THE EVALUATION OF ACTIVE RESISTANCE TO PNEUMOCOCCUS INFECTION IN RABBITS

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(Received for publication, May 19, 1933)

The utilization of the dermal pneumococcus infection to determine the active resistance of rabbits has been discussed in an earlier paper (1). Since the publication of that report the method has been applied in the study of an extensive series of animals, so that it has now become possible to evaluate the results obtained by the use of this method.

It is commonly held that if an animal survives following an injection of virulent pneumococci, it is immune, or, conversely, if the animal dies, it is non-immune. Careful study reveals, however, that there are differences in the rate at which animals die, and certainly even greater differences in response to infection among those which survive. An animal's resistance may be of such a character as to bring about recovery, but, nevertheless, the animal may suffer from a severe and protracted infection differing in no considerable respect from one in which the result is fatal. On the other hand, the degree of resistance may be such that the animal develops only a slight localized area of infection and shows no temperature elevation. Thus, while it is possible to stipulate arbitrarily that animals which die are non-immune, and those which survive are immune, it would seem more reasonable to hold that survival and death merely divide the gradient of response to infection into two phases, in each of which considerable differences may be encountered, and that the much abused terms "immune" and "non-immune" describe the two extremes of the scale.

The present paper deals with this gradient of resistance to infection as observed in a large series of rabbits which, previous to infection, had received courses of injection of pneumococci or their derivatives, and in rabbits which had recovered from dermal pneumococcus infections spontaneously or following specific therapeutic measures.
The method of test infection with pneumococci of the various types has been used in a large number of rabbits during the last 8 years. It is manifestly impossible to present in detail all the data which form the basis for the system of evaluation of resistance. The grades of active resistance which will be enumerated have been determined upon after a careful analysis of these data. Even if it were possible to recount all of the experiments, it is doubtful if such data would contribute more than do the illustrative instances presented in this paper. Further evidence will be reported subsequently in connection with studies on the duration of active resistance following immunization.

Methods Used for Test Infections

The general technical procedures are those described in a previous paper on the dermal pneumococcus infection in rabbits (2).

Cultures.—All strains of pneumococci were cultivated and frequently transferred in rabbit blood broth. Repeated rabbit passages were used to maintain the virulence of the organisms. The following strains were used.

Pneumococcus Type I, original Neufeld strain; virulence such that 0.000,000,01 cc. produces a fatal infection in rabbits following intradermal inoculation.

Pneumococcus Type II, Strain D 39; the virulence for rabbits fluctuates slightly, but 0.000,01 cc. given intradermally causes death in a majority of normal rabbits.

Pneumococcus Type III, Strain PH; regularly produces a fatal infection in amounts as small as 0.000,1 cc.

Method of Infective Inoculation.—Each animal to be tested received 0.2 cc. of undiluted 18 hour blood broth culture of Pneumococcus in the skin midway on the flank area.

Observations.—Daily determinations of rectal temperature were made. The appearance of the lesion was carefully followed with regard to the area involved, the amount of edema, and the character of the inflammatory color. In many cases these findings have been supplemented by blood cultures to determine the degree of blood invasion.

The System of Grading of Active Resistance

When the results of a long series of these infective tests are considered, it becomes immediately apparent that in terms of active resistance each animal may be assigned to one of several distinct groups, of which at one extreme is a group including the animals which died promptly, and at the other a group embracing those rabbits which showed greatest resistance to infection. Between these two extremes animals of inter-
mediate resistance fall into groups which may be arranged in a sequential order.

The different degrees or grades of active resistance to infection are listed in Table I, together with the distinguishing criteria.

In Text-fig. 1 are shown temperature charts of rabbits illustrating the characteristic reactions of animals exhibiting the various grades of resistance to dermal infection with Type I Pneumococcus. Entirely comparable results are obtained in animals infected with Pneumococcus Types II and III.

**TABLE I**

*System of Grading of Active Resistance*

This system of classification is based on the reactions which follow the intradermal injection in rabbits of 0.2 cc. of an 18 hour blood broth culture of virulent pneumococci.

<table>
<thead>
<tr>
<th>Grade</th>
<th>Reaction after infective inoculation</th>
<th>Extent of lesion</th>
<th>Ultimate result</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Febrile reaction</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Maximum temperature</td>
<td>Duration</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>High</td>
<td>Until death</td>
<td>Widespread</td>
</tr>
<tr>
<td>2</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>3</td>
<td>&quot;</td>
<td>5 days or longer</td>
<td>&quot;</td>
</tr>
<tr>
<td>4</td>
<td>&quot;</td>
<td>Less than 5 days</td>
<td>&quot;</td>
</tr>
<tr>
<td>5</td>
<td>Elevated but less than 105.5°F.</td>
<td>&quot; 5 &quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>6</td>
<td>No significant elevation</td>
<td>—</td>
<td>Moderate to widespread</td>
</tr>
<tr>
<td>7</td>
<td>No elevation</td>
<td>—</td>
<td>Local</td>
</tr>
</tbody>
</table>

The characteristics of the various grades of resistance may be defined as follows:

*Grade 1.*—This grade is assigned to all animals which develop a typical edematous dermal infection and exhibit a high temperature persisting until death, the latter occurring within 4 days. This arbitrary time period has been selected because it covers the course of the infection in most normal controls.

*Grade 2.*—This grade is allotted to those rabbits which die after 4 days. Many such cases are encountered, especially with Type III infections. These animals are apparently able to withstand the infection or hold it in check for many hours, only to die as the resistance is gradually overcome.
Grade 3.—Animals which show a typical widespread lesion and a high temperature persisting for 5 days or longer but which eventually survive, are given the rating of Grade 3. In some cases the recovery is abrupt, while in others the febrile reaction is of long duration and only gradually subsides to normal levels.

Text-Fig. 1. Temperature charts of rabbits which have been chosen as representative of the various grades of active resistance to pneumococcus dermal infection.

Grade 4.—Those rabbits which develop a very high rectal temperature (105.5°F. and above), but in which fever does not persist as long as 5 days, are given Grade 4. These cases also show a widespread edematous lesion. All animals in this group survive. The shorter febrile course is regarded as a significant feature.

Grade 5.—The grading of 5 is assigned to those animals which show a tempera-
ture of transient character ranging up to 105.4°F., 24 hours after infection. The tendency to early termination of the disease process is even more pronounced than in Grade 4.

**Grade 6.**—This grade is assigned to those animals which develop a lesion of moderate to considerable spread, but which fail to show a significant elevation of body temperature (temperatures below 104.0°F. are regarded as within the normal range). The lack of a marked febrile reaction distinguishes this from Grade 5, and the presence of a spreading lesion sets it apart from Grade 7.

**Grade 7.**—The rabbits designated by this grade show an entire absence of temperature elevation 24 hours after infection. There is always a slight localized area of inflammation at the point of inoculation, but this shows no tendency to spread.

Two other grades or degrees of resistance may be postulated, one at either extreme of the general scale:

The first additional grade, which may be designated as No. 0, is assigned to those previously infected animals in which the general physiological resistance has been so lowered by severe intoxication, poisons, and other deleterious influences, that the presence of the infecting organisms excites almost no local reaction, and there occurs a rapid, generalized invasion by the bacteria. The body temperature is depressed rather than elevated and the animal quickly dies. This condition is due to extraneous influences and is not of importance in a consideration of the natural gradient of resistance. Examples of this order have been cited in a previous paper (3).

The second additional grade is largely hypothetical, and is designated as No. 8. In a very few instances, in over 500 test infections, no inflammation of any sort occurred at the point of inoculation. These few animals had received many courses of injections with heat-killed pneumococci prior to the test infection, and might be thought of as hyperimmune. Although a rarity, and perhaps an accident, such a state of immunity must be considered as a possibility.

