE NUCLEIC ACID BY STREPTODORNASE

In Figure 4, the results of an experiment are summarized that was conducted for the purpose of determining whether acid soluble phosphorus and acid soluble nitrogen were liberated by the action of streptodornase.

0.2 cc. of a streptococcal concentrate containing 180 units of streptodornase per cc. was incubated at 37° C with 67 cc. of a 0.2% solution of purified desoxyribose nucleic acid (calf thymus) containing 0.003 M of Mg^{++} in M/40 barbital buffer at pH 7.4.

From the findings recorded in Figure 4 it may be seen that analyses of trichloroacetic acid filtrates revealed a progressive liberation of organic acid soluble P and acid soluble N and that the activity continued after depolymerization, as determined by viscosity measurements, was completed. Since no significant increase in inorganic P was noted, the rise in total acid soluble P may be interpreted as being entirely dependent on an increase in phosphate esters.

No evidence of deamination was observed. These findings, coupled with the observation of marked increases in the extinction coefficient at 2600 Å of trichloroacetic acid filtrates of desoxyribose nucleic acid incubated with streptodornase, indicate a liberation of acid soluble nucleotides.

A search for inhibitors has been made using a wide variety of reagents that might be pertinent to

the broad problems of this study including the possible significance of inactivation in relation to the effective treatment of patients.

The following substances were found to have no effect on the depolymerase activity of streptodornase: salicylate, gentisate, ascorbate, thiourea, paraminobenzoic acid, parachlorophenol, oxalate, colchicine, and penicillin. In conducting the above experiments 0.1 cc. solution of the test reagents was mixed with the nucleic acid substrate. 0.1 cc. of streptodornase solution containing 1 unit of enzyme was then added and the rate of depolymerization over a ten minute interval observed. The quantities of each reagent employed were estimated as being of sufficient concentration to give an indication of an inhibitory effect if such were present. Since no evidence of inhibition has been found, the observations have not been extended beyond the preliminary stages.

Definite evidence of an inhibitory effect has, however, been noted in the use of citrate and heparin.

The results briefly stated are shown in Table I.

2. The action of streptodornase on the purulent exudates of patients, *in vivo* and *in vitro*

Nine patients with empyema have been studied. The diseases giving rise to the empyema were caused by tuberculosis in four instances, anaerobic

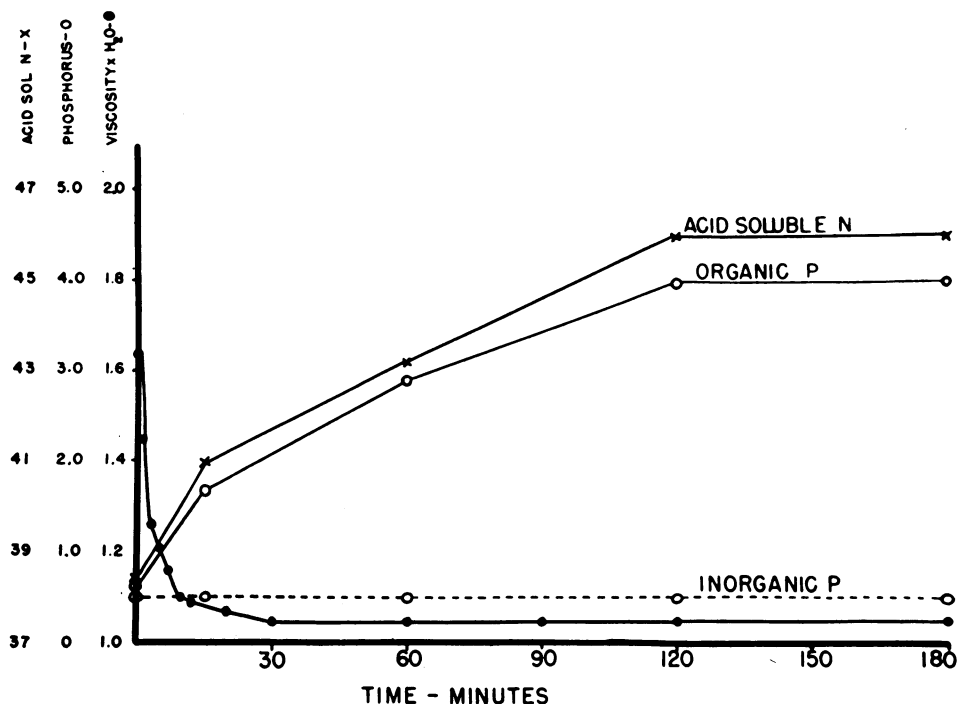


FIG. 4. THE EFFECT OF STREPTODORNASE ON THE DEPOLYMERIZATION AND LIBERATION OF ACID SOLUBLE NITROGEN AND PHOSPHORUS FROM PURIFIED DESOXYRIBOSE NUCLEIC ACID

streptococcus in two cases, pneumococcus pneumonia with sterile empyema in two cases, and infection with *B. pyocyaneus* in one case.

In the study of each case a sample of pleural exudate was removed for analyses prior to the injection. The streptococcal concentrates in amounts of fluid varying from 2 to 10 cc. and containing from 5000 to 50,000 units of streptodornase were introduced into the pleural site of the infection. Subsequent specimens were removed by

thoracentesis during periods of one or more hours in the first day and then usually at daily intervals thereafter for the duration of the observations.

The required dosage of streptodornase in relation to the type and amount of pus present in each case has not yet been determined on an exact quantitative basis. However, the doses employed, usually averaging 20,000 units per dose, have regularly proved effective as evidenced by the definitive changes to be described.

It may be noted at this point that no serious untoward effects occurred in any of the patients. The reactions were limited to pyrogenic ones of a transient nature together with febrile malaise such as that previously described (4). A report of the therapeutic effects of the injections together with the clinical details of the course of the patients is now in preparation.

In six cases of empyema measurements were made of the liberation of organic acid soluble P at the site of the pleural exudation following the local introduction of the streptococcal concentrates containing streptodornase.

Determinations of acid soluble P and N were made on the supernatant portion of centrifuged

TABLE I

Inhibition of streptodornase depolymerase activity by various reagents

Substance tested	Amount in test mgms.	Inhibition %
Salicylate	0.02	0
	0.2	10
	2.0	0
Gentisate	0.1	1
	0.2	5
	1.0	0
Thiourea	1.0	1
Oxalate	1.0	0
Colchicine	0.011	0
Paraaminobenzoate	1.0	0
Parachlorophenol	0.25	8
Ascorbate	1.0	16
Penicillin	(10,000 units)	19
Heparin	1.0	61
Citrate	1.0	82

samples of exudate removed at hourly intervals. From the data in Figure 5 it may be seen that the peak of the rise in liberated P was reached one to four hours after the injection. The values then returned to the pre-injection level during the next 24 hours.

The liberation of acid soluble N was also evident coincidental with the rise in P. The acid filtrates of the supernatant specimens showed marked increases in absorption at 2600 Å. Further breakdown to inorganic P or uric acid was not demonstrable, as shown in Figure 6.

Experiments comparable to those just described have been conducted, *in vitro*, by incubating mixtures of streptodornase and pus, and making determinations of acid soluble P and N liberated into the supernatant fluid. The results of such a study

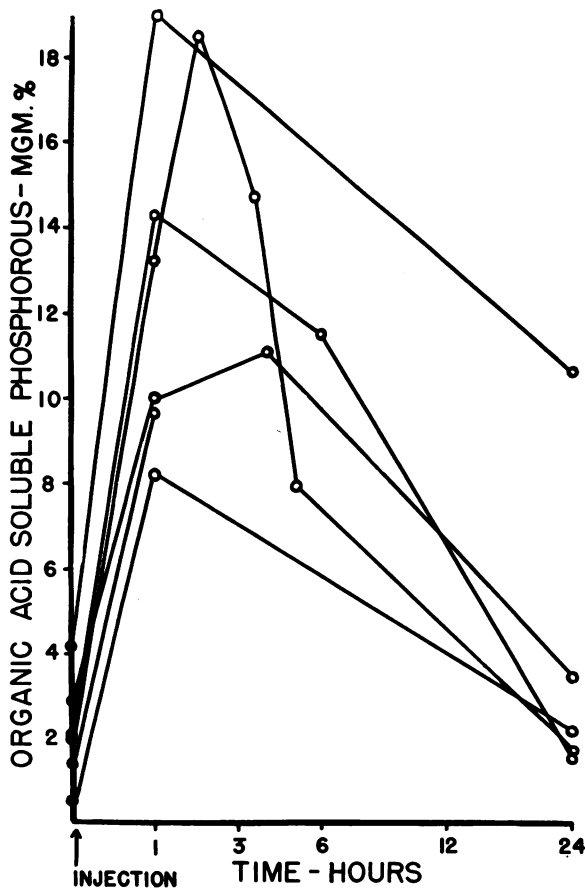


FIG. 5. THE LIBERATION OF ORGANIC ACID SOLUBLE PHOSPHORUS INTO THE SUPERNATANT OF PLEURAL EXUDATES AT VARYING INTERVALS FOLLOWING THE LOCAL INTRODUCTION OF STREPTODORNASE INTO THE SITE OF DISEASE IN SIX PATIENTS WITH EMPYEMA

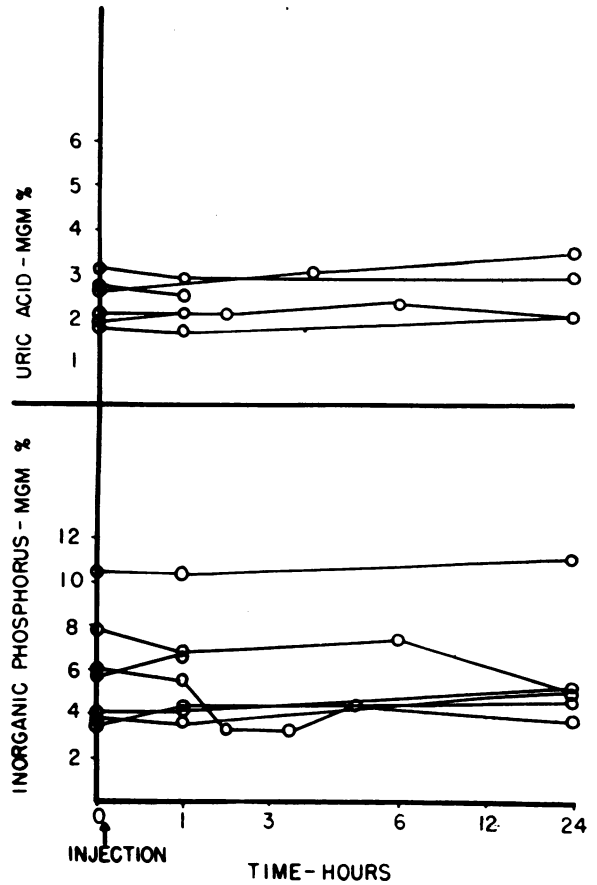


FIG. 6. THE CONCENTRATION OF URIC ACID AND INORGANIC PHOSPHORUS OF THE SUPERNATANT OF PLEURAL EXUDATES AT VARYING INTERVALS FOLLOWING THE LOCAL INTRODUCTION OF STREPTODORNASE INTO THE SITE OF DISEASE IN PATIENTS WITH EMPYEMA

are recorded in Table II. The purulent exudate was obtained from a case of penicillin treated pneumococcus Type II pneumonia who developed a sterile empyema. The pH of the fluid was 6.5. The sample of pus was divided into three aliquots. One aliquot served as control. A second aliquot was incubated with a streptococcal concentrate previously heated at 56° C for one and one-half hours to destroy the desoxyribonuclease present. This concentrate still contained considerable streptokinase. The third aliquot was incubated with the unheated streptococcal concentrate containing both streptokinase and desoxyribonuclease.

