THE EXTENT OF THE ENZYMATIC DEGRADATION OF DESOXYRIBONUCLEIC ACID (DNA) [1] IN PURULENT EXUDATES BY STREPTODORNASE [2, 3]

By SOL SHERRY and JOHN P. GOELLER

(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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It has been demonstrated that desoxyribonucleoprotein (DNA protein) [4] is a major constituent of the purulent exudates of patients, and plays a significant part in determining the exudate's character, viscosity, and amount of sediment (1, 2).

Biochemical and cytological studies from our laboratories have localized the DNA protein of the purulent exudate as occurring in the nuclei of living and degenerating cells; and extracellularly, both as an insoluble increment of the sediment and as a soluble, partially depolymerized component of the supernatant (2–4). Recent studies have indicated that the extracellular DNA protein (both in supernatant and sediment) is derived from the degeneration of the cellular elements of the exudate, and accumulates to a significant extent in areas of infection which are poorly draining and not undergoing extensive bacterial enzymatic breakdown (3, 4).

The local injections of streptococcal enzyme concentrates containing streptodornase or SD (streptococcal desoxyribonuclease) (5) into sites of purulent exudation, produces a rapid degradation of the DNA protein (2, 6) and is often associated with a striking clinical improvement of the local area of infection (7). Streptodornase rapidly depolymerizes the extracellular DNA protein of the exudate and the DNA protein of the degenerating cell, but does not affect the DNA protein of the living cell (3). It has been postulated that streptodornase probably does not penetrate the viable cell, but readily diffuses into the degenerating cell.

Since streptodornase has its action on desoxyribonucleic acid (DNA) the purpose of the present study was to investigate the extent of the degradation of DNA brought about by this material in purulent exudates.

Figure 1 illustrates schematically the postulated enzymatic degradation of DNA. This degradation involves several definite steps, each requiring catalysis by a different enzyme.

First, highly polymerized DNA is broken down by a depolymerase to acid soluble oligonucleotides. The beef pancreatic DNASE purified by McCarty and crystallized by Kunitz acts in this fashion (8, 9). Next the oligonucleotides are split into mononucleotides by a polynucleotidase (10). The mononucleotides are complexes of a purine or pyrimidine base joined to desoxyribose, which in turn is linked to phosphate. Inorganic phosphorus is then split off the mononucleotide by a nucleotidase, with the liberation of nucleosides (11). The latter are attacked by a purine nucleosidase with the liberation of the free purine bases, adenine and guanine; and by a pyrimidine nucleosidase with the liberation of the pyrimidine bases, cytosine and thymine (12). The sugar moiety is believed to be split off by a phosphorolytic cleavage (13).

Previous studies of the action of streptodornase on both purified DNA and DNA in purulent exudates, demonstrated a rapid depolymerization with the liberation of acid soluble phosphorus, similar to the action of pancreatic DNASE (2). However, it was found that when large amounts of enzyme concentrate were employed *in vitro*, 30–70% of the available phosphorus of the DNA (purified or in exudates) was liberated as inorganic phosphorus over a 24 hour period. This implied a splitting of the DNA in whole or in part below the mononucleotide stage and suggested the

[1] DNA has been employed in this article as an abbreviation of desoxyribonucleic acid.

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[3] Read at the Forty-Second Annual Meeting of the American Society for Clinical Investigation, Atlantic City, May 1, 1950.

[4] DNA protein has been employed in this article as an abbreviation of desoxyribonucleoprotein.

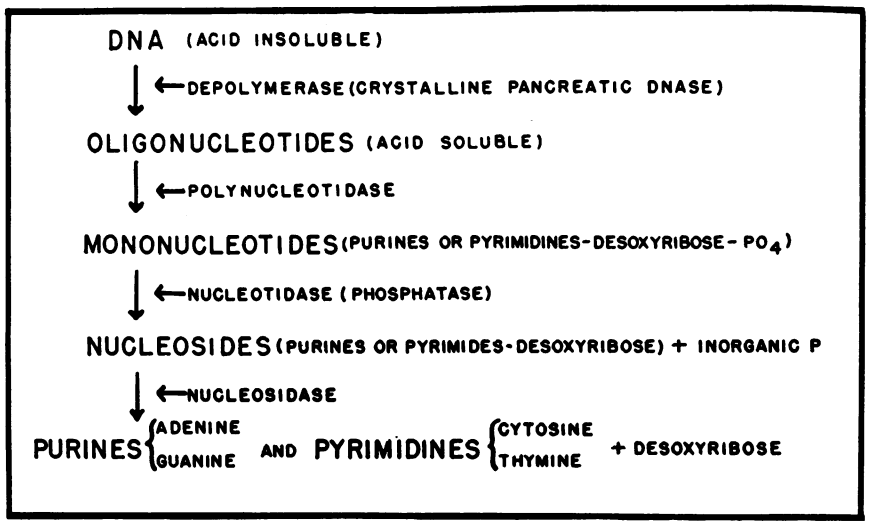

FIG. 1. SCHEMATIC REPRESENTATION OF ENZYMATIC DEGRADATION OF DNA

presence of several nucleolytic enzymes in streptodornase. The extent of the degradation of DNA by the nucleolytic enzymes of streptodornase was elucidated by paper chromatography, and the results of these experiments form the basis of this report.

MATERIALS AND METHODS

Streptodornase. This enzyme was contained in the partially purified streptococcal enzyme concentrates that have been used throughout these studies, and are being employed for clinical work.[5] They contained streptokinase in addition to streptodornase. However, the effects to be described in this article are limited to those caused by streptodornase. The samples employed had approximately 10,000 units of streptodornase per mgm. of concentrate. The actual titers used are given in the individual protocols and were determined by previously published methods (14).

Pancreatic desoxyribonuclease (pancreatic DNASE).[6] A crystalline product prepared from beef pancreas by the method of Kunitz (9).

. *DNA.* This was prepared in our laboratories by modifications of the methods of Mirsky and Pollister (15), McCarty (16), and Gulland (17). It was a white fibrous material, readily soluble in water, with an N : P ratio of 1.6 : 1. A 0.15% solution in M/40 veronal buffer had a viscosity of 4–5 × water at 37° C.

Perchloric acid hydrolysate of DNA. A complete hydrolysis of DNA into free purine and pyrimidine bases, prepared according to the method of Marshak and Vogel (18).

Acetate buffer. A 0.1 N sodium acetate solution pH 7.0 was employed as a buffer since it did not significantly absorb in the U.V.

Feulgen stain. Carried out on smears of exudates by a previously described method (2).

Paper chromatography. Methods and modifications developed by Vischer and Chargaff (19, 20), Hotchkiss (21), and Carter (22) were employed. Both Whatman No. 1 and S. and S. No. 597 filter papers were satisfactory. Ten μl. of the fluid to be studied, were deposited from a calibrated pipette at the starting point of a lane, and allowed to dry.[7] A n-butanol : 1 N ammonia (7 : 1) solvent system was used. The solvent front was allowed to run approximately 30 cm. before the papers were withdrawn from the chamber and hung up inverted, to dry. Only nucleosides and free purine and pyrimidine bases moved out from the starting point with this solvent system. They were recognized as ultraviolet absorbing spots by scanning the paper in the dark with a filtered ultraviolet lamp emitting at 2537Å, according to the method of Carter (22). The papers were photographed in the ultraviolet for permanent records (23).

The individual spots were marked under the ultraviolet, and then carefully cut out. Equal size strips were cut out from an adjacent control lane and used as a blank. Each strip was then cut into many small pieces and extracted with 4 cc. 0.1 N HCl for two to three hours. After centrifugation of the eluate, its ultraviolet absorption spectrum from 220–300 mμ was determined in the Beckmann spectrophotometer. The control lane eluate was used as a blank. The eluate was then adjusted to

[5] These preparations were supplied to us by Lederle and Co.

[6] Obtained from the Worthington Biochemical Laboratory.

[7] Where comparisons were being made to perchloric acid hydrolysates of DNA, an equivalent amount of perchloric acid was first placed in those lanes not containing the acid hydrolysate. The acid hydrolysate and the perchloric acid were then neutralized by exposure of the paper to fumes of concentrated NH_3 for one-half hour.

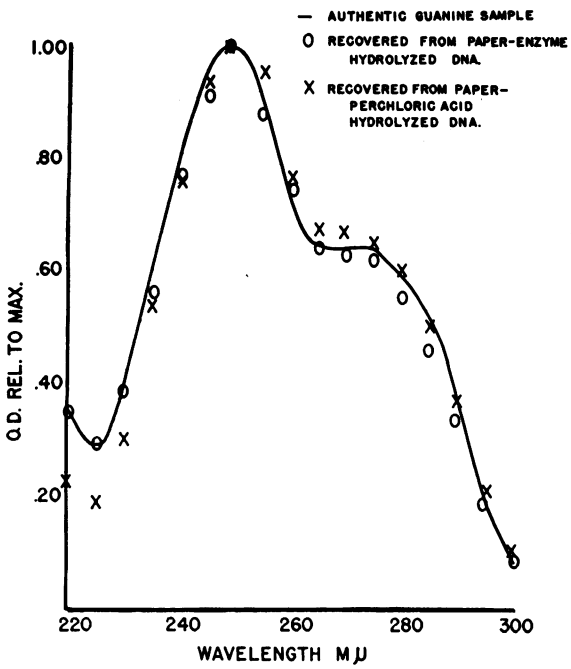

FIG. 2. ULTRAVIOLET ABSORPTION SPECTRUM OF GUANINE IN 0.1 N ACID

Note the close agreement to the values obtained on the eluate of the guanine position spot of a paper chromatogram of an enzyme (O) and an acid (X) hydrolyzed DNA solution.

0.1 N alkali by the addition of NaOH and the absorption spectrum redetermined.

Identification of the substance in each of the spots seen on the paper chromatogram was made by:

a) Its position relative to the solvent front (Rf value)
b) Its complete acid and alkali absorption spectrum in the ultraviolet.

Figure 2 depicts the characteristic absorption spectrum in 0.1 N acid of a highly purified authentic guanine sample. The values obtained by elution from a paper chromatogram of the guanine position spot of a perchloric acid hydrolysate of DNA (crosses), and an enzymatic DNA hydrolysate (circles) are also shown. The close agreement of the three sets of data is quite obvious and defines the accuracy of the method employed.

Hotchkiss' detailed data on the characteristic U.V. absorption spectra of purines, pyrimidines and nucleosides, in 0.1 N acid and alkali served as a reference (21). Reference Rf values for the various bases and nucleosides were determined on commercial preparations.

RESULTS

A. The action of streptodornase on purified DNA

The ultraviolet photograph of the paper chromatogram shown in Figure 3 illustrates the complete enzymatic action of streptodornase on DNA.

For this experiment 10 μl. of the following solutions were deposited on paper for study:

a) Control-4 cc. of 1% DNA solution in 0.1 N Na acetate incubated for 24 hours at 37°C.—lane 1.
b) 4 cc. of 1% DNA solution in 0.1 N Na acetate containing 500,000 units streptodornase in 10 mgm. of streptococcal enzyme concentrate.[8] Incubated for 24 hours at 37°C.—lane 2.
c) Perchloric acid hydrolysate of a 1% DNA solution —lane 3.

[8] Powdered merthiolate was added to a concentration of 1:1000, and the Mg concentration adjusted to 0.005 M by the addition of $MgCl_2$. Bovine albumin was added as a stabilizer to a final concentration of 2%. The albumin was supplied to us by Dr. L. L. Lachat of Armour and Co. as a 35% solution of crystalline bovine albumin.

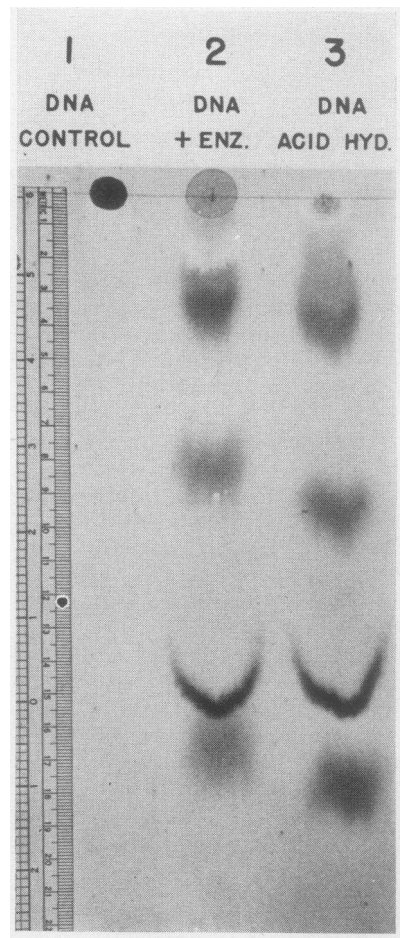

FIG. 3. ULTRAVIOLET PHOTOGRAPH OF A PAPER CHROMATOGRAM OF DNA (LANE 1), DNA + STREPTODORNASE (LANE 2), AND ACID HYDROLYZED DNA (LANE 3)

It will be noted that four spots have moved out from the acid and enzyme hydrolysates. In the complete acid hydrolysate, in lane 3, these spots have been identified from above downwards by their Rf values and absorption spectra as guanine, cytosine, adenine, and thymine. Note the typical adenine crescent in the presence of perchlorate. In lane 2 which contained the enzyme substrate mixture, the first and third spots have the same Rf values and absorption spectra as guanine and adenine. The second and fourth spots do not move as far as cytosine and thymine, and their absorption spectra were found to be characteristic of the pyrimidine nucleosides, cytidine and thymidine.

Many experiments of this type have been made with incubation periods varying up to 72 hours. The end products have been consistently similar to those described above, though the amount liberated has varied directly with the time of enzyme substrate interaction. The DNA molecule appears to be split into guanine, adenine, cytidine, and thymidine by streptodornase.

B. The action of streptodornase on DNA in purulent exudates

Although exudates, removed from sterile areas or sites of low grade infection, have been found to have little to no effect on highly polymerized DNA, the action of streptodornase on DNA in purulent exudates is accelerated and modified by other enzymes present in the exudate itself. By using ribose mononucleotides as substrates, we have been able to demonstrate that sterile exudates removed from the thorax contain several enzymes capable of degrading these mononucleotides. A nucleotidase has been consistently found even in relatively acellular exudates, and can be readily demonstrated in the blood serum as well. Specific nucleosidases and deaminases have been observed, but have varied with the exudate studied. The latter enzymes appear to be associated with the cellular elements since they are not demonstrable in the supernatant of the exudate, or in blood serum; but they are present in cellular exudates and in whole blood. Similar enzymes are present in exudates for degrading the breakdown products of streptodornase action.

The combined action of streptodornase and the enzymes of a thin sero-purulent exudate[9] on the degradation of DNA is illustrated in the ultraviolet photograph shown in Figure 4.

