

REACTIONS OF RABBITS TO INTRACUTANEOUS IN-
JECTIONS OF PNEUMOCOCCI AND THEIR
PRODUCTS

VIII. THE INTERRELATIONSHIPS OF HYPERSENSITIVENESS TO
PNEUMOCOCCUS AND STREPTOCOCCUS

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The observations of certain investigators have called attention to the possession by some bacteria of common serological and immunological properties. On the one hand, it has been shown that this condition may occur in remotely separated species because of the chemical similarity of the soluble specific substances or carbohydrates elaborated by the organisms as in the case of Friedländer's bacillus and Pneumococcus (1); while, on the other hand, the presence of similar proteins in closely related species as in the case of the Gram-positive cocci (2, 3) endows the bacteria with common antigenic characters.

In the previous papers of this series, studies have been reported which indicate that rabbits develop a heightened skin (4) and eye (5) sensitivity to pneumococcus protein following repeated intracutaneous injections of heat-killed pneumococci. The increased skin reactivity (6) was found to be associated with the presence of circulating antibodies for the protein (anti-P) while the eye reactivity apparently occurred independently of serum antibodies.

On the basis of these observations, it seemed possible that pneumococci and streptococci possess the ability to stimulate common manifestations of hypersensitiveness by virtue of similar protein antigens present in both organisms.

Methods

The pneumococci used were S strains of Type I and Type III, and an R strain derived from Type II (S). The Streptococcus was a strain of indifferent¹ Streptococcus (Q 155) obtained from Dr. H. F. Swift. Suspensions of Pneumococcus and Streptococcus were prepared as previously described (7).

Injections of the bacterial suspensions were made into the depilated skin once a week and the reactions following each injection were recorded as described elsewhere (6). The number of injections varied from 6 to 10.

3 weeks after the last intracutaneous injection of bacterial suspension, the animals were bled and the sera were tested for the presence of antibodies. Skin tests and eye tests were performed at this time and in some of the animals, resistance to infection was also determined.

The Reactions at the Sites of Injection of Bacteria

The reactions at the site of injection of both S and R pneumococci have been described in detail in a previous paper (8). For purposes of comparison, however, it seems advisable to review briefly the descriptions of the reactions to this organism.

Following the repeated intracutaneous injection of heat-killed pneumococci, the skin acquires an increasing reactivity to fixed quantities of bacteria until 4 to 6 injections have been made after which the skin reactivity diminishes. The reaction to the first injection appears as a circumscribed slightly raised and indurated nodule measuring 2 or more cm. in diameter. With repeated injections the reactions increase in intensity until the skin lesion is markedly elevated, deep red or purplish in hue, and may reach about 6 cm. in diameter. Surrounding the raised area is an areola of erythema and beyond this, the skin is edematous. Not infrequently, necrosis of it occurs with discharge of purulent material. In a general way, the time required for disappearance of the lesion parallels the severity of the reaction. In a number of instances, after the reaction to the first injection has subsided, a recrudescence of the reaction occurs, a phenomenon first noted by Andrewes, Derick and Swift (9) and designated by them the secondary reaction. The skin reacts similarly to successive injections of R pneumococci, although the reactions are ordinarily not so intense or severe as are those stimulated by S strains.

When heat-killed suspensions of indifferent streptococci are injected into the skin at weekly intervals, the successive reactions at the sites of injections also undergo changes in size, character, intensity and duration. The changes how-

¹By indifferent Streptococcus is meant *Streptococcus anhemolyticus*, a distinctive character of which is the inability to cause either hemolysis or green coloration of blood.

ever, are not so marked as those accompanying injections of Pneumococcus and the tendency of the reaction to be circumscribed and dome-shaped readily differentiates it from the reaction to pneumococci.