The control exudate, and the exudate mixed with concentrate free of streptodornase, liberated acid soluble N and P into the supernatant at about the same rate. The concentrate containing strepto-

TABLE II

The in vitro effect of streptodornase on the liberation of acid soluble P and N into the supernatant of a purulent exudate

Experimental	Streptodornase added	Streptokinase added	Time of incubation	Acid soluble N		Total acid soluble P		Inorganic P		Organic acid soluble P	
				Total	Change	Total	Change	Total	Change	Total	Change
	units	units	min.	mgm. %	mgm. %	mgm. %	mgm. %	mgm. %	mgm. %	mgm. %	mgm. %
49 cc. pus + 1 cc. M/40 barbitol buffer	0	0	0	57.5	—	13.0	—	9.6	—	3.4	—
			180	75.0	17.5	14.9	1.9	11.0	1.4	3.9	0.5
49 cc. pus + 1 cc. heated streptococcal concentrate in M/40 barbitol buffer	0	10,000	15	61.6	4.1	13.1	0.1	9.9	0.3	3.3	0.0
			30	61.6	4.1	13.8	0.8	9.8	0.2	4.0	0.6
			60	64.0	6.5	13.9	0.9	10.0	0.4	3.9	0.5
			120	68.6	13.1	13.9	0.9	10.4	0.8	3.5	0.1
			180	68.6	13.1	13.8	0.8	10.5	0.9	3.3	0.0
49 cc. pus + 1 cc. streptococcal concentrate in M/40 barbitol buffer	10,000	50,000	15	73.0	15.5	21.2	8.2	9.5	0.0	11.7	8.3
			30	75.0	17.5	23.3	10.3	9.5	0.0	13.8	10.4
			60	79.6	22.1	25.6	12.6	9.7	0.1	15.9	12.5
			120	95.6	38.1	28.2	15.2	10.2	0.6	18.0	14.6
			180	106.0	48.5	29.2	16.2	10.5	0.9	18.7	15.3

dornase produced a striking progressive liberation of organic acid soluble P and acid soluble N into the supernatant. The liberation of inorganic P was at the same rate in all three instances.

The above data point to the fact that since no liberation of inorganic P or uric acid was detectable in the supernatant portions of the exudates,

either *in vivo* or *in vitro*, the breakdown of the nucleoprotein of the exudates probably did not proceed further than the nucleotide stage.

A readily obvious physical change caused by the action of streptodornase on the intrapleural purulent exudates is the transformation of the viscid, thick, coarsely granular material to a thin, milky type of fluid. Photographs contained in previous articles (2, 4) demonstrate the extent of the visible change.

Quantitative estimations of the physical changes have been made by determining in the serial samples of exudate derived from the patients the decreases in both the per cent of sediment and viscosity in association with the biochemical changes described above.

Figure 7 demonstrates the effect of the injection on the viscosity of exudates in nine patients.

The range of fall was from 2–2400 viscosity units. Where the initial viscosities were highest because of large amounts of extracellular desoxyribose nucleoprotein, the effect was most striking. This correlation will be demonstrated in the subsequent description of the findings obtained by the use of Feulgen stained preparation.

The range of the per cent fall in one hour in the amount of sediment following injection in nine patients is shown in Figure 8. The observations were made by centrifuging aliquot parts of the serial samples of exudates at approximately 2500 revolutions per minute for 60 minutes.

The results are again closely correlated with

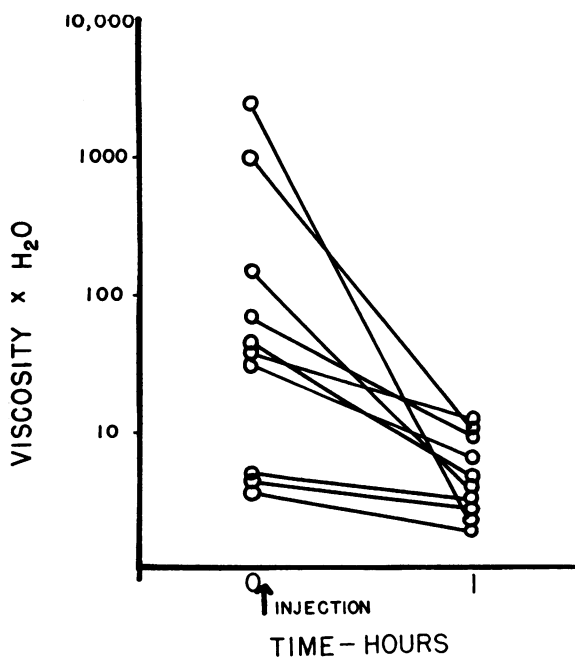


FIG. 7. THE EFFECT OF STREPTODORNASE, INTRAPLEURALLY INJECTED, ON THE VISCOSITY OF THE PLEURAL EXUDATES IN NINE CASES OF EMPYEMA

One patient was injected twice.

both the changes in viscosity and the microscopic demonstration of the lysis of extracellular desoxyribose nucleoprotein.

By the Feulgen method of staining, the presence of desoxyribose nucleoprotein has been identified microscopically as being present extracellularly in considerable abundance. Morphologically it appears, as has been previously described (1), in the form of small granules, fibrous-like strands, and amorphous plaques. Intracellularly, desoxyribose nucleoprotein is noted as a significant part of the nuclei. The extracellular material is obviously derived from destroyed cells, chiefly from the leucocytes of the exudate and to an undetermined degree from local fixed tissue cells. From the cytological studies, leucocytes in various stages of degeneration have also been noted.

The small granular forms of the desoxyribose nucleoprotein have been found to be characteristic of the freshest exudates, the reticular and amorphous forms to be present in greatest abundance in protracted cases of purulent infection such as tuberculous empyema.

When the streptococcal concentrates containing streptodornase have been introduced intrapleurally

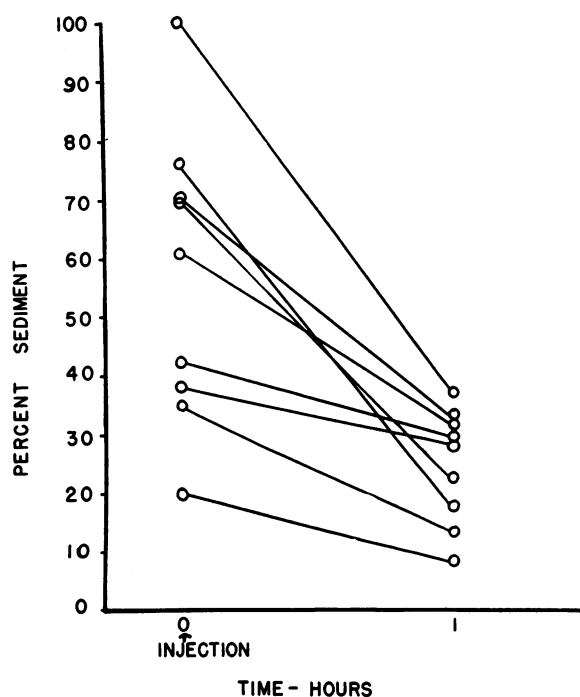


FIG. 8. THE EFFECT OF STREPTODORNASE, INTRAPLEURALLY INJECTED, ON THE PERCENTAGE SEDIMENT OF PLEURAL EXUDATES IN NINE CASES OF EMPYEMA