**Illustrative Examples of the Application of the System of Grading**

In order to make this system of grading of resistance more comprehensible and to demonstrate the method of application, two series of tests for active resistance in rabbits will be cited. Each experiment will be considered as an independent entity which, although undertaken in connection with other work, is used here as an illustrative example.

**Experiment 1.** Active Resistance to Infection in Rabbits Previously Given Intravenous Injections of Heat-Killed Suspensions of Pneumococcus Types I, II, and III.—Six rabbits were each given identical courses of intravenous injections of a heat-killed bacterial suspension consisting
of Types I, II, and III pneumococci in equal proportions. 10 days after the last immunizing injection two of these rabbits were infected intradermally with Type I pneumococci, two with Type II, and two with Type III. Normal controls were likewise infected. The results of these test infections are shown in Table II.

**TABLE II**

Results of Test Infections in Rabbits Previously Given Intravenous Injections of 
Heat-Killed Suspensions of Pneumococcus Types I, II, and III

Each of the first six rabbits had received identical immunizing courses of intravenous injections of a vaccine composed of equal numbers of heat-killed pneumococci of Types I, II, and III. Test infections as indicated, 10 days after last injection of bacterial suspension.

<table>
<thead>
<tr>
<th>Rabbit</th>
<th>Character</th>
<th>Test infection with Pneumococcus Type</th>
<th>Reaction after infective inoculation</th>
<th>Grade assigned</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Febrile reaction</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Maximum temperature</td>
<td>Duration</td>
</tr>
<tr>
<td>A</td>
<td>Immunized</td>
<td>I</td>
<td>103.4</td>
<td>—</td>
</tr>
<tr>
<td>B</td>
<td>&quot;</td>
<td>II</td>
<td>103.4</td>
<td>—</td>
</tr>
<tr>
<td>C</td>
<td>&quot;</td>
<td>II</td>
<td>103.5</td>
<td>—</td>
</tr>
<tr>
<td>D</td>
<td>&quot;</td>
<td>II</td>
<td>103.3</td>
<td>—</td>
</tr>
<tr>
<td>E</td>
<td>&quot;</td>
<td>III</td>
<td>104.4</td>
<td>1 day</td>
</tr>
<tr>
<td>F</td>
<td>&quot;</td>
<td>III</td>
<td>104.1</td>
<td>1 day</td>
</tr>
<tr>
<td>G</td>
<td>Normal control</td>
<td>I</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>H</td>
<td>&quot;</td>
<td>II</td>
<td>105.1</td>
<td>Until death</td>
</tr>
<tr>
<td>I</td>
<td>&quot;</td>
<td>III</td>
<td>108.0</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

S = survival.
D = death, at indicated number of days after infective inoculation.

From the results of this experiment, it will be noted that the animals infected with Type I pneumococci developed only a local lesion and showed no febrile response. The resistance has been given the grade of 7. The rabbits infected with Type II developed a widespread lesion, but did not show a febrile reaction, and the grade of 6 has been assigned. The rabbits infected with Type III also developed a widespread edematous lesion, but in addition developed a fever which persisted for a day. These have been classed as showing resistance of the fifth grade. All normal controls died within 4 days and their resistance has consequently been rated as that of Grade 1.
That these animals possessed a higher resistance against Type I pneumococci than against Type II, and higher resistance to Type II than to Type III pneumococcus infection, is not surprising in view of the fact that the antigenic capacities of these types range in the same order. While there is evidence that these animals were really immune to Type I pneumococcus infection, since there was no spread of the infectious process and no systemic reaction, it is doubtful if the same term can be correctly applied in the other cases, since the infections with Type II and Type III pneumococci gave rise to processes

### Table III

**Active Resistance to Infection in Rabbits Previously Given Intravenous Injections of Pneumococcus Autolysate**

Each of the first six rabbits had received identical courses of intravenous injections of bacterial material; three of these being treated with heat-killed Type I pneumococci, and three with an autolysate of an equivalent amount of bacteria. Test infections as indicated were carried out 10 days after last injection of bacterial material.

<table>
<thead>
<tr>
<th>Rabbit No.</th>
<th>Immunizing courses with</th>
<th>Reaction after infective inoculation</th>
<th>Grade assigned</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Febrile reaction</td>
<td>Extent of lesion</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Maximum temperature</td>
<td>Duration</td>
</tr>
<tr>
<td>1</td>
<td>Heat-killed Type I pneumococci</td>
<td>I 102.9</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>“ “</td>
<td>I 102.5</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>“ “</td>
<td>III 106.6</td>
<td>11 days</td>
</tr>
<tr>
<td>4</td>
<td>Autolysate of equivalent suspension</td>
<td>I 106.3</td>
<td>Until death</td>
</tr>
<tr>
<td>5</td>
<td>“ “</td>
<td>I 106.4</td>
<td>8 days</td>
</tr>
<tr>
<td>6</td>
<td>“ “</td>
<td>III 106.3</td>
<td>Until death</td>
</tr>
<tr>
<td>7</td>
<td>Normal control</td>
<td>I 106.6</td>
<td>“ “</td>
</tr>
<tr>
<td>8</td>
<td>“ “</td>
<td>III 106.6</td>
<td>“ “</td>
</tr>
</tbody>
</table>

S = survival.
D = death, at indicated number of days after infective inoculation.
which were not limited to the point of inoculation, and in the animals infected with Type III Pneumococcus there was a definite systemic reaction.

Experiment 2. Active Resistance in Rabbits Previously Given Intravenous Injections of Pneumococcus Autolysate.—This experiment was designed to determine the degree of active resistance brought about by injections of a heat-killed suspension of Type I pneumococci as compared to that produced by the injection of an autolysate of the same organisms.

A suitable suspension of washed Type I pneumococci was divided into two equal parts. The first portion was immediately heated at 65°C, for 30 minutes, and the second was allowed to autolyze until no formed elements remained and then was heated in a similar manner. Identical courses of intravenous injections of these materials were then given to rabbits, three animals receiving the heat-killed organisms, and three the autolysate. 2 weeks after the last injection two rabbits of each group were infected with Type I pneumococci, and one from each group was infected with Type III. Normal controls were infected with each type. The results of this experiment are shown in Table III.

Those animals which received the intact bacterial cells were immune against Type I infection, as shown by the failure to develop a febrile reaction and the fact that the infective process remained entirely localized. The rabbit infected with Type III survived the infection, but only after a long febrile period of 11 days. On the other hand, the animals which had been given injections of pneumococcus autolysate showed almost no resistance against either Type I or Type III. One rabbit survived infection with Type I, but only after a long febrile disease. The normal controls died promptly.

This experiment particularly emphasizes the failure of the criteria of survival and death as a basis for judgment of active immunity. By the application of the scale of grading it is easily possible to conclude (a) that following immunization with heat-killed suspensions of pneumococci the resistance is high and specific, and (b) that previous injections of pneumococcus autolysates confer little if any active resistance.

Many similar instances of the inherent fallacies of the survival-death system of evaluating immunity or resistance have been observed. Animals immunized with a given type of pneumococci, if tested at the proper time, show relatively high resistance to infection with the homologous type, and almost invariably survive. If infected with pneumo-
cocci of an heterologous type, the degree of resistance is always low, but some of the animals may survive and thus give an impression, if the circumstances are not understood, of non-type-specific active immunity. Similarly, if animals are immunized with rough pneumococci, some may survive test infection with virulent smooth forms, but the degree of resistance, if determined in the manner described, is low in every instance.

The question of the relation of the degree of resistance to the titer of specific antibodies of the serum of the test animal will be discussed at length in a subsequent paper, but it may be stated that these two factors do not necessarily parallel each other.

DISCUSSION

In discussing the gradient of resistance to infection, it may be well to present the evidence now available as to the significance which can be attached to the various degrees of resistance as defined in this paper.

While it is generally assumed that normal rabbits possess little active resistance to pneumococcus infection, we have observed that an occasional normal animal may recover spontaneously from Type I and Type III pneumococcus infections, although only after a typical febrile course (2, 4). These cases would be classed as showing a Grade 3 resistance. A few normal animals react in such a way that their resistance would be rated as Grade 2. By far the greater number (about 98 per cent) show a complete lack of resistance (Grade 1). Thus, normal animals which by commonly accepted definition are non-immune and possess no specific antibodies, may be found in each of the first three groups or grades. The type of reaction illustrated by Grade 3 is also frequently encountered in animals immunized to heterologous types of pneumococci, and to derivatives of pneumococci. These animals show no type-specific antibodies.

Grade 7 represents the highest active resistance demonstrable under these conditions in a majority of instances. The infection fails to spread and there is no systemic reaction. Examples of this degree of resistance are found only among those animals which have been specifically immunized, either actively or passively. This high degree of resistance is invariably associated with relatively high specific antibody titers.
Grade 6 also must represent a high degree of resistance, for, although
the lesion spreads and in this respect resembles the less immune ani-
mals, there is almost a total absence of systemic reaction.

Grades 4 and 5 present a much more difficult subject for analysis.
These grades have some features in common: a widespread edematous
lesion and definite febrile reaction. In neither instance does this
temperature persist as in Grade 3, a feature of special significance.
Grades 4 and 5 are differentiated arbitrarily by the degree of this fe-
brile response. The fever level in rabbits is about 104.0°F., and
points over this are regarded as significant. In Group 5 the tem-
perature always exceeds this level, but by definition does not exceed
105.4°F. nor persist longer than 4 days. The mechanism involved
in bringing about the abbreviation of these infections is not under-
stood.

Although it is obvious that the virulence and the dosage of the in-
vading organisms play a considerable part in the matter of the establish-
ment of an infectious process, these complicating factors have been
eliminated in the present experiments by the use of constant doses of
pneumococci of almost constant virulence for the animals employed.
It may be pointed out that at least two elements are concerned in
these evaluations of active resistance. The first is the element of
specific resistance, usually thought of as associated with antibodies,
either cellular or circulating. The second factor is that of the capacity
of the animal body to react in such a way as to make possible the
utilization of these specific elements; this appears to be the underlying
basis which conditions the functioning of the influences which find
expression in the phenomena of specific resistance.

Aside from the primary purpose of this report, another matter of
possible importance may be referred to. It has been shown that in
animals possessing varying degrees of active resistance to pneumococ-
cus infection any one of a series of reactions, from the most mild to the
most severe, may be encountered. These reactions parallel in many
instances the varieties of lobar pneumonia seen in man,—the fulminat-
ing type; the cases which die after a long febrile course; those which
undergo recovery by crisis or lysis; the abortive type; the cases which
have definite pulmonary involvement but little systemic reaction; and
other varieties. It is suggested that these degrees of clinical severity
of lobar pneumonia may likewise indicate differences in the individual potentialities of active resistance.

The data presented in this paper serve to emphasize that the terms "immunity" and "resistance," so frequently used in a positive or negative sense, can only be considered as relative. The mere fact that an animal survives a test infection leads to a wholly inadequate conception that this animal is immune or resistant. For example, if the commonly used experimental terms were applied to clinical practice, any patient who survives lobar pneumonia, no matter how severe the case, might be said to be actively immune at the beginning of the infection. Obviously, the term active immunity, in the positive sense, must refer to the ability to prevent or at least sharply limit infection, and the term non-immune, in the negative sense, to the total inability to cope with an infectious agent. A majority of cases of natural infection in man fall between these two extremes. It would seem that our understanding of infection and resistance must involve other criteria besides the question of survival and death in bacterial disease.

SUMMARY

The dermal pneumococcus infection has been employed to determine the active resistance of rabbits against infection, and an evaluating scale of the gradient of resistance has been established. The significance of the various degrees of resistance has been discussed, and the possible general inferences of the findings indicated.

BIBLIOGRAPHY