THE PROTECTIVE ACTION OF A SPECIFIC ENZYME AGAINST TYPE III PNEUMOCOCCUS INFECTION IN MICE

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PLATE 4

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The cultural characteristics of a bacillus capable of decomposing the capsular polysaccharide of Type III Pneumococcus were described in the preceding paper (1). The decomposition of the specific carbohydrate was shown to be due to the action of an intracellular enzyme which may be extracted in active form from the bacilli. It was pointed out that the enzyme acts only on the capsular polysaccharide of Type III pneumococci and does not affect the specific carbohydrates of certain other encapsulated bacteria. In this respect, the enzyme exhibits a selective action which is as unique in its specificity as is the serological reaction between the capsular polysaccharide and homologous antibody. Furthermore, it was shown that the breaking down of the complex sugar by the enzyme is accompanied by a loss of the serological specificity of the carbohydrate. This form of decomposition by a biological agent represents a splitting which is less drastic than that accomplished by chemical hydrolysis with acid and heat. The fact that the breaking down of the sugar molecule is, in each instance, accompanied by the loss of its immunological specificity furnishes convincing evidence that the capsular polysaccharide itself is the substance responsible for the type specificity of pneumococci.

The observations recorded in the preceding paper concerned the action of the enzyme on the chemically purified polysaccharide removed and separate from the bacterial cells. In its native state the capsular substance forms a morphological structure which conditions the antigenic and serological reactions of the cell as a whole, as well as its power to invade and multiply in the animal body. It was of special interest, therefore, to ascertain what effect this specific enzyme

would have upon the encapsulated cells growing *in vitro* and *in vivo*; whether in a medium containing the active enzyme Type III pneumococci would fail to grow, or would grow merely deprived of their capsules; whether in the body of a susceptible animal the administration of the enzyme would in any way modify the course of experimental infection with virulent Type III pneumococci. It is with these two questions that the present paper deals.

Before discussing the experimental results to be reported in this paper, it may be well at this point to mention briefly the observations of other investigators who have attempted to modify or inhibit the activity of bacteria by the use of certain enzymes.

Reference has already been made in previous papers (1–3), to the work of Toenniessen (4) who isolated in the form of galactan a gum-like material from an encapsulated strain of Friedländer's bacillus. Of special interest in this regard is his observation on the utilization of this capsular substance by *Bacillus vulgatus*, when both organisms were grown together in symbiosis. Under these conditions he observed that the Friedländer bacilli progressively lost their capsules, although the viability of the decapsulated forms was in no way impaired. This investigator attached no immunological significance to the capsular carbohydrate, nor did he attempt to apply the principle involved in this symbiotic relationship to the problems of experimental infection. Rather, Toenniessen regarded the substance of the capsule as a reserve food stuff, similar to starch in plants and glycogen in animals. From this viewpoint, however, he emphasized the significance of the capsule as a physiologically important part of the bacterial cell and showed that it may be decomposed by an unrelated species of microorganism.

In 1910 Vaudremer (5) pointed out that tuberculin, when added to filtered extracts of *Aspergillus fumigatus*, loses in great part its activity. This decomposition he attributed to the presence of various enzymes in the unheated extracts. He also showed that tubercle bacilli were modified by maceration in extracts of the fungus, and that under these conditions they became granular and less acid-fast. The injection of the bacilli, altered by growth in the fungus extracts, served to stimulate in animals an increased resistance to subsequent infection with virulent tubercle bacilli. Filtered extracts of the *Aspergillus* alone were used in the treatment of guinea pigs previously infected with tubercle bacilli. In these instances, the progress of the disease was often much retarded and the life of the treated animals prolonged. When Vaudremer had shown that unheated extracts of *Aspergillus fumigatus* were able to destroy tuberculin, to diminish the acid resistance of tubercle bacilli, and to retard infection in experimental animals, he applied the same methods to the treatment of tuberculosis in man.

That tubercle bacilli lose their acid fastness after 3 months incubation in a

culture of an unidentified mold was also observed by Machado (6) who traced this action to the activity of the fungus enzymes.

The suggestion that the lipoid substances of tubercle and lepra bacilli may be acted upon by the esterases of the tissues is found in the observations of Citron and Reichen, Peritz and others (7), who have expressed the view that an increased content of these enzymes in the serum of patients is associated with an increased resistance to infection. The relative resistance of the skin to tuberculosis, as Porter pointed out, may possibly be related to the presence of the tissue lipase, although as Sexsmith and Petersen (8) suggest this resistance cannot be due wholly to the activity of this ferment since lepra bacilli, also rich in lipoids, invade the skin. The observation by Robinovitch, Stiles, and Payne (9) that tuberculosis of the pancreas is relatively uncommon in tuberculous animals led these workers to attempt to influence the disease in experimentally infected guinea pigs by the injection of pancreatic extracts containing active lipase.

The active principle, pyocyanase, present in autolysed cultures of *B. pyocyaneus* was found by Emmerich and his associates (10) to bring about rapid dissolution of many different species of living bacteria. They considered the active agent to be an enzyme belonging to the class of nucleases. Active preparations of pyocyanase were used in the treatment of animals experimentally infected with anthrax bacilli. The protection afforded by pyocyanase was attributed to the direct action of the enzyme on the infecting bacilli.

EXPERIMENTAL

Culture.—Young, plain broth cultures of a strain of Type III Pneumococcus were used in the following experiments. The virulence of the strain, maintained by repeated mouse passage, was such that 0.00000001 cc. of culture injected intraperitoneally into white mice caused death within 36 to 72 hours. The dilutions of the culture were made in broth in such manner that the infecting dose was always contained in a constant volume of 0.5 cc.

Enzyme.—Filtered solutions of the enzyme extracted from the S III bacillus were prepared as described in the preceding paper.

Protection Tests.—White mice, 18 to 20 gm. in weight, served as test animals. All injections were made intraperitoneally and the technique commonly used in protection tests with antipneumococcus serum was employed. Unless otherwise stated, the infecting organisms and enzyme were injected simultaneously. In all instances the virulence of the culture was controlled by injecting normal mice with minimal amounts of the culture alone as shown in the protocols.

I. Action of the Enzyme on Encapsulated Type III Pneumococci in Vitro

Reference has already been made to the observations of Toenniessen (4) on the disappearance of the capsules of Friedländer bacilli when

these organisms are grown in symbiosis with *B. vulgatus*. Similar relationships have been found to exist in the case of the symbiotic growth of an encapsulated strain of Type III Pneumococcus together with the enzyme-producing bacillus. The specific enzyme elaborated by the bacilli brings about the decomposition of the capsular polysaccharide formed by the Pneumococcus. In symbiotic cultures of these two organisms, the pneumococci lose their agglutinability in type-specific serum, and the soluble specific substance can no longer be demonstrated free in the culture fluid. Obviously these results are conditioned by certain variable factors, such as the relationship between the rate of decomposition and the rate of production of capsular substance, and the maintenance of cultural conditions suitable for the physiological activity of both species of microorganisms. However, there is no difficulty in demonstrating the reaction when, instead of the living bacilli, a sterile extract of the enzyme itself is added in suitable concentration to the culture medium. Under these conditions the decomposition of the capsular polysaccharide by the enzyme can be demonstrated during growth of the pneumococci, by failure of the culture to react specifically when added to Type III antipneumococcus serum.

Experiments of this nature reveal the fact that the enzyme by itself is neither bactericidal nor bacteriolytic; that by decomposing the specific carbohydrate, the enzyme merely deprives the bacteria of their capsules without impairing the viability of the cells. It is also evident that the action of the enzyme does not result in a loss of the function of elaborating the capsular substance, since organisms so treated regain their capsule and form the specific polysaccharide when transferred to a medium free of the enzyme.

The experiments reported in the preceding paper (1) showed that an enzyme derived from an unrelated species of microorganisms acts upon the Type III capsular polysaccharide of Pneumococcus when this substance, separated from the bacterial cells, is used as the specific substrate. The experiments just described afford evidence that the enzyme also brings about the decomposition of this same capsular material in the native form in which it exists as a structural part of the living organism. Since it has been shown that the enzyme has a definite effect upon the capsular component of the cell growing *in*

vitro, the question naturally arose, whether it would exert a similar action on the encapsulated forms growing in the animal body; to seek the answer to this question the following experiments were designed.

II. The Action of the Enzyme on Encapsulated Type III Pneumococci in Vivo

Specificity of the Action of Enzyme in Mice.—To determine whether the enzyme would protect mice against infection, and to what extent the specificity exhibited by the enzyme *in vitro* would be reflected in its action in animals, three separate groups of mice were infected with Pneumococcus Types I, II, and III, each animal receiving at the same time a constant amount of the Type III specific enzyme. The experiment was carried out as follows:

TABLE I

Specificity of the Protective Action of Enzyme

Infecting dose of Pneumococcus	Enzyme (Lot 4-*a*) 0.5 cc.			No enzyme		
				Virulence controls		
	Pneumococcus Type I	Pneumococcus Type II	Pneumococcus Type III	Type I	Type II	Type III
cc.						
0.1	—	—	S	—	—	—
0.01	—	—	S	—	—	—
0.001	—	—	S	—	—	—
0.0001	D 20	D 34	S	—	—	—
0.00001	D 24	D 34	S	D 22	D 36	D 34
0.000001	D 34	D 34	S	D 34	D 36	D 34
0.0000001	—	—	—	D 34	D 20	D 72

S = survived.

D = death of animal; the numeral indicates the number of hours before death, or the time at which the animal was found dead.

— = not done.

The technique of the protection test was the same as that already described. Three mice were injected intraperitoneally with 10^{-4}, 10^{-5}, and 10^{-6} cc. of a broth culture of Type I Pneumococcus; three other mice received the same amounts of a culture of Type II; six mice were given much larger doses of a culture of Type III pneumococci ranging in amounts from 10^{-1} to 10^{-6} cc. Simultaneously with the bacteria each mouse of all three groups received 0.5 cc. of the same preparation of

enzyme (Lot 4-*a*). The virulence of the three types of pneumococci was controlled in each instance by the infection of normal mice with 10^{-5}, 10^{-6}, and 10^{-7} cc. of the respective culture alone.

The results of this experiment are recorded in Table I. The evidence clearly shows that the enzyme afforded mice protection against 1 million times the fatal dose of a virulent culture of Type III Pneumococcus. It is further apparent that the protective action is type-specific, since mice receiving the same enzyme but infected with amounts as small as 0.000001 cc. of a culture of a heterologous type promptly succumbed to infection. Just as in the test tube, the enzyme acts only on the Type III polysaccharide, so in the animal body it is effective only against infection with Type III Pneumococcus.

Heat Inactivation of the Enzyme.—Experiments previously reported (1) showed that the activity of the enzyme, as measured by the decomposition of the free carbohydrate, was destroyed by exposure to a temperature of 60°C. for 10 minutes. In the following experiment, an active preparation of the enzyme was heated, at a temperature known to destroy completely its action in the test tube, in order to determine the effect of heat upon the protective power of enzyme in mice.

5 cc. of enzyme preparation (Lot 4-*a*) were heated in a water bath at 70°C. for 10 minutes. Each of three mice was injected intraperitoneally with 0.5 cc. of the *heated* enzyme together with a culture of Type III Pneumococcus in amounts of 10^{-4}, 10^{-5}, and 10^{-6} cc. respectively. Each of five other mice received similar amounts of the same preparation of the *unheated* enzyme simultaneously with an amount of the cultures varying from 10^{-1} to 10^{-5} cc., respectively. Three mice infected with culture alone in doses of 10^{-5}, 10^{-6}, 10^{-7} cc. were used as virulence controls.

The results of this experiment show that the active principle responsible for the protection of mice against infection is destroyed by exposure to 70°C. for 10 minutes. Mice receiving the *heated* enzyme succumbed to infection with the smallest dose of culture used, 0.000001 cc. On the other hand, mice injected with an equal amount of the same enzyme *unheated* survived as much as 0.1 cc. of the same culture of which 0.0000001 cc. alone proved fatal for the untreated controls. The inactivation by heat of the protective power of the enzyme in the animal body, parallels the loss of its activity *in vitro* after exposure to

temperatures of 60°C. or higher. The fact that heat destroys the activity of the enzyme both *in vitro* and *in vivo*, supports the assumption that the same principle is involved in the mechanism of both reactions.

Relation between the Concentration and Protective Action of Enzyme.— Repeated tests have demonstrated that within the limits of the reaction capacity of the mouse, the protective action of the enzyme is a function of the concentration of the active principle in any given preparation. It is also apparent that the protection afforded by different

TABLE II

Inactivation of Enzyme by Heat

Pneumococcus Type III	Enzyme (Lot 4-*a*) 0.5 cc.		No enzyme
	Unheated	Heated at 70°C. 10 min.	Virulence control
cc.			
0.1	Survived	—	
0.01	Survived	—	
0.001	Survived	—	
0.0001	Survived	D 34	
0.00001	Survived	D 72	D 34
0.000001	—	D 72	D 34
0.0000001*	—	—	D 72

D = death of animal; the numeral indicates the number of hours before death.
— = not done.
* The inoculum at this dilution of culture (10^{-7} cc.) yielded 13 colonies on growth in blood agar.

preparations of the enzyme bears a relation to their capacity to decompose a known quantity of the capsular polysaccharide *in vitro*.

The comparative protective action in mice of two separate preparations, one of which was much less active *in vitro* is shown in Table III.

From Table III it is evident that Preparation 3, which was much less active than Preparation 4-*a* in decomposing the polysaccharide in the test tube, is also the less effective in the animal body. Although the results obtained by the two methods of titration are not wholly comparable, they indicate the existence of a correlation between the *in vitro* and *in vivo* activity of the enzyme. The comparison of these two

different preparations also suggests that the concentration of the enzyme in Preparation 3 is close to the minimum threshold value below which the enzyme ceases to function in protecting against even minimal infecting doses. From this and other similar experiments, the impression is gained that to be effective in animal protection, the concentration of enzyme in the body must be in excess so that the rate of decomposition is greater than the rate of production of the capsular substance by the living bacteria. The evidence also indicates that the greater the activity of the enzyme *in vitro* the greater is its protective

TABLE III

Protective Action of Enzyme against Infection with Pneumococcus Type III

Difference in protection titre of two preparations of enzyme which show marked differences in their activity *in vitro*.

Pneumococcus Type III	Enzyme 0.5 cc.		No enzyme
	Lot 3	Lot 4-*a*	Virulence controls
cc.			
0.1	—	S	
0.01	D 45	S	
0.001	D 72	S	
0.0001	D 72	S	
0.00001	S 6 days	S	D 34
0.000001	S	S	D 34
0.0000001	—	—	D 72

S = survival of animal.
D = death of animal; the numeral indicates the number of hours before death.
— = not done.

action in mice. Both of these deductions, however, suffer the limitations imposed by the variations which occur in the cellular response of the host, as will be pointed out later in discussing the importance of phagocytosis in the protective reaction.

Titration of Protective Action.—The concentration of enzyme in any given preparation as measured by the protection test in mice may be ascertained in two ways; (1) by determining the protective action of varying amounts of enzyme against a fixed quantity of culture, and (2) by determining the maximum amount of culture against which a constant quantity of the enzyme will protect. The results of experi-

ments of this nature are given in Tables IV and V. Although in the present instance both methods of titration were not carried out on the same preparation, the examples given illustrate the general principle.

Table IV shows that 0.1 cc. of enzyme preparation protected mice against 0.01 cc. of a virulent culture, an amount 1 million times greater than the minimal dose fatal for the normal controls.

TABLE IV

Titration of Protective Action of Enzyme against Infection with Pneumococcus Type III

1. Varying amounts of enzyme and constant amount of culture.

Enzyme (Lot 7)	Pneumococcus Type III (1 million fatal doses)		Virulence controls
Amount	Amount	Result	
cc.	*cc.*		
1.0	0.01	S	
1.0	0.01	S	
0.5	0.01	S	
0.5	0.01	S	
0.25	0.01	S	
0.25	0.01	S	
0.1	0.01	S	
0.1	0.01	S	
0	0.000001 cc.		D 45
0	0.0000001 cc.*		D 35
0	0.00000001 cc.†		D 46

S = survival of animal. Observation period 10 days.

D = death of animal; the numeral indicates the number of hours before death of animal.

* Inoculum of this amount of culture (10^{-7} cc.) yielded 30 colonies in blood agar.

† Inoculum of this amount of culture (10^{-8} cc.) yielded 1 colony in blood agar.

The results presented in Table V illustrate the maximum amount of culture against which 1 cc. of enzyme preparation (4-*a*) protected mice. Under the experimental conditions, this particular preparation protected against infection with 0.2 cc. of culture, an amount which represents 2 million fatal doses. Death was delayed in mice infected with 0.3 and 0.4 cc. of culture, the animals surviving as long as 4 days. Large infecting doses of this order however apparently represent the

upper limit against which a single dose of the enzyme fails to protect. Whether repeated injections of enzyme during the course of the infection, or whether the perfecting of methods for concentrating and purifying the enzyme will enhance its protective action, cannot now be stated. Moreover the maintenance of an effective concentration of enzyme in the body during the course of infection appears necessary to achieve the maximum of protection.

In order to ascertain how long the enzyme remains active after injection into normal mice, the following experiment was carried out.

TABLE V

Titration of Protective Action of Enzyme, against Infection with Pneumococcus Type III

2. Constant amount of enzyme and varying amounts of culture.

Pneumococcus Type III	Enzyme (Lot 4-*a*)	
	Amount	Result
cc.	*cc.*	
0.5	1.0	D 18
0.4	1.0	D 4 days
0.3	1.0	D 4 days
0.2	1.0	S
0.1	1.0	S
0.01	1.0	S
0.000001	0	D 25
0.0000001	0	D 42

D = death of animal; numeral indicates number of hours before death.
S = survival of animal.

Mice were injected intraperitoneally with 1 cc. of a sterile solution of enzyme. At intervals thereafter varying from 20 to 43 hours, the mice were infected by the intraperitoneal injection of varying amounts of a virulent culture of Type III Pneumococcus.

The results given in Tables VI and VII show that there is a gradual diminution in the protective action of the enzyme after its injection into the animal body. To determine more accurately the length of time during which the enzyme remains active in normal animals will require further detailed study. From the evidence available at present, it seems not unlikely that the occasional death of an infected

TABLE VI

Protective Action of Enzyme against Infection with Pneumococcus Type III

Action of enzyme given 24 hours before and simultaneously with the infecting dose of culture.

Pneumococcus Type III	Enzyme (Lot 7) 1 cc.		No enzyme
	Simultaneously	24 hrs. before	Virulence controls
cc.			
0.2	S	D 6 days	
0.1	S	S	
0.01	S	S	
0.001	S	S	
0.0001	S	S	
0.000001	—	—	D 45
0.0000001	—	—	D 35
0.00000001	—	—	D 45

D = death of animal; the numeral indicates the number of hours before death.
S = survival of animal—observation period 10 days.
— = not done.

TABLE VII

Protective Action of Enzyme Given before Infection with Pneumococcus Type III

Action of enzyme given 20 to 43 hours before infection.

Pneumococcus Type III	Enzyme (Lot 6) 1 cc.	Enzyme (Lot 4-*a*) 1 cc. given		Virulence controls
	20 hrs. before infection	24 hrs. before infection	43 hrs. before infection	Culture alone
cc.				
0.1	S	D 24	D 48	—
0.01	S	S	S	—
0.001	S	S	S	—
0.0001	S	S	D 8 days	—
0.00001	S	S	S	—
0.000001	S	S	S	D 46
0.0000001	—	—	—	D 46
0.00000001	—	—	—	D 46

S = survival of animal.
D = death of animal; the numeral indicates the number of hours before death.
— = not done.

animal several days after the administration of a single dose of the enzyme (Table VII) may be attributable to the loss or inactivation of the active agent during the course of the infection, thereby allowing a few organisms to escape and reestablish themselves in the absence of an effective concentration of the protective principle.

Curative Action of the Enzyme in Mice.—The preceding experiments were designed to demonstrate the protective action of the enzyme when administered simultaneously with the infecting microorganisms. In a few experiments the enzyme was given several hours before the bacteria were injected, in order to ascertain the duration of the activity of enzyme in the animal body. The degree of protection afforded under these circumstances is noteworthy, when the susceptibility of mice to pneumococcus infection, and the virulence of the strain employed are taken into consideration. It seemed of even greater interest, however, to determine whether the enzyme would have any effect when injected into mice that had been previously infected with a fatal dose of culture. The following experiment illustrates the so called curative action of the enzyme in the presence of an infection already established at the time of treatment.

Nine normal mice were infected with 10^{-6} cc. of broth culture of a virulent strain of Pneumococcus Type III. 12 hours later three of these animals were given an intraperitoneal injection of 1 cc. of enzyme (Preparation 4-*a*). Three other mice of the infected group were treated with the same amount of enzyme 18 hours after the infecting organisms had been given. The remaining three mice received no enzyme and served as untreated controls. As further controls of virulence two normal mice were injected with the culture alone in amounts of 10^{-7} and 10^{-8} cc. respectively. To estimate the number of organisms, these inocula were plated in blood agar and the number of colonies developing were counted. 10^{-7} cc. of culture yielded twenty-five colonies in the poured plates, and 10^{-8} cc., three colonies.

The results of this experiment on the curative action of the enzyme (Table VIII) show that mice receiving a single injection of the enzyme 18 hours after the onset of infection, recovered, whereas the untreated controls all died. Under the experimental conditions, the survival of the treated animals represents recovery from an infecting dose of culture 100 times greater than that fatal for untreated mice. While the conditions of this test were not the most severe, the outcome indi-

cates that the enzyme is effective when administered early in the course of an infection which otherwise invariably proves fatal. From other experiments, evidence has been gained that the administration of the enzyme several hours after infection with larger amounts of culture may favorably influence the course of the infection in mice.

Mechanism of the Protective Action.—It was thought possible that some idea of the mechanism involved in the protective action of the enzyme in infected mice might be gained by following the course of the bacteremia by means of blood cultures, and by a study of the

TABLE VIII

Curative Action of Enzyme in Mice Infected with Pneumococcus Type III

Pneumococcus Type III (100 fatal doses)	Enzyme 1 cc. (Lot 4-*a*) given		Controls
	12 hrs. after infection	18 hrs. after infection	No enzyme
cc.			
0.000001	S	S	D 35
0.000001	S	S	D 59
0.000001	S	S	D 59
0.0000001*	—	—	D 84
0.000000001†	—	—	D 60

D = death of animal; the numeral indicates the number of hours before death.
S = survival of animal.
* = Inoculum of this dilution of culture (10^{-7} cc.) gave 25 colonies in blood agar.
† Inoculum of this dilution of culture (10^{-8} cc.) gave 3 colonies in blood agar.

cellular reactions in the peritoneal exudates of treated and untreated animals. A typical experiment follows:

Twelve mice were injected intraperitoneally with 10^{-2} cc. of a culture of Pneumococcus Type III, the virulence of which was such that 10^{-8} cc. caused death in from 36 to 72 hours. Six of the mice also received at the time of infection 0.5 cc. of an active preparation of the enzyme. The other six infected animals served as untreated controls. At hourly intervals following infection, one mouse of each group was sacrificed and autopsied. Cultures of the heart's blood were made in broth and on blood agar plates. Films of the peritoneal exudates were stained by the Gram method, and examined to compare differences in the morphology of the organism and in the occurrence of phagacytosis in the treated and untreated animals.

The results of blood cultures taken during the course of the infection show that the enzyme has a distinct effect in checking the bacteremia which invariably occurs in infected mice. In both the treated and untreated animals pneumococci are present in the blood stream within the 1st hour following infection. However, the subsequent course of events, with reference to the persistence of bacteremia, differs in the two series of animals. For, while the bacteria progressively increase in the blood of the control mice until death, they invariably diminish in numbers in the circulation of the treated animals until by the 4th or 5th hour they can no longer be demonstrated by culture. The sterilization of the blood under these conditions is objective evidence of the protective action of the enzyme.

The fate of the pneumococci under the influence of the enzyme is strikingly revealed by microscopic study of the peritoneal exudate during the course of the infection. Without giving in detail the results of the serial examinations at hourly intervals, the progress of events is shown in the accompanying photomicrographs which illustrate the differences in the cellular reactions of treated and untreated mice 2 and 4 hours after the injection of 1 million fatal doses of Pneumococcus Type III. Two hours after infection the peritoneal exudate of the untreated control mouse (Fig. 1) shows numerous well encapsulated cocci free in the fluid. In contrast to this, the pneumococci in the exudate of the enzyme-treated animal at this time (Fig. 2) are devoid of capsules and only the naked bacteria are visible, many of which are already engulfed by the polynuclear leucocytes. At the end of 4 hours, the number of encapsulated pneumococci have increased in the peritoneum of the untreated control (Fig. 3), there is no evidence of phagocytosis, and cultures of the heart blood indicate a progressively increasing bacteremia. In the treated mouse at the end of 4 hours (Fig. 4) only an occasional unencapsulated organism is seen outside the accumulated leucocytes and frequently at this time and almost invariably by the 5th hour, pneumococci are no longer demonstrable by blood cultures. These findings have been repeatedly confirmed in a series of similar experiments and they support the view that the protective action of the enzyme lies in its capacity to decompose the capsular polysaccharide of Type III Pneumococcus.

DISCUSSION

The present study emphasizes the importance of the capsule in the biological reactions of the pneumococcus. It is, indeed, a significant fact, that no matter whether one regards this organism from the viewpoint of type specificity, antigenicity, or its capacity to undergo variation, or whether, as in the present instance, one considers the pneumococcus with reference to its virulence and fate in the animal body, the one dominant factor influencing all these phenomena is the function of the cell to elaborate the specific capsular polysaccharide. These relationships, however, are not to be interpreted as meaning that virulence is dependent merely upon differences in the structural morphology of the bacterial cell. For it is a common observation that an encapsulated strain of Pneumococcus may be virulent for one species and not for another. However, it is equally true that the function of elaborating the specific capsular polysaccharide is most highly developed in pneumococci that are best adapted to growth in the animal body. From this point of view, virulence and capsule formation, although not causally related, are at least intimately associated. When the function of forming the capsular substance is suppressed or inhibited, as in the case of the R variants, or when, as in the present instance, although this function is unimpaired the capsule itself is destroyed by an enzyme, the naked bacteria are thereby exposed directly to attack by the phagocytes of the host.

In this sense, the action of the enzyme may be said to result in preparing the encapsulated bacteria for phagocytosis; not, as in the case of antibodies, by specific sensitization, but by the process of decapsulation. In the former instance, the reaction is an immunological one, whereby the capsular material is altered by union with the type-specific antibody; in the latter case, the reaction is a chemical one in which the capsular polysaccharide is actually decomposed by the enzyme. Although the mode of action of both these specific agents is different in each instance, the end result, so far as the fate of the microorganism is concerned, is the same in both cases.

It is of interest that although neither the enzyme nor the specific antibody is by itself bactericidal or bacteriolytic, yet each by reacting specifically with the capsular substance exposes the virulent organisms to the phagocytic action of the body tissues. The enzyme, like the

specific antibody, serves merely to initiate the protective reaction, the completion of which is ultimately dependent for its successful issue upon the effective cellular response of the host.

The present study also suggests that the capsule—long recognized as a defense mechanism on the part of virulent bacteria—is a decisive factor in determining the fate of pneumococci in the animal body, and that this structure is vulnerable to attack by specific agents other than antibodies.

SUMMARY

The bacterial enzyme which decomposes the purified capsular polysaccharide of Type III Pneumococcus *in vitro* also destroys the capsules of the living organisms growing in media and in the animal body.

Potent preparations of this same enzyme protect mice against infection with virulent Type III Pneumococcus. The protective action is type-specific.

The protective activity of the specific enzyme is destroyed by heat (70°C. for 10 minutes).

The enzyme remains in an effective concentration 24 to 48 hours after its injection into normal mice.

The enzyme has been found to exert a favorable influence on the outcome of an infection already established at the time of treatment.

A definite relationship has been found to exist between the activity of the enzyme *in vitro* and its protective power in the animal body.

The mechanism of the protective action is discussed with special reference to the relation between the decapsulation of the bacteria by the enzyme and the phagocytic response of the host.

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EXPLANATION OF PLATE 4

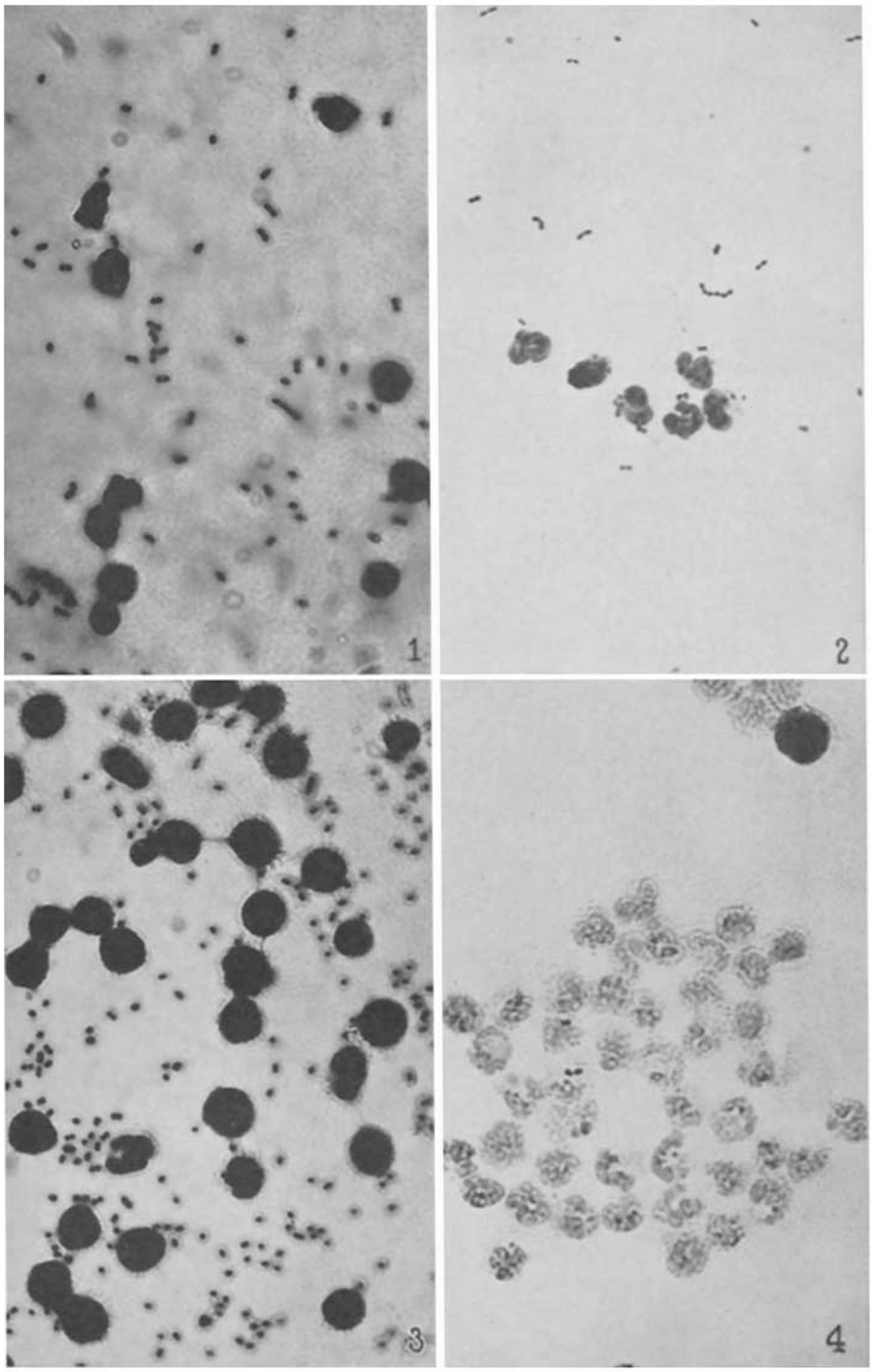
FIG. 1. Photomicrograph of a stained preparation of the peritoneal exudate of a mouse 2 hours after the intraperitoneal injection of 0.01 cc. of a virulent culture of Type III Pneumococcus. The bacteria show well defined capsules and no evidence of phagocytosis is seen. Many polymorphonuclear and a moderate number of mononuclear leucocytes are present. Gram stain, ×1000.

FIG. 2. Photomicrograph of a corresponding preparation of the exudate of a mouse 2 hours after receiving the same amount of culture together with 0.5 cc. of a preparation of the specific enzyme. The bacteria are devoid of capsules. Polymorphonuclear leucocytes predominate and phagocytosis is evident. Gram stain, ×1000.

FIG. 3. Photomicrograph of a stained film of the peritoneal exudate of a mouse 4 hours after injection with 0.01 cc. of culture alone. The bacteria are increased in number, encapsulated, and extracellular. The cellular elements are polymorphonuclear and mononuclear leucocytes in about equal numbers. Gram stain, ×1000.

FIG. 4. Photomicrograph of a corresponding preparation of the exudate of a mouse 4 hours after receiving the same amount of culture together with 0.5 cc. of a preparation of the specific enzyme. Marked phagocytosis has occurred and only an occasional organism is seen outside the accumulated leucocytes, nearly all of which are of the polymorphonuclear type. Gram stain, ×1000.

Differences in the density of the backgrounds of the four figures are due to the use of color screens in the photographic reproductions. This technique, however, alters none of the essential details observed in the original microscopic preparations.



Photographed by Louis Schmidt

(Avery and Dubos: Enzyme against Type III Pneumococcus)