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Presence and Significance of Desoxyribose Nucleoprotein in the Purulent Pleural Exudates of Patients.*

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In an investigation which has consisted of determining the effect of injections of partially purified streptococcal fibrinolysin (streptokinase) on fibrinous and purulent exudations, detailed biochemical assays of the solid and fluid content of samples of the exudations have been made before and after the injections. In the course of the study evidence has been accumulated that there is present as a conspicuous part of the thick inflammatory exudates a considerable amount of a fibrous protein which is not fibrin or its denaturation products. The coarse characteristic coagulum and thick sediments of purulent material has been generally interpreted as being due to its cellular and fibrinous content. However, it is the purpose of this report to describe and define the presence in considerable amounts of a nucleoprotein as a constituent part of purulent exudations and to indicate its importance in accounting for such characteristics of pus as thickness, sliminess, viscosity, and stringiness.

In addition, in an accompanying article,[1] the elaboration by hemolytic streptococci of a product which rapidly liquefies the fibrous nucleoprotein will be described. This property of the organisms although different from the fibrinolysin (streptokinase) also produced by hemolytic streptococci, parallels the presence of the latter in an interesting although not inseparable manner.

The fact that the solid sediment of purulent empyemal material was not solely fibrin was first suggested by observations made on samples of pus derived from a case of empyema due to Friedlander bacillus infection. It was noted that when the pH of this sample was raised to above 10, by the addition of alkali, a major proportion of the sediment went into solution which now became diffusely viscid, and that when the solution was brought back to neutrality by the slow addition of 0.1 N HCl, a fibrous stringy material formed, which redissolved again in alkaline mediums. Additional procedures were performed as follows: 25 ml of a tenfold dilution of the same sample of pus was added to 100 ml of 95% alcohol, stirred, and allowed to stand overnight. The precipitate that formed was filtered, washed with alcohol, and dried with ether. The dried powder was insoluble in water, normal saline solution, and 0.1 N HCl but was readily soluble in 0.1 N NaOH, forming a viscid solution. This solution was found after acid hydrolysis to reduce Benedict's reagent. On the basis of the results just mentioned it was apparent that the characteristics were not those of fibrin. On the other hand the presence of a reducing sugar, the solubilities, and the stringy fibrous physical character led to an exploration of the possibility that the substance was nucleoprotein.

Isolation of Nucleoprotein from Purulent Exudates of Patients. The sediments from 2 cases of purulent empyema, based on mixed infections superimposed on tuberculosis, were analyzed in the following manner: The specimens, adjusted to neutral pH, were thoroughly washed with physiological saline, and then extracted with 1 M NaCl. The supernatant portion became viscid and opalescent. The addition of 6 volumes of distilled water to the NaCl extract resulted in the immediate ap-

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

† Supported by a grant to the Department of Microbiology, from the Life Insurance Medical Research Fund.

[1] Tillett, W. S., Sherry, S., and Christensen, L. R., PROC. SOC. EXP. BIOL. AND MED., 1948, **68**, 184.

pearance of a heavy fibrous precipitate, which redissolved in 1 M NaCl and reprecipitated in 0.14 M NaCl. Materials obtained after 6 reprecipitations from M NaCl extracts were shown to contain N, P, and to give a positive biuret reaction. After acid hydrolysis positive purine and Molisch tests were obtained.

A similar analysis yielding identical results has been carried out on 10 samples of pleural exudation derived from patients having pulmonary infections of different etiologies. In some instances causative bacteria, such as pneumococci, aerobic and anaerobic streptococci, and others, were present in the exudates and in other instances the specimens were sterile. It is apparent, therefore, that the material derived from the specimens was not a product of the organisms but resulted from the inflammatory reaction. Although not described in detail in this article, a substance with comparable chemical and physical characteristics has been derived from the buffy coat of human blood.

