scopic preparations lends support to evidence obtained by chemical means that desoxyribose nucleoprotein constitutes a considerable proportion of purulent sediment that has been generally accepted as being fibrin or denatured serum proteins. The accompanying photographs illustrate the microscopic appearance of exudates of a freshly formed inflammatory effusion (Fig. 2), and of a tuberculous empyema of long standing (Fig. 3).

Up to the present time, no determinations of the presence or significance of ribose nucleoprotein have been made.

Summary. Desoxyribose nucleoprotein has been identified as a significant constituent of purulent and inflammatory exudates derived from patients. Its chemical characteristics have been described and it has been isolated in quantities ranging from 30 to 70% of the total purulent sediment.

Its physical characteristics of stringiness, viscidity, and coagulated gel, indicate its significance in contributing to the sedimented elements of exudations. Protein-free desoxyribonucleic acid has been derived from the nucleoprotein.

The liquefaction of both the nucleoprotein and the nucleic acid has been found to occur following the addition of beef desoxyribonuclease and preparations of hemolytic streptococcal filtrates containing desoxyribonuclease.

By the Feulgen method of staining the abundance of desoxyribose nucleoprotein in exudates has been demonstrated microscopically.

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Streptococcal Desoxyribonuclease: Significance in Lysis of Purulent Exudates and Production by Strains of Hemolytic Streptococci.*

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As has been indicated in the previous report,[1] purulent pleural exudates contain desoxyribose nucleoprotein as an important constituent which, together with depositions of fibrin, accounts for the gelatinous coagulum and coarse sediment that characterizes such material. In an investigation directed toward observing the action in patients of streptococcal fibrinolysin (streptokinase), the streptococcal product, substantially purified by one of us (L.R.C.) according to methods previously described,[2] was found to cause a striking and rapid change in the physical qualities of exudates both *in vitro* and also within the pleural cavities of patients suffering from various forms of purulent and fibrinous pleurisy. When it became evident that the characteristics of the specimens being altered were due to the presence of considerable amounts of nucleoprotein as well as fibrin, studies were undertaken which have revealed that hemolytic streptococci produce, as they grow, a potent desoxyribonuclease which is readily demonstrable as a secretory or excretory product in the fluid medium in which the organisms are cultivated. The effect of the streptococcal preparations has, therefore, been found to be dual in action, the purulent

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1 Sherry, S., Tillett, W. S., and Christensen, L. R., PROC. SOC. EXP. BIOL. AND MED., 1948, **68**, 179.

2 Christensen, L. R., *J. Gen. Phys.*, 1947, **30**, 465.

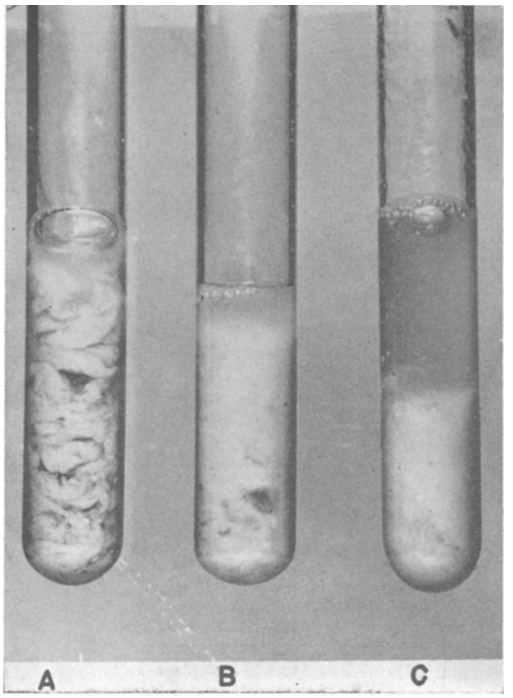


FIG. 1.
Tubes A, B, and C each contain approximately 5 cc of pus from a case of pneumococcal empyema. Tube A is an untreated control. To Tube B was added 2-3 mg of beef desoxyribonuclease. To Tube C was added 2-3 mg of crude streptococcal concentrate. The content of the tubes was mixed, shaken frequently, and observed at room temperature. Beginning changes in the character of the sediment were evident immediately. Photograph was taken after ½ hour. Tubes were uncentrifuged.

material containing two substrates, nucleoprotein and fibrin, and the streptococci yielding two substances, each acting simultaneously in a specific catalytic or enzymic manner.

Data primarily pertinent to the production of desoxyribonuclease by strains of hemolytic streptococci and the action of the enzyme on substrates of nucleic acid composition from purulent materials as well as purified preparations of animal origin‡ constitute the body of this report.

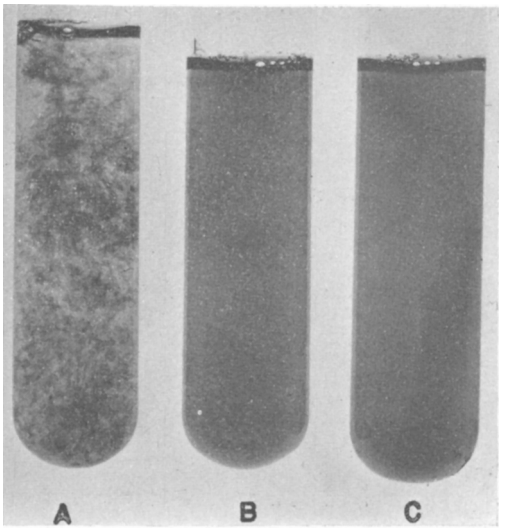


FIG. 2.
Tubes A, B, and C each contain in 7 cc the nucleoprotein isolated from 1 cc of sedimented pus by repeated extractions with 1 M NaCl. Tube A is untreated control. To Tube B was added 0.1 cc of 0.15% solution of beef desoxyribonuclease. To Tube C was added 0.1 cc of 0.15% solution of crude streptococcal concentrate. The tubes were mixed, shaken frequently, and observed at room temperature. Beginning changes in the solid strands were noted immediately and complete lysis occurred in 10 minutes. Photograph was taken after ½ hour.

Changes Induced in Pus by Desoxyribonuclease. Fig. 1 illustrates the action of: 1) beef desoxyribonuclease§ and, 2) streptococcal concentrate,¶ on the sediment of pus obtained from a case of pneumococcal empyema.

The details are given in the legend which accompanies Fig. 1.

The beef desoxyribonuclease was a well purified preparation while the streptococcal concentrate contained both desoxyribonuclease and streptokinase (streptococcal fibrinolysin). Substantial qualitative and quantitative changes in the sediment may be noted in each tube (to which the nuclease containing reagents had been added.) Following the

‡ Sodium thymonucleate (calf thymus) kindly supplied by Dr. Milton Levy, Department of Biochemistry, New York University College of Medicine.

§ The purified beef pancreas desoxyribonuclease (preparation R1MR) kindly supplied by Dr. Maclyn McCarty of the Hospital of the Rockefeller Institute for Medical Research.

¶ Lederle Laboratories have kindly prepared and supplied the concentrated filtrate from large volume broth cultures of hemolytic streptococci (Strain H46A).

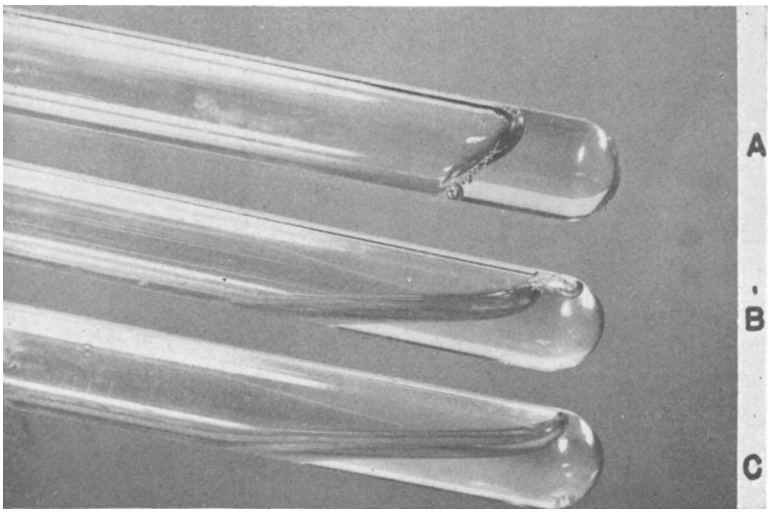


FIG. 3.

Tubes A, B, and C each contain 2 cc of 4% solution of purified desoxyribose nucleic acid (calf thymus). Tube A is untreated control. To Tube B was added 0.1 cc of 0.15% solution of beef desoxyribonuclease. To Tube C was added 0.1 cc of 0.15% solution of crude streptococcal concentrate. The tubes were mixed, shaken frequently, and observed at room temperature. Beginning changes in the gel were noted immediately. Photograph, taken with tubes on slant to indicate gelatinous quality of control substrate and complete liquefaction in other tubes, was made after ½ hour.

addition of the enzymatic substances, in each instance there was immediate thinning of the specimen, a rapid breaking up of the mucoid coagulum, a clouding of the supernatant fluid, and an obvious decrease in sediment.

Since the preparation of streptococcal origin contained at least two enzymatic elements the reduction in the sediment in Tube C is greater than that exhibited in Tube B, fibrin being liquefied in the latter as well as the nucleoprotein.

Changes Induced in Desoxyribose Nucleoprotein Derived from Purulent Exudation of a Patient with Empyema. Fig. 2 illustrates the action of: 1) beef desoxyribonuclease and, 2) a streptococcal concentrate on a sample of desoxyribose nucleoprotein isolated from human pus. The details are given in the legend which accompanies Fig. 2.

Since no fibrin was present in this specimen, the lysis of the nucleoprotein was complete on the addition of the beef desoxyribonuclease as well as the streptococcal preparation.

Alterations in the appearance of the solid strands was noted to begin immediately after the addition of the enzyme-containing preparations and dissolution was complete in a few minutes time.

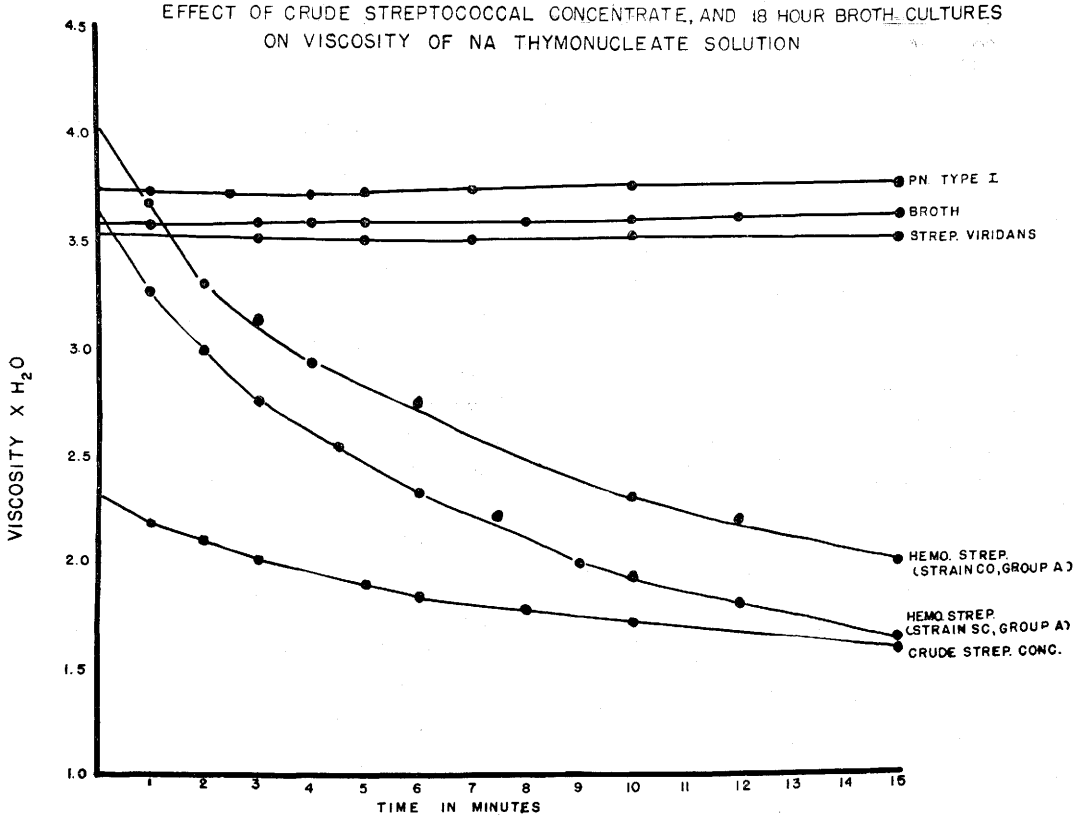
Changes Induced in Desoxyribose Nucleic Acid (Calf Thymus). Fig. 3 illustrates the action of: 1) beef desoxyribonuclease and, 2) a streptococcal concentrate on purified desoxyribose nucleic acid.

The similarity of the liquefying effect in each instance is self-explanatory. With respect to the physical character of the specimen, the gel-like quality of the desoxyribose nucleic acid may be noted while the fibrous stringy quality of the desoxyribose nucleoprotein is evident in Fig. 2.

Effect of Crude Streptococcal Concentrate on Viscosity of 1% Solution of Na Thymonucleate. From the above experiments it was apparent that the preparation of crude streptococcal concentrate contained a highly active desoxyribonuclease. In Chart 1 is shown the depolymerase activity of 0.25 cc of a 0.15% solution of the streptococcal preparation, dissolved in saline phosphate buffer, on 5 cc of a 1% desoxyribosenucleic acid solution in

CHART I

EFFECT OF CRUDE STREPTOCOCCAL CONCENTRATE, AND 18 HOUR BROTH CULTURES ON VISCOSITY OF NA THYMONUCLEATE SOLUTION



0.02 M saline containing 0.0016 M PO_4 at pH 7.20 at 37°C.

Tests For the Production of Desoxyribonuclease by Hemolytic Streptococci, Green Streptococci, and Pneumococci. The finding of desoxyribonuclease in large amounts in preparations made from filtrates of cultures of hemolytic streptococci (Strain H46A, Group C), which had been concentrated for the purpose of isolating the fibrinolytic principle (streptokinase) elaborated by the organisms, led to a study of other strains in order to determine their capacity to produce this additional enzyme. Initial interest primarily centered around the production of desoxyribonuclease in relation to streptokinase.

For this purpose several selected strains of hemolytic streptococcus were available in our laboratory in a lyophilized form which were formerly studied for the fibrinolytic activity.[3] Their source, biochemical and cultural characteristics are given in detail in Reference 3. A simple method of testing cultures directly was as follows: 5 cc of a 1% solution of a highly purified desoxyribose nucleic acid (calf thymus) in M/40 barbital buffer (pH 7.4) was placed in an Ostwald viscosimeter. Preliminary readings of viscosity were made in a water bath (37-37.5°C) for approximately 15 minutes until constant rates of flow were obtained. Then 1 cc of an 18-hour broth culture of the test strain was added and mixed. Subsequent viscosimetric readings were then made at 2, 5, 10, 15 minutes. Examples of nuclease and non-nuclease producing strains are given in Chart I.

A total of 22 strains have been tested in the manner described. When the result listed in the tabulation below is described as positive the rate and degree of change in viscosity was comparable to that given for the 2 positive strains graphically recorded in Chart I.

[3] Tillett, W. S., *J. Bact.*, 1935, **29**, 111.

Strain	Streptokinase	Nuclease
Streptococcus hemolyticus (Group A), Co, Se, Ra, Mac, OT, C108, E22, V10. (8 strains)	All positive	All positive
Streptococcus viridans from cases of bacterial endocarditis. (6 strains)	All negative	All negative
Streptococcus hemolyticus (Group B), K158A (1 strain)	Negative	Positive
Streptococcus hemolyticus (Group C), H46A, K104. (2 strains)	H46A positive K104 negative	H46A positive K104 negative
Streptococcus hemolyticus (Group E), K128. (1 strain)	Negative	Negative
Pneumococcus (1 strain each, Types I, II, VIII, XIX)	"	"

The strains tested are shown above.

From the above data it may be seen that all of Group A, fibrinolytic strains which are classified as human pathogens were also highly active in the production of desoxyribonuclease. In scattered tests with other strains of hemolytic streptococci of other serological groups, one strain of Group B proved to be active in nuclease production but without fibrinolytic activity. The strains of green streptococci and pneumococci were negative under the conditions of the method employed. In studies of the type transforming principle McCarty and Avery[4] have reported the presence of desoxyribonuclease in autolysates of pneumococcus. Under the conditions of the experimental procedure employed in this study, however, the nuclease was not found to be elaborated into the fluid medium in which the organisms were cultivated.

1. When mixtures of the two agents present in the streptococcal concentrate were heated at 56° C for one hour, the nuclease was destroyed and the streptokinase activity remained unchanged.

2. Preparations have been obtained which showed 10 fold and even greater differences in fibrinolytic titre but a constant level of depolymerase activity per unit of time.

3. Beef desoxyribonuclease was without fibrinolytic activity against either human or beef plasma clots.

4. Mg++ markedly accelerated the nuclease activity of the streptococcal concentrate but did not affect the streptokinase in a similar manner. This result is similar to the findings of others with depolymerases of animal origin.[5,6]

Summary. In experiments in which the following substrates were employed: 1) specimens of purulent exudations from patients, 2) desoxyribose nucleoprotein isolated from the exudates and, 3) desoxyribose nucleic acid derived from nucleoprotein, striking and rapid lytic changes were demonstrable in the coarse sediment of substrate 1, in the fibrous material of substrate 2, and in the gel of substrate 3, following the addition of concentrated filtrates obtained from cultures of hemolytic streptococci. The lytic action was found to be due to the presence of desoxyribonuclease which is elaborated during the growth of strains of hemolytic streptococci and is freely demonstrable in the fluid medium in which the organisms are cultivated.

Among the strains tested, hemolytic streptococci of Group A which also possessed fibrinolytic properties were uniformly potent in the production of desoxyribonuclease. By the methods of simple testing that were employed, strains of *streptococcus viridans* and pneumococcus were not found to possess a comparable property.

Desoxyribonuclease and streptokinase, produced by the same strains were found to be different substances. When streptococcal concentrates were added to whole samples of inflammatory exudates, the rapid lysis of both fibrin and desoxyribose nucleoprotein occurred.

[4] McCarty, M., and Avery, O. T., *J. Exp. Med.*, 1946, **83**, 97.

[5] McCarty, M., *J. Gen. Phys.*, 1946, **29**, 123.

[6] Carter, C. E., and Greenstein, J. P., *J. Nat. Cancer Inst.*, 1946, **7**, 29.