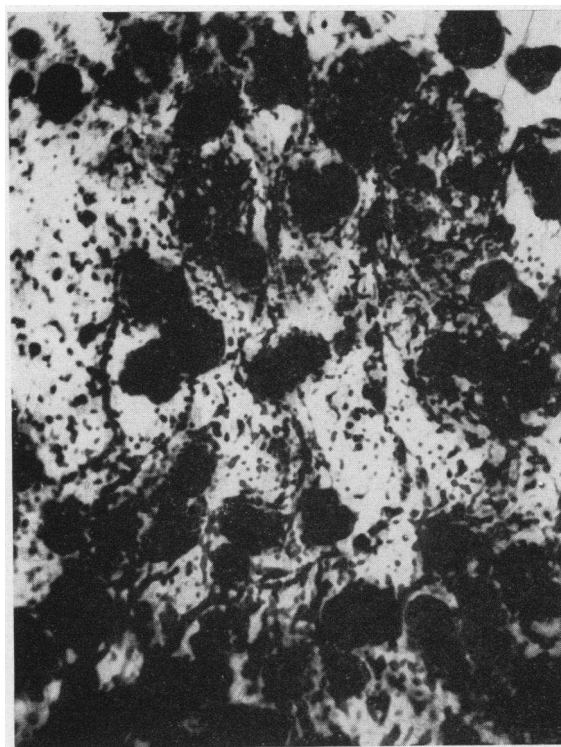


FIG. 9. FEULGEN STAIN OF PLEURAL EXUDATE ASPIRATED FROM A PATIENT WITH A *B. pyocyaneus* EMPYEMA IMMEDIATELY BEFORE THE LOCAL INJECTION OF STREPTOCOCCAL CONCENTRATE

The nuclei of the leucocytes, and many extracellular granules and fibrous strands take the Feulgen stain. $\times 882$.

into patients and stained preparations made of serial samples of the exudate, the rapid and complete disappearance of the extracellular material has been observed. The details of such findings in two patients are given in association with the descriptions of the accompanying photographs which are illustrative of the rapidity and completeness of the disappearance of the extracellular desoxyribose nucleoprotein at the site, *in vivo*, of the exudation.

Figures 9 and 10 depict results obtained in a patient with relatively recent exudation consisting chiefly of granules and fibrous strands. Figures 11-13 illustrate the type of Feulgen staining material in a more protracted case of empyema. In both instances the injection of streptodornase resulted in complete disappearance of the extracellular desoxyribose nucleoprotein, within one hour in the first patient, and within two hours in the second patient.

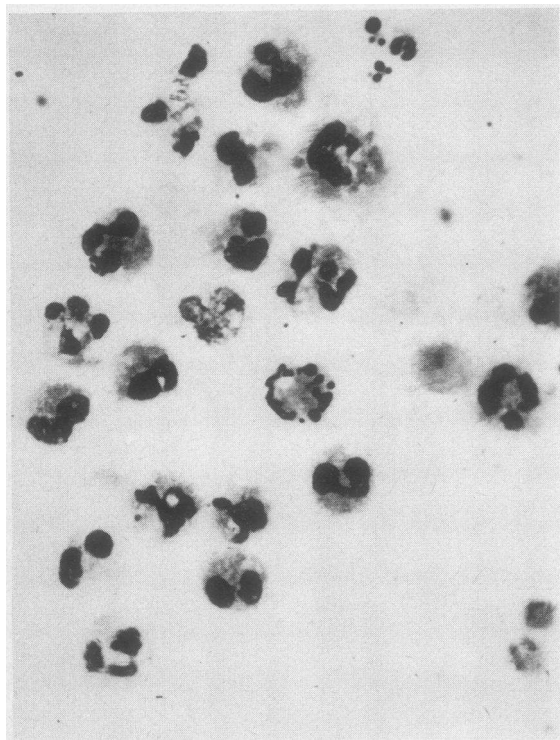


FIG. 10. FEULGEN STAIN OF PLEURAL EXUDATE ASPIRATED FROM THE PATIENT WITH *B. pyocyaneus* EMPYEMA, ONE HOUR AFTER THE LOCAL INJECTION OF STREPTOCOCCAL CONCENTRATE CONTAINING 9000 UNITS OF STREPTODORNASE

The extracellular matrix has been swept clean of the Feulgen staining material. $\times 882$.

The leucocytes remaining visible in the photographs taken following the lytic action represent to the greatest extent, cells that have not degenerated and are morphologically undamaged by the nuclease. A progressive fall in the percentage of degenerating cells, as determined by the Feulgen stain, was regularly observed for at least 24 hours after the injection of the streptococcal concentrate. As shown in Figure 14 the effect is greater, the larger the percentage of degenerating cells in the exudate prior to the injection of the concentrates.

The effect of streptodornase on motility and other functions of leucocytes will be the subject of a separate communication.

SUMMARY

1. The action of streptodornase contained in the streptococcal concentrates on purified desoxyri-

bose nucleic acid from calf thymus has been found to have the following characteristics:

- a. A linear relationship between enzyme concentration and depolymerase activity.
- b. Temperature inactivation of the enzyme exceeds the thermal enhancement of reaction velocity above 45°C . Temperature inactivation is apparent above 30°C .
- c. Depolymerase activity occurs over a wide pH range, and is greatest between 7.0 and 8.5.
- d. In addition to depolymerization, there is a progressive liberation of acid soluble P and N from the substrate. The liberated P appears entirely in the organic form.
- e. Salicylate, gentisate, ascorbate, thiourea, paraminobenzoic acid, parachlorophenol, oxalate, colchicine, and penicillin had no signifi-



FIG. 11. FEULGEN STAIN OF PLEURAL EXUDATE ASPIRATED FROM A PATIENT WITH A THICK TUBERCULOUS EMPYEMA, IMMEDIATELY BEFORE THE LOCAL INJECTION OF STREPTOCOCCAL CONCENTRATE

The nuclei of the leucocytes, and extensive extracellular amorphous plaques and fibrous strands take the Feulgen stain. $\times 882$.

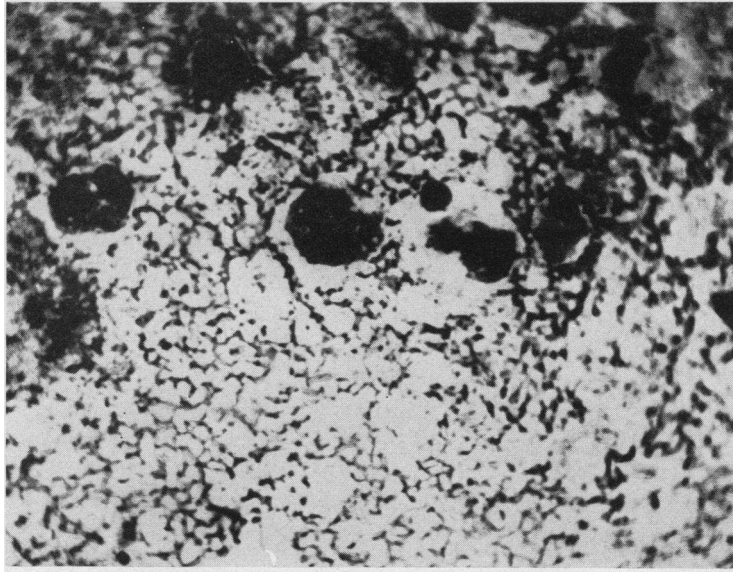


FIG. 12. FEULGEN STAIN OF PLEURAL EXUDATE ASPIRATED FROM THE PATIENT WITH A THICK TUBERCULOUS EMPYEMA, ONE HOUR AFTER THE LOCAL INJECTION OF STREPTOCOCCAL CONCENTRATE CONTAINING 7200 UNITS OF STREPTODORNASE

The amorphous plaques and fibrous strands have begun to break up and disappear. $\times 882$.

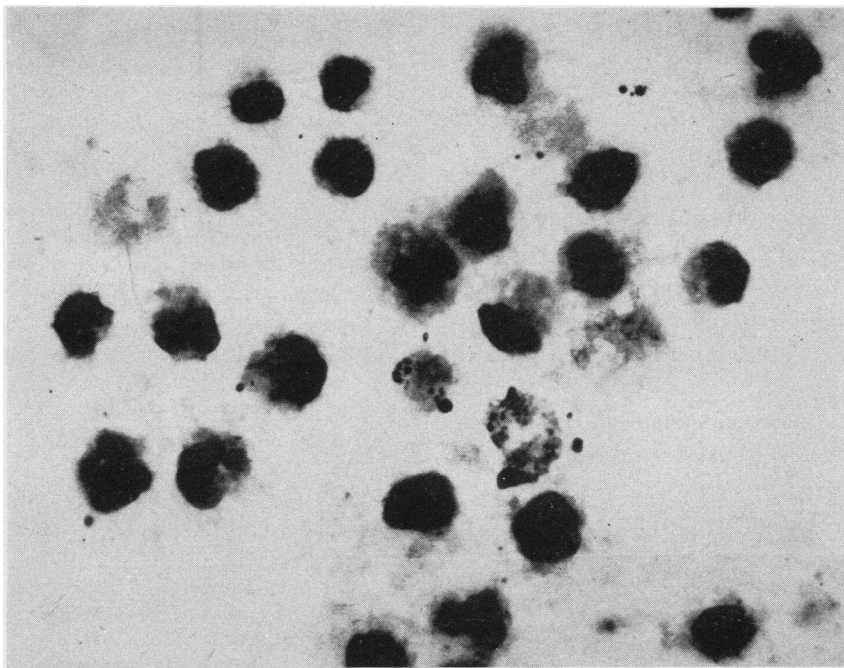


FIG. 13. FEULGEN STAIN OF PLEURAL EXUDATE ASPIRATED FROM THE PATIENT WITH TUBERCULOUS EMPYEMA, TWO HOURS AFTER THE LOCAL INJECTION OF STREPTOCOCCAL CONCENTRATE

The extracellular matrix has been swept clean of the Feulgen staining material. $\times 882$.

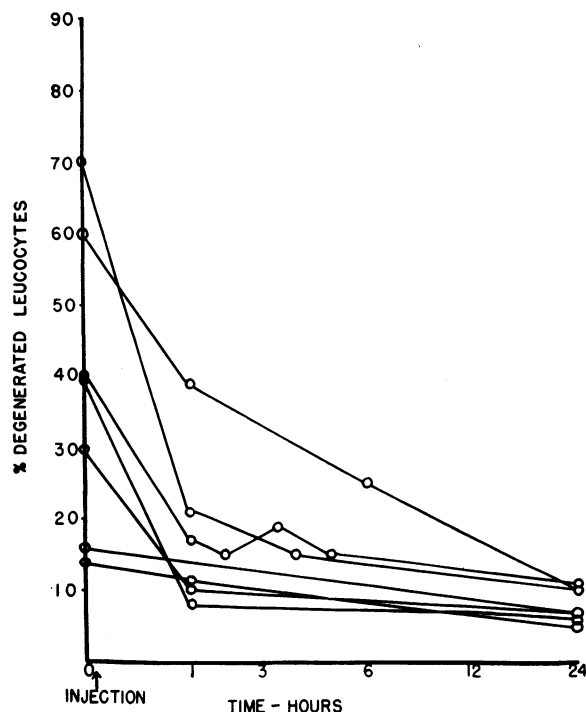


FIG. 14. THE EFFECT OF STREPTODORNASE, INTRA-
PLEURALLY INJECTED, ON THE PERCENTAGE OF DEGEN-
ERATED LEUCOCYTES OF THE PLEURAL EXUDATE IN SEVEN
PATIENTS WITH EMPYEMA

cant inhibitory effect on depolymerase activity. Citrate and heparin were found to have an inhibitory action.

2. Streptodornase has been found to be operative when introduced locally into the pleural cavity of patients with empyema by effecting the rapid depolymerization of the desoxyribose nucleoprotein that has been found to constitute a considerable proportion of the solid sediment of coarse purulent exudates.

The following physical, chemical, histological, and cytological changes have been described in this article.

- a. Grossly visible changes of the coarse sediment to a thin milky type of fluid.
- b. Striking fall in viscosity.
- c. Striking fall in per cent sediment.
- d. Sharp rise in organic acid soluble phosphorus and acid soluble nitrogen.
- e. No rise in inorganic phosphorus.
- f. No increase in uric acid.
- g. Striking decrease in number of degenerated leucocytes.

h. Rapid disappearance of all extracellular Feulgen staining material. (desoxyribose nucleoprotein).

3. The changes mentioned above were demonstrable in a significant degree within one hour following the injection of streptodornase. Following a single dose, they continued for varying periods of time ranging from several hours to several days, but were self terminating.

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