The above chemical findings which identify the substance from exudates as being a protein and containing purines, phosphorus, and reducing sugars serve to demonstrate its nucleoprotein characteristics. The physical characteristics of the nucleoprotein derived from the exudates is also of interest in connection with its significance in contributing to the total gross appearance of pus. The material which has been isolated is fibrous and stringy. Its nature and abundant yield from a purulent exudate, is shown in Fig. 1. When the pH of the solution was raised to 8-9 by the addition of 0.1 N NaOH, the fibrous portion became slimy, swollen and formed a coagulum. When the pH was raised above 10 the substance dissolved forming a viscid solution. Mirsky[2] refers to the earlier report of Hoppe-Seyler made in 1865 in which the author described the recovery of a fibrous material from pus cells but did not identify it further.

Estimations of Amount of Nucleoprotein in Samples of Purulent Exudations. All of the preliminary experiments have indicated

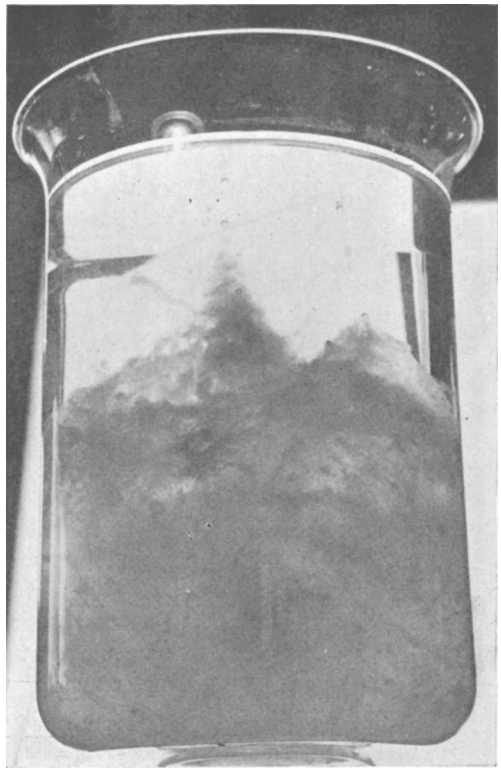


FIG. 1.

The fibrous stringy nucleoprotein derived from 25 g of purulent exudate is shown suspended in 3 liters of 0.1 M NaCl. In this instance the nucleoprotein comprised 70% of the dried solids and 50% of the nitrogen of the total sediment.

that the nucleoprotein increment of the exudates constituted a surprisingly large amount of the total sediment. Accordingly quantitative estimations were carried out on 3 specimens. Using the dry weight and N content of the total sediment and that of the isolated nucleoprotein, it was found that the latter comprised 30-70% of the total solids and contained 26-50% of the nitrogen. This finding takes on additional interest and importance in connection with the studies on the production of desoxyribonuclease by hemolytic streptococci,[1] and in our investigation on the effect of the injection of partially purified, nontoxic streptococcal concentrates into patients, a subject which will be reported in a subsequent communication.

Isolation of Nucleic Acid from Nucleoprotein Derived from Pus. Three different samples

[2] Mirsky, A. E., and Pollister, A. W., *J. Gen. Physiol.*, 1947, **30**, 117.

