THE EFFECT OF PNEUMOCOCCUS AUTOLYSATES UPON PNEUMOCOCCUS DERMAL INFECTION IN THE RABBIT

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(Received for publication, March 14, 1933)

The injection of virulent pneumococci into the skin of a rabbit's flank gives rise to an edematous lesion which spreads ventrally until the entire midabdominal area of the skin is involved (1). Previous studies (2) have shown that the edema fluid may be drained from this lesion, and that, if taken during the earlier phases of the infection, this fluid not only fails to clot but has the property of retarding the coagulation of normal rabbit blood. The origin of the antithrombic property of this edema fluid was not definitely proven but it was shown that pneumococcus autolysates also possessed a similar property and the suggestion was made that the bacteria might be the source of the anticoagulant substance in the edema fluid. It is not unreasonable to suspect that some of the less well protected microorganisms may be damaged in the earlier phases of the pneumococcus infection, and that the resulting death and dissolution of these less resistant bacterial cells may take place with the liberation of autolytic products which, by retarding coagulation and altering vascular permeability, may promote the invasion of the surviving organisms.

In a continuation of the study of this problem, the experiments to be reported in this paper were undertaken to determine whether bacterial autolysates might promote the invasiveness or enhance the virulence of pneumococci. Although in the course of these experiments a variety of different strains of pneumococci of various specific types have been studied, only the results obtained with Pneumococcus Type III are included in the present paper, since strains of this type exhibiting different degrees of virulence for rabbits offer excellent opportunity for the study of infections under selective and experimentally reproducible conditions.
**PNEUMOCOCCUS DERMAL INFECTION**

**EXPERIMENTAL**

*Cultures.*—The strains used for infection were as follows:

Pneumococcus Type III (Strain PH), virulent for both mice and rabbits.

Pneumococcus Type III (Strain A66), highly virulent for mice but never leading to the death of rabbits when injected intravenously or intradermally.

Pneumococcus (Strain M 3 R), rough organism, derived from a strain of Type III Pneumococcus. Avirulent for both mice and rabbits.

*Autolysates.*—The bacteria from 18 hour broth cultures were collected by centrifugation and suspended in amounts of saline equivalent to 1 per cent of the original culture volume. The bacterial suspensions were quickly frozen and thawed eight times and then allowed to stand in the ice box for 1 week. A suitable preparation of autolyzed Pneumococcus produces purpura in mice when injected intraperitoneally in 0.5 cc. amounts, and, when added to freshly drawn rabbit blood, causes a marked prolongation of clotting time. Before injection, all bacterial autolysates were heated at 70°C. for 15 minutes to insure the death of all viable pneumococci.

*Infections.*—Rabbits were infected intradermally by the method previously described (1). Varying amounts of sterile autolysate and living culture were mixed and immediately injected into the skin of the flank area of rabbits.

The use of the dermal method of infection permits a thorough observation of the progress of the infective process and allows the differentiation of what is commonly called virulence into (a) lethal capacity and (b) invasiveness, the latter meaning capacity to provoke infection, as indicated by the production of a local lesion, and the stimulation of non-fatal systemic reactions. Ordinarily in experimental work, it is customary to refer to virulence as the ability of the microorganism to bring about the death of the infected animal, but a strict application of the term virulence must also include the capacity of an organism to invade the tissues without causing death, since there are obviously many organisms capable of inciting a disease which under ordinary conditions is seldom if ever fatal.

**Enhancement of Virulence of a Virulent Strain**

Experiments were designed to determine whether pneumococcus autolysates enhance the virulence of a strain which when injected alone may produce disease and death in rabbits. The strain of Type III Pneumococcus designated PH usually produces a fatal dermal infection in rabbits when injected in amounts as small as 0.00001 cc. of broth culture, but never causes death when less than this amount of culture is used. Varying amounts of this culture together with fixed quantities of pneumococcus autolysates derived from the same or other strains were injected intradermally in the flank areas of rabbits.
Table I shows the results of such an experiment in terms of survival
and death of the infected rabbits.

In this instance, the culture alone brought about death of the
rabbit when 0.000, 1 cc. was injected. Animals receiving amounts of
culture less than this survived. On the other hand, when mixed
with 0.2 cc. of an autolysate of the homologous strain, 1/10,000 the
lethal number of the same organism sufficed to induce a fatal infec-
tion. Colony counts showed that the number of pneumococci con-
tained in 0.000,000,01 cc. of the culture was less than 10. Thus the

<table>
<thead>
<tr>
<th>Amount of broth culture</th>
<th>Materials added to pneumococcus culture</th>
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|                         | 0.2 cc. of saline (control) | 0.2 cc. of autolysate homolo-
gous strain (PH) | 0.2 cc. of heterologous auto-
ylate (Pneumocc-
coccus Type I) | 0.2 cc. of autolysate of an R strain of Pneumococcus
derived from Type III |
| 0.000,000,01 | - | D | S | S |
| 0.000,000,1 | - | D | D | D |
| 0.000,001 | S | D | D | D |
| 0.000,01 | S | - | - | - |
| 0.000,1 | D | - | - | - |

S = survival.
D = death.
- = not done.

virulence has been so enhanced that whereas with the culture alone
approximately 10,000 pneumococci were required to bring about a
fatal infection, less than 10 organisms caused death when injected
together with autolysate.

Experiments, not shown in the table, demonstrate that autolysate,
when injected alone, causes only a mild reaction and is incapable of
bringing about infection or death in rabbits.

Another group of rabbits in this series received varying amounts
of the same culture of Type III pneumococci, together with heterol-
ogous autolysate prepared from organisms of Type I. Under these conditions, as expressed in terms of initial number of infecting organisms, the virulence was also markedly enhanced although not to the same degree as that occurring when the homologous autolysate was used. Whether this slight variation is within the limits of experimental error or represents an actual difference in the action of the two autolysates is not entirely clear, but it is apparent that a marked, if not equal degree of enhancement of virulence can be obtained by the use of an autolysate of Pneumococcus of a type different from that causing the infection.

In a fourth series of animals included in the same experiment the injections were similar except that the autolysate was prepared from a rough strain of Pneumococcus which was entirely avirulent. Here again, results of the same type were obtained. The fact that this particular autolysate was prepared from a culture of R pneumococci seems to indicate that the presence of the soluble specific substance in the autolysate is not essential for enhancing virulence and further, that the factor responsible for this property may be quite independent of those factors upon which virulence ordinarily depends.

These results indicate that pneumococcus autolysates can enhance the virulence of a strain of Pneumococcus which is already somewhat virulent for rabbits, since without the autolysate, 10,000 or more bacterial cells were necessary to bring about fatal disease, whereas when the autolysate was added, less than 10 organisms caused an infection which terminated fatally. Furthermore, these results show that the enhancing factor is not type-specific and that it may be derived equally well from the entirely avirulent, rough strains of pneumococci.

**Enhancement of Invasive Properties of a Strain Incapable of Inducing Fatal Infection in Rabbits**

The A66 strain of Type III Pneumococcus used in these experiments under ordinary conditions does not bring about death in rabbits when injected intravenously or intradermally in large amounts. On the other hand, it possesses marked virulence for mice, for in these animals amounts as small as 0.000,000,1 cc. of culture invariably prove fatal. This provided the means for determining whether the addition
of autolysates, and particularly autolysates from rabbit-virulent strains, might lead to an enhancement of lethal virulence of a strain ordinarily considered to be non-virulent for rabbits.

Preliminary experiments indicated that this was not the case for even when injected together with autolysates prepared from highly virulent pneumococci this strain remained incapable of causing fatal infection. However, the addition of the autolysate did lead to greater invasiveness in the sense that under these conditions fewer organisms

| TABLE II |
| Effect of Autolysate on the Infective Power of a Strain of Pneumococcus Which Is Non-Lethal for Rabbits |

Culture: Pneumococcus Type III, Strain A66. Does not kill rabbits when given intradermally. Autolysate and culture mixed at time of injection. All injections intradermal.

<table>
<thead>
<tr>
<th>Amount of broth culture</th>
<th>Materials added to pneumococcus culture</th>
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<tbody>
<tr>
<td>cc.</td>
<td>0.2 cc. of saline</td>
</tr>
<tr>
<td>0.000,000,1</td>
<td>+</td>
</tr>
<tr>
<td>0.000,001</td>
<td>+</td>
</tr>
<tr>
<td>0.000,01</td>
<td>+</td>
</tr>
<tr>
<td>0.000,1</td>
<td>+</td>
</tr>
<tr>
<td>0.001</td>
<td>+++</td>
</tr>
<tr>
<td>0.01</td>
<td>+++</td>
</tr>
<tr>
<td>0.1</td>
<td>+++</td>
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</tbody>
</table>

+ = local area of inflammation.
+++ = area of inflammation extending to the ventral midline.
++++ = widespread area of inflammation over midabdominal area.

were required to produce a widespread area of inflammation. An experiment illustrating this effect is shown in Table II.