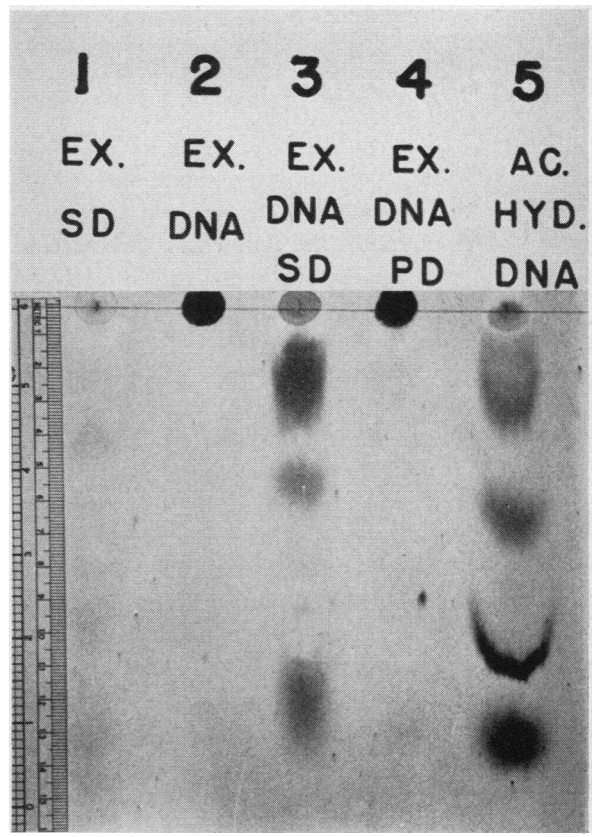

FIG. 4. ULTRAVIOLET PHOTOGRAPH OF A PAPER CHROMATOGRAM OF SERO-PURULENT EXUDATE + STREPTODORNASE (LANE 1), EXUDATE + DNA (LANE 2), EXUDATE + DNA + STREPTODORNASE (LANE 3), EXUDATE + DNA + PANCREATIC DNASE (LANE 4), AND ACID HYDROLYZED DNA (LANE 5)

For this experiment, the following mixtures were incubated for 24 hours at 37°C. and then chromatographed by the procedure previously described:

a) 5 cc. of 0.1 N acetate buffer plus 120,000 units of streptodornase in 2.5 cc. of exudate—lane 1.
b) 5 cc. of 1% DNA in 0.1 N acetate buffer plus 2.5 cc. of exudate—lane 2.
c) 5 cc. of 1% DNA in 0.1 N acetate buffer plus 120,000 units of streptodornase in 2.5 cc. of exudate —lane 3.

[9] This exudate was obtained from the thorax of a patient with a loculated post-pneumonic effusion which was sterile on culture. It contained no extracellular Feulgen positive material on smear.

d) 5 cc. of 1% DNA in 0.1 N acetate buffer plus 100,000 units of crystalline pancreatic beef desoxyribonuclease in 2.5 cc. of exudate—lane 4.

e) Perchloric acid hydrolysate of a 1% DNA solution —lane 5.

Lanes 1 and 2, containing exudate and streptodornase, and exudate and DNA, acted as controls. Lane 5 demonstrates the four characteristic bases obtained from complete acid hydrolysis of DNA. Lane 3, which represents an incubated mixture of DNA, exudate, and streptodornase contains three spots. From above downwards, they have been identified as hypoxanthine, cytidine, and thymidine. The adenine crescent which we would have expected from the action of streptodornase on DNA is missing, since deamination has occurred and hypoxanthine formed. There is evidence in our studies that the guanine precursors have also been deaminated and reduced under these conditions, with the final formation of hypoxanthine. Note that in the fourth lane, which represents an incubated mixture of DNA, exudate, and crystalline pancreatic DNASE, no free bases or nucleosides have been liberated. However, extensive depolymerization was grossly apparent as measured by viscosity change. Apparently pancreatic DNASE does not carry the enzymatic degradation of DNA far enough for the enzymes of the exudate to complete the digestion, and is in sharp contrast to the action of streptodornase.

Figure 5 represents a reasonable schema summarizing the results of our studies on the enzymatic degradation of DNA in purulent exudates by the combined action of streptodornase and the enzymes of the exudate itself. It is constructed from eight observations on five exudates. Three of the exudates were thin, sero-purulent, sterile and were obtained from penicillin treated postpneumonic loculated effusions. They contained little to no extracellular DNA on Feulgen stain. The other two were thick tuberculous exudates, one obtained from a chronic empyema and the other from a suppurative cervical node. These two exudates contained large amounts of extracellular DNA on Feulgen stain. The schema illustrates the most complete breakdown we have observed both of added DNA, in the case of the thin exudates, and of the DNA already present in the thick purulent tuberculous exudates. The completeness of the breakdown has varied with the exudate studied. An example of an intermediate type breakdown has been cited in Figure 4. The enzymatic degradation of DNA by streptodornase in these exudates is also compared in Figure 5 to

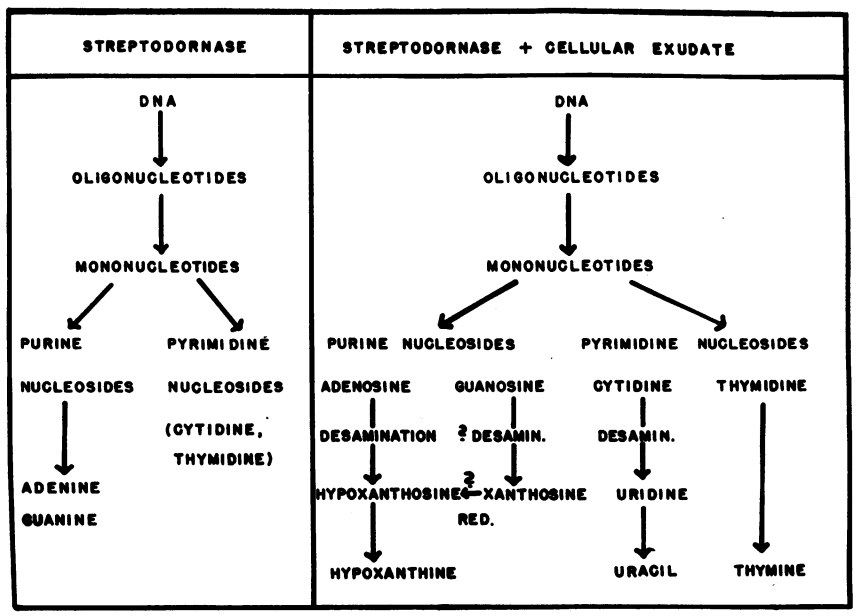

FIG. 5. SCHEMA CONSTRUCTED FROM DATA TO ILLUSTRATE THE ACTION OF STREPTODORNASE ALONE ON DNA, AND THE COMBINED ACTION OF STREPTODORNASE AND THE ENZYMES OF PURULENT EXUDATES ON DNA

the action of streptodornase on purified DNA alone.

Streptodornase breaks the highly polymerized complex DNA molecule into the purine bases and the pyrimidine desoxynucleosides as previously noted. In the presence of SD and an exudate, it has been found that the purine desoxynucleosides may be converted into hypoxanthine; and of the pyrimidine desoxynucleosides, cytidine may be degraded into uracil and thymidine split to thymine.

DISCUSSION

The extensive splitting of DNA by streptodornase *in vitro,* when compared to the schema shown in Figure 1, implies that streptodornase consists of a group of enzymes rather than a single enzyme. This is not an unreasonable viewpoint when one considers the various types of cleavages involved in releasing free purine bases from DNA. This is further substantiated by unpublished observations of differences in pH and Mg concentration optima for depolymerase and nucleotidase activity of the streptococcal concentrate. It may be concluded that streptodornase contains a DNA depolymerase, a desoxypolynucleotidase, a desoxynucleotidase, and a purine desoxynucleosidase. No attempt at separating these different enzymes has as yet been made.

We have no evidence to date that the end products described in this report as resulting from streptodornase action *in vitro,* accumulate to any significant extent *in vivo.* Our studies in empyemas suggest that the acid soluble nucleotides formed early in the course of streptodornase action are fairly readily absorbed from the site of inflammation (2). However, further studies of the products accumulating at the site of disease following streptodornase therapy are contemplated with a view toward determining whether they are related to the beneficial effects observed.

SUMMARY AND CONCLUSIONS

1. Biochemical studies, employing paper chromatography and ultraviolet absorption spectra, have been made of the extent of degradation of DNA by streptodornase.

2. DNA is extensively split by streptodornase, with the liberation of free purine bases (adenine and guanine), and pyrimidine desoxynucleosides (cytidine and thymidine).

3. It is concluded that streptodornase consists of several nucleolytic enzymes rather than a single enzyme.

4. In purulent exudates, the enzymatic action of streptodornase on DNA is enhanced and modified by the nucleotidases, nucleosidases, and deaminases which may be present in the exudate itself.

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