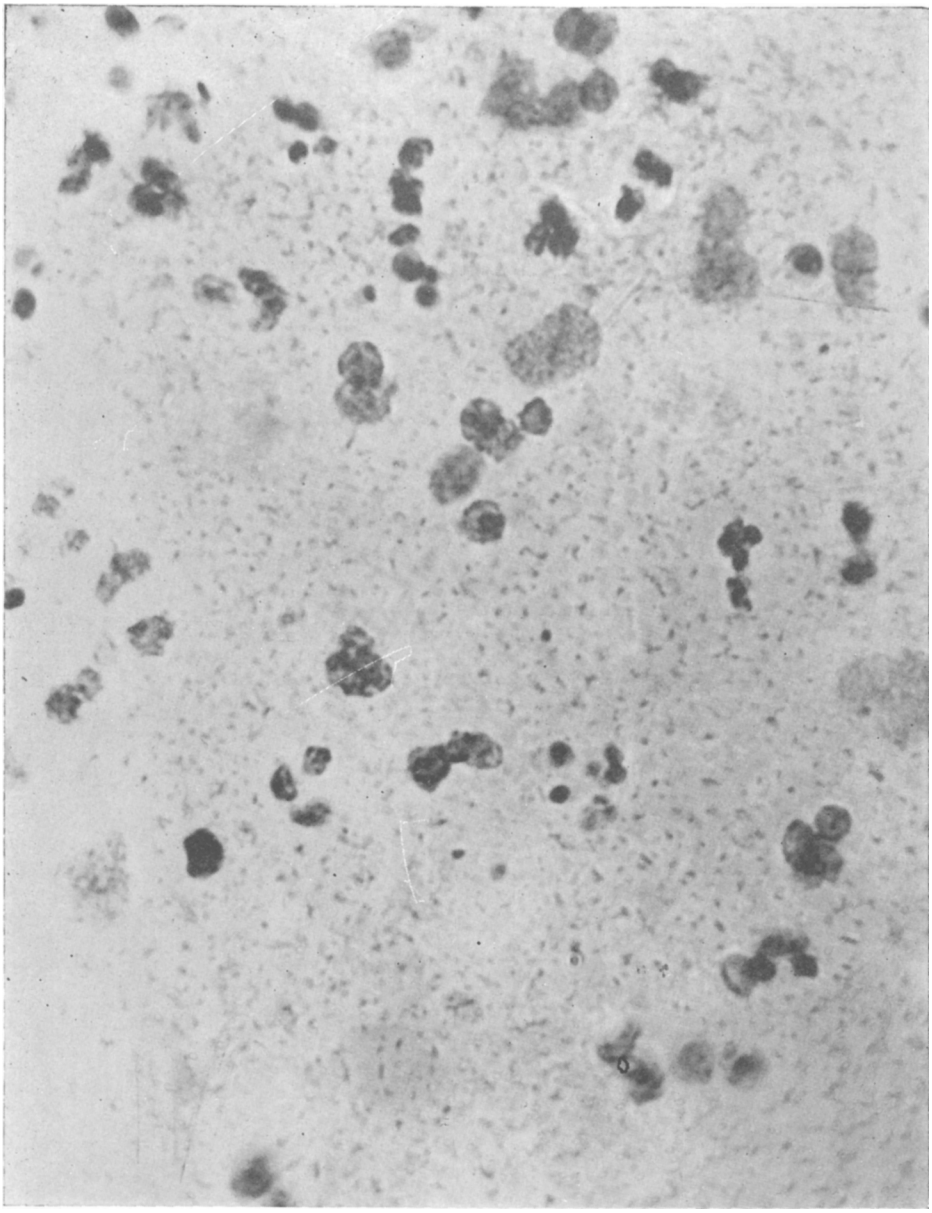


FIG. 2.
Specimen of early pleural exudate (sterile) from a patient with pneumonia. Stained by Feulgen method. 1766 × magnification. Nuclei of W.B.C., some collections of lattice work stroma of an undetermined nature, and large numbers of extracellular granules are stained.

of purulent sediment were extracted with 1 M NaCl after thorough washing with physiological saline. The viscid opalescent supernatant portions were centrifuged clear of debris, and the nucleoprotein precipitated by addition of six volumes of distilled water. After several reprecipitations, the precipitate was finally dissolved in alkalinized M NaCl, and protein removed by shaking with a chloroform-octyl alcohol mixture (4:1) followed by centrifugation. After about 8 such shakings, and when no more precipitate formed on 2

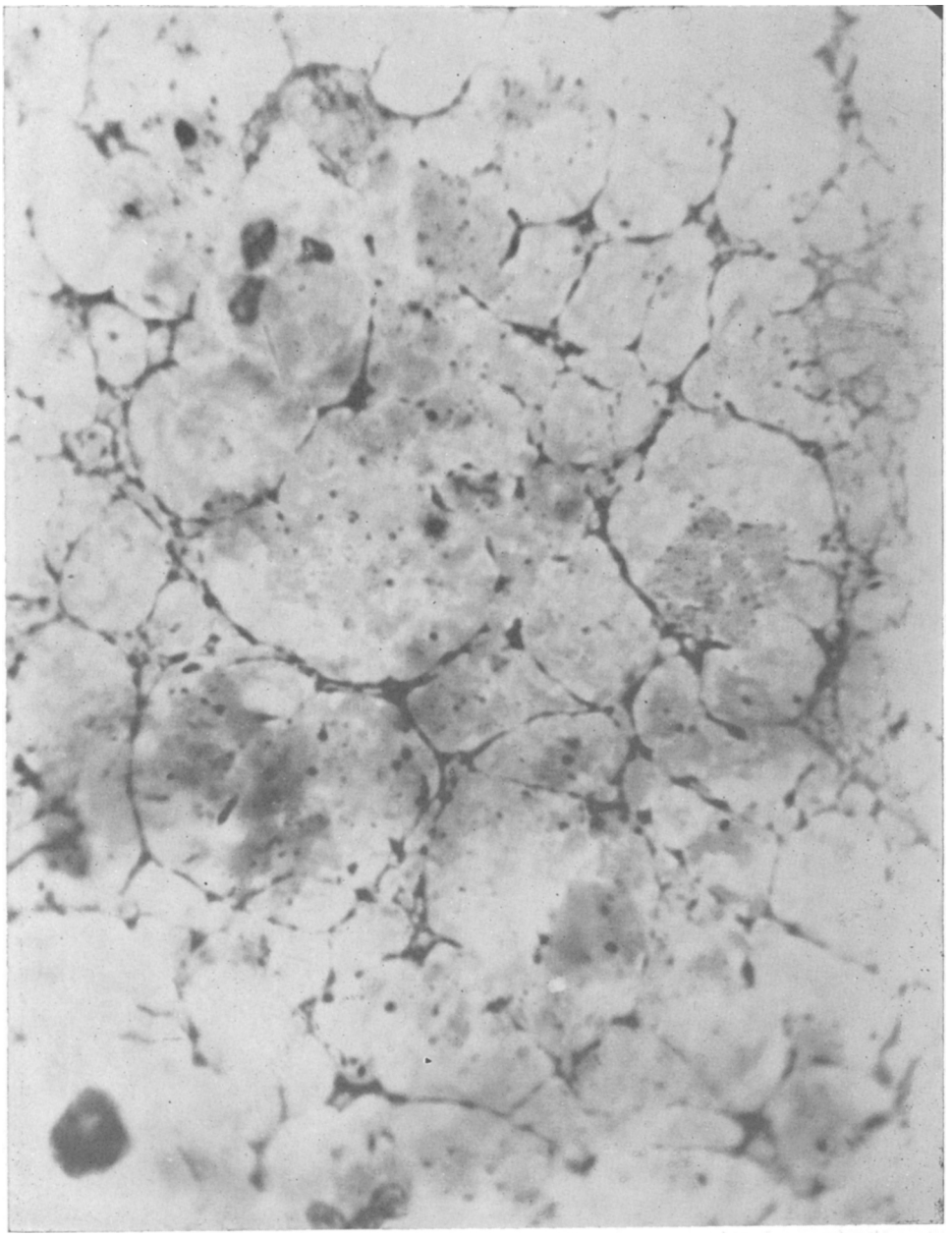
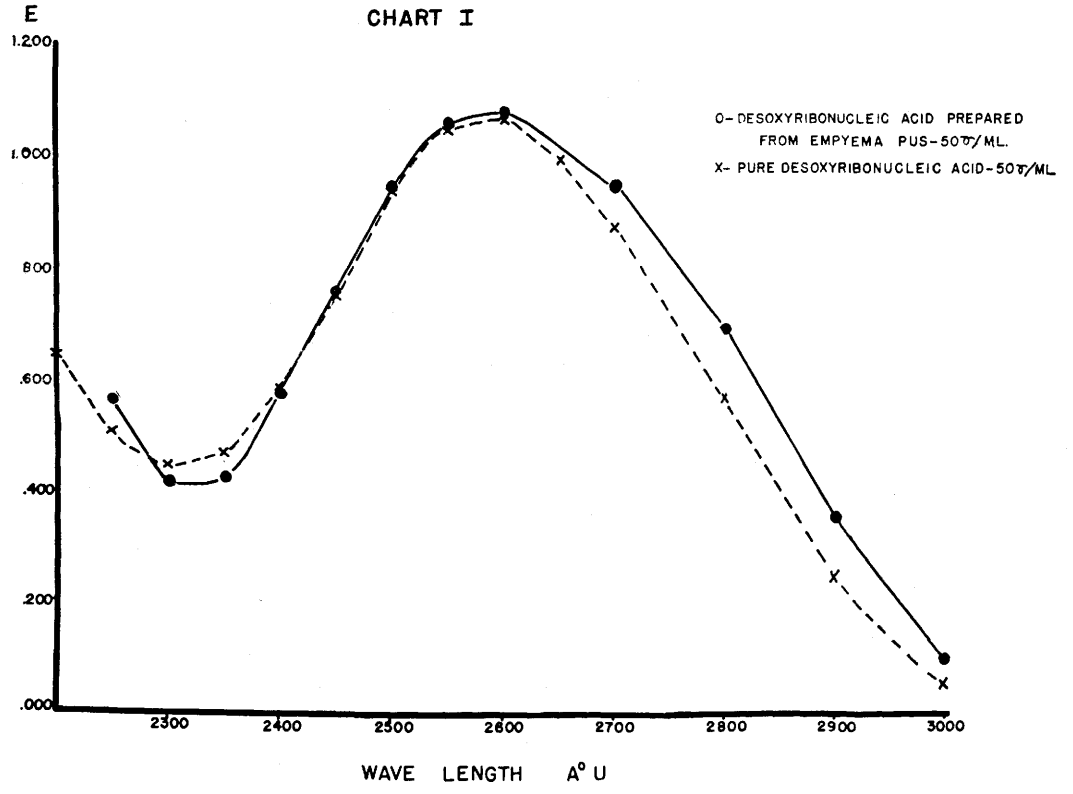


FIG. 3.

Specimen from protracted case of tuberculous empyema. Stained by the Feulgen method. 1766 × magnification. Only occasional leucocyte present. Extensive extracellular reticulum, together with some granules and areas of amorphous formation have taken up the stain.

successive treatments, the supernatant was neutralized and added to 2 vols. of alcohol. The precipitate that formed overnight was dried with ether. The powders obtained were water soluble and yielded very viscous solutions, which showed birefringence of flow indicating a high degree of polymerization. The absorption spectrum of one of the samples is shown in Fig. 2. Its absorption at 2600A° in a concentration of 50 γ/ml was



ULTRAVIOLET ABSORPTION SPECTRUM OF DESOXYRIBONUCLEIC ACID PREPARED FROM EMPYEMA PUS COMPARED WITH DATA ON PURE NUCLEIC ACID (2)

within 3% of that characteristic of pure nucleic acid.[2] The N content was 14.95%, P 8.1%, N/P ratio 1.84. It was biuret negative and gave a strongly positive Dische diphenylamine reaction.[3] Because of the methods of extraction that were successful, the results of the chemical analyses and the essential identical similarity of its properties to a highly purified preparation of sodium thymonucleate prepared by the Hammarsten method from calf thymus which was used as a control for comparison,‡ the materials were identified as a desoxyribose nucleic acid. The identity of the substance as desoxyribonucleic acid was further established by the demonstration of its depolymerization with both desoxyribonuclease and also with concentrates of hemolytic streptococcal filtrates containing desoxyribonuclease which are described in the subsequent article.[1]

For further observations on the presence of desoxyribose nucleoprotein in inflammatory exudates in large amounts, preparations have been stained by the Feulgen method and examined microscopically.

In such preparations the stained nuclei of the leucocytes have a characteristic appearance. In addition, granules in freshly formed exudates and fibrous strands in cases with long standing effusion are abundantly present in the extracellular areas. Since the Feulgen staining is considered a highly specific histochemical reaction for desoxyribose nucleoprotein,[4,5] the abundance of stained material both intra- and extracellular in the micro-

[3] Dische, Z., *Mikrochemie*, 1930, **8**, 4.

‡ Dr. Milton Levy, Department of Biochemistry, New York University College of Medicine, kindly supplied the Na thymonucleate available for this experiment.

[4] Mirsky, A. E., *Advances in Enzymology*, 1943, **3**, 1.

[5] Stowell, R. E., *Stain Technology*, 1946, **21**, 137.

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.