In no instance did the addition of the autolysate to the non-virulent strain bring about the death of the animal. However, a definite effect was observed in the ability of the autolysate to enhance the spread of the lesion. The results are expressed in terms of the size of the lesion induced by the organisms alone, contrasted with those provoked by the same organisms in the presence of autolysate. It
will be noted that when pneumococci were injected alone, an amount of culture as great as 0.1 cc., representing about 100,000,000 bacterial cells, was capable of causing a lesion which spread just to the ventral midline. When a smaller amount, such as 0.000,1 cc. was used, only a circumscribed local area of inflammation was produced. On the other hand, when mixed with autolysate an amount of culture as small as 0.000,001 cc. produced a lesion considerably greater in extent than that resulting from the injection of 0.1 cc. of the culture alone.

The results of this experiment show that, while the invasiveness of a non-virulent organism may be enhanced by the addition of the autolysate of a virulent strain of Pneumococcus, the essential virulence of the organism, in the sense of lethal capacity, is not enhanced in any way.

**Effect of Autolysates on the Infectivity of R Pneumococci**

The intradermal injection of rough strains of pneumococci in relatively large amounts produces only a local area of inflammation. When autolysates of virulent or non-virulent pneumococci are injected together with this living rough culture, the area of involvement is invariably larger, but never of great consequence. There is little elevation of body temperature and the inflammatory reaction at the site of inoculation is transient.

The experimental evidence obtained under these conditions shows that the invasiveness of the non-virulent rough forms of pneumococci is not appreciably altered in the presence of autolysates which possess the property of enhancing the infectivity of potentially virulent strains of "S" pneumococci.

**DISCUSSION**

The results of this study show that pneumococcus autolysates have the property of enhancing the potential virulence which a particular strain of pneumococcus may possess. This enhancement is entirely quantitative since there is no qualitative change in the nature of the virulence possessed by the organism. The autolysates seem merely to permit fewer pneumococci to produce the same results as those ordinarily brought about by a much greater number of micro-organisms.
The nature of the active substance in the autolysate is not known. Autolysates active in this respect also produce purpura in mice and when added to freshly drawn rabbit blood have the property of inhibiting coagulation. That the virulence-enhancing effect is not type-specific is shown by the fact that an autolysate from organisms of a heterologous type is almost equally effective. Furthermore, an autolysate prepared from a culture of R pneumococci possessed the same power. The result, therefore, is apparently not dependent upon the presence of specific capsular polysaccharide in the autolysate. From the evidence thus far available, it seems not unlikely that the enhancing effect of the autolysate may be due to the presence of a product of protein degradation.

These experiments do not prove, but they lend some weight to the probability that under natural conditions pneumococcus infection may be promoted by the autolysis which some of the infecting organisms undergo. Thus for the rabbit-virulent Type III Pneumococcus it seems likely that the death and autolysis of the less resistant microorganisms may enhance the invasiveness of the few which survive. The relative ease with which pneumococci undergo autolysis suggests the possibility that this mechanism is more likely to be operative in infection with pneumococci than in infections due to many other species of bacteria.

One argument which might be advanced against this view is that with Type I Pneumococcus, as previously reported (1), a minimal number of organisms suffices to produce a fatal infection. In such an instance, however, the long latent period may represent the time required by a few organisms to multiply while others are undergoing autolysis.

Although the results of the present studies suggest certain similarities in action between autolysates, bacterial aggressins (Bail (3)), and virulin (Rosenow (4)), the evidence indicates that in the case of Pneumococcus, at least, the autolysates serve to enhance a property or function potentially present in a given strain rather than to confer a new property.

SUMMARY

In pneumococcus dermal infections in rabbits, the addition of pneumococcus autolysate to an infective inoculum favors the invasive-
ness of the particular strain employed, but does not alter the kind of virulence possessed by that strain.

Autolysates exhibiting this enhancing property also induce purpura in mice and inhibit the coagulation of rabbit blood. The relation of these properties to the infectivity of Pneumococcus and the possible rôle of bacterial autolysis in natural infection are discussed.

BIBLIOGRAPHY