The reaction to the first injection of Streptococcus is usually sharply defined, circular, dome-shaped, pink and firm. It reaches maximum intensity in about 48 hours, when the area of skin involved measures about 1.5 cm. in diameter. Occasionally, the reaction is surrounded by a zone of erythema which disappears within 2 days time. The reaction retrogresses gradually and usually disappears within 10 to 12 days, although in some instances complete disappearance may require weeks. In certain rabbits, the primary reaction is followed by an exacerbation or secondary reaction. Subsequent injections give rise to reactions which

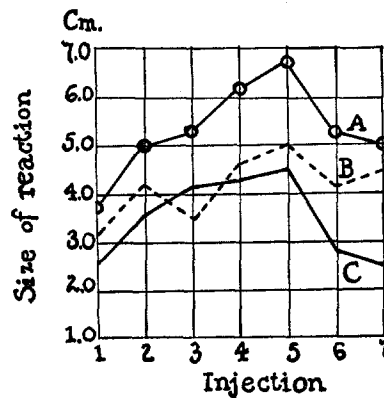


FIG. 1. The size of the skin reactions following each of a series of injections of heat-killed pneumococci or indifferent streptococci. Each curve represents the size as expressed by the sum of two diameters for a group of 6 rabbits. A represents sizes of reaction to Pneumococcus S. B represents sizes of reaction to Pneumococcus R. C represents sizes of reaction to indifferent Streptococcus.

gradually become more intense in character. Maximum reactivity usually occurs after 4 to 6 injections have been made. At this time, the reaction is still circular and dome-shaped but not quite so well defined and the edema is more marked and the diameter of skin involved is about 3 cm. The reaction is usually surrounded by an areola of erythema measuring in width from 0.5 to 1.5 cm. and frequently the skin undergoes necrosis with discharge of purulent material. Reactions of this intensity require 7 to 8 weeks for complete disappearance. After the skin reactivity has reached the maximum the lesions become less severe and irregular.

Secondary reactions occur only following the first injection of bacteria. In this study of 36 rabbits injected with S pneumococci 10 showed secondary reactions; of 10 injected with R pneumococci, 4 showed secondary reactions; and of 36 injected with indifferent Streptococcus, 6 showed secondary reactions. The

frequency of occurrence of the secondary reaction is not so great as that observed in the previous studies. Hitchcock and Swift (10) have already pointed out the occurrence of secondary reactions when rabbits are injected intracutaneously with live cultures of indifferent streptococci.

In 3 of the rabbits injected with indifferent streptococci, it was observed that about 2 weeks after the first injection a number of reactions appeared in various places over the skin without necessarily causing a recrudescence of the original reaction. These lesions varied in size, but did not differ in general appearance from the usual reactions. The phenomenon has never been observed in rabbits receiving injections of pneumococci.

To illustrate comparatively the changes which occur in the intensity of the reactions following repeated injections of R and S pneumococci and indifferent streptococci, a chart has been made which gives the area of skin involved in each reaction (see Fig. 1). The measurements given represent the sum of the length and breadth of the surface of skin involved in the reaction.

The Increased Skin Reactivity to the Bacterial Proteins

About 3 weeks after the last intracutaneous injection of bacteria all the rabbits were tested for increased skin reactivity to the nucleoproteins derived from *Pneumococcus* and from indifferent *Streptococcus*. The pneumococcus protein was prepared and standardized as already described (8). The streptococcus protein was obtained by first grinding the bacterial cells, and then precipitating out the protein with N/acetetic acid. In the quantities used neither protein caused reactions in normal rabbits.

The intracutaneous injection of both pneumococcus and streptococcus protein in rabbits which had previously received heat-killed suspensions of S or R pneumococci or streptococci was followed by a reaction at the site of injection. The lesion usually reached its height within 48 hours when it began to fade and generally disappeared within 4 to 5 days. The area of skin involved was sharply defined, reddened, raised, thickened and edematous and measured 2 to 3 cm. in diameter. Frequently, the reactions were surrounded by an erythematous band, but breaking down of the skin was not seen. It is important to point out that the character of the reaction was the same, irrespective of the protein injected, or the organism employed in the preparatory injections.

The Development of Eye Reactivity to the Bacteria and Their Proteins

The technique for the eye test and a description of the reaction have been given in an earlier paper (5). It was pointed out at that time that eye reactions

during hypersensitiveness to *Pneumococcus* were elicited by the proteins. Swift and his associates (10) on the other hand have shown that in hypersensitiveness to the different varieties of *Streptococcus*, eye reactions are elicited by whole cultures, not by the protein. It was, therefore, necessary to perform eye tests with both cultures and both proteins. Consequently each eye was tested twice. In order to test with living pneumococci, it was found necessary to use R cells, since the S forms induce infection which obscures the reaction sought for, if present.

The number of rabbits manifesting hypersensitiveness to either *Pneumococcus* or *Streptococcus* was somewhat less than that reported

TABLE I
Summary of Reactions in Rabbits Following Intracutaneous Injections of Pneumococci or Indifferent Streptococci

Reactions	Following repeated intracutaneous injections of		
	<i>Pneumococcus</i> S	<i>Pneumococcus</i> R	Indifferent <i>Streptococcus</i>
Skin reaction to protein of			
(a) <i>Pneumococcus</i>	+	+	+
(b) <i>Streptococcus</i>	+	+	+
Eye reaction to protein of			
(a) <i>Pneumococcus</i>	+	+	-
(b) <i>Streptococcus</i>	-	-	-
Eye reaction to living suspension of			
(a) <i>Pneumococcus</i>	-	-	-
(b) <i>Streptococcus</i>	-	-	+
Development of agglutinins for			
(a) <i>Pneumococcus</i> S.....	-	-	-
(b) " R.....	+	+	+
(c) <i>Streptococcus</i>	+	+	+
Resistance to pneumococcal infection.....	+	+	-

in earlier communications and by Hitchcock and Swift (10). As is seen in Table I, the eyes of rabbits injected with pneumococci did not react after corneal scarification and the instillation of live suspensions of R pneumococci or streptococci. When reactions did occur in these rabbits they were stimulated only by the protein of *Pneumococcus*. Conversely, the eyes of rabbits injected with indifferent

TABLE II
Cross-Agglutinations with Sera of Rabbits Injected Intracutaneously with Pneumococcus or Streptococcus

Serum from rabbits injected with	S Pneumococcus homologous type		R Pneumococcus								Indifferent Streptococcus							
			Dilution of sera															
	1:5	1:10	1:20	1:5	1:10	1:20	1:40	1:80	1:160	1:320	1:5	1:10	1:20	1:40	1:80	1:160	1:320	
Indifferent Streptococcus 1.....	-	-	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
" 2.....	-	-	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
" 3.....	-	-	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
" 4.....	-	-	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
S Pneumococcus 1.....	-	-	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
" 2.....	-	-	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
" 3.....	-	-	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
" 4.....	-	-	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
R Pneumococcus 1.....	-	-	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
" 2.....	-	-	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
" 3.....	-	-	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
" 4.....	-	-	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++

streptococci did not react to the protein of either *Pneumococcus* or *Streptococcus* or to living cells of *Pneumococcus*. The reactions that were observed occurred only when living streptococci were introduced into the conjunctival sac. It seems, therefore, that the eye reactivity exhibits a specificity which is not shared by the skin reactivity.

The Specificity of the Circulating Antibodies

The sera of all the rabbits which had received intracutaneous injections of pneumococci or streptococci were studied for the presence and specificity of antibodies. Since it has already been shown (7) that even when S pneumococci are injected intracutaneously, the antibody response is essentially species-specific or anti-R and only a small percentage of rabbits develop anti-S agglutinins, agglutination tests were conducted with R pneumococci. For the sake of brevity, a typical protocol of the agglutinations is presented in Table II. It will be seen that the sera of rabbits injected with S pneumococci while lacking in type-specific agglutinins, showed a high antibody titre for R pneumococci and for indifferent streptococci. The sera of rabbits injected with indifferent streptococci also possess agglutinins for both R pneumococci and indifferent streptococci. It will be noticed however, that the antibody titre in either case is slightly higher for the species employed in the intracutaneous injections.

*Resistance to Infection by *Pneumococcus**

In a previous report (12) it was shown that repeated intracutaneous injections of S or R pneumococci evoke a state of resistance to infection which is equally effective against infection by organisms of homologous and heterologous types. It was interesting to determine whether previous injections of indifferent streptococci would render rabbits resistant to pneumococcal infection. The protocols are summarized in Table III. It will be seen that only those rabbits injected with *Pneumococcus* acquired an active resistance to infection by *Pneumococcus*. This lends further confirmation to the earlier observation that while skin reactivity to pneumococcus protein occurs in resistant and non-resistant animals, only those animals resistant to infection may manifest eye sensitivity.

TABLE III
Resistance of Rabbits to Infection by Pneumococcus Following Six Intracutaneous Injections of Pneumococci or Streptococci

Organism used for injections	Rabbit No.	Dilution of pneumococcus culture injected (Type I)	Result
Indifferent Streptococcus	20	cc.	Died
	21	10 ⁻¹	"
	22	10 ⁻²	"
	23	10 ⁻³	"
	24	10 ⁻⁴	"
	25	10 ⁻⁵	"
Pneumococcus Type III (S)	26	10 ⁻¹	Survived
	27	10 ⁻¹	Died
	28	10 ⁻²	"
	29	10 ⁻²	Survived
	30	10 ⁻³	"
	31	10 ⁻³	"

M.L.D. of the Pneumococcus Type I culture for normal rabbits was 10⁻⁷ cc.

DISCUSSION

The experiments reported in the present communication reveal certain reactions in common of rabbits repeatedly injected with heat-killed pneumococci or anhemolytic streptococci. There occurs in these animals an increased reactivity of the skin to the nucleoprotein of either organism. It has been demonstrated, moreover, that the sera of these rabbits contain antibodies which agglutinate either R pneumococci or indifferent streptococci irrespective of which of these organisms was employed in the previous intracutaneous injections. The evidence presented in a preceding report supported the concept that the skin sensitivity to pneumococcus protein is referable to the presence of circulating species-specific antibody (anti-P). It is not unlikely, therefore, that the skin reactivity to nucleoprotein of both organisms by the rabbits injected intracutaneously with Pneumococcus or Streptococcus is rendered possible by the possession of a similar or common antibody.

It has also been shown, in contrast to the foregoing, that hypersensitiveness as manifested by sensitivity of the eye to bacteria or their

products is specific for the organism employed in the preparatory intracutaneous injections. Thus, inoculations of *Streptococcus* stimulate eye reactivity only to cultures of *Streptococcus*, and inoculations of *Pneumococcus* stimulate eye reactivity only to the nucleoprotein of *Pneumococcus*. Measured in terms of bacterial specificity, therefore, the eye reactivity exhibits a specificity not seen in the case of skin sensitivity. The results of the earlier studies on *Pneumococcus* indicated that eye hypersensitiveness, while species-specific, is independent of the usual agglutinating, precipitating and protective antibodies found in the serum. The bacterial specificity of eye reactivity in the present instance may be looked upon as confirmative of this finding.

Following intracutaneous injections with *Pneumococcus* or *Streptococcus*, resistance to infection is specific. Only those animals injected with pneumococci acquire resistance to pneumococcal infection. This confirms the view that (1) skin hypersensitiveness is elicited equally well in resistant and non-resistant animals, and (2) eye hypersensitiveness, while not necessarily related, is found only in those animals resistant to infection.

SUMMARY AND CONCLUSIONS

1. Following repeated intracutaneous injections of heat-killed S or R pneumococci, rabbits acquire an increased skin reactivity to the nucleoprotein of both *Pneumococcus* and indifferent *Streptococcus*.
2. Rabbits acquire a skin reactivity to the proteins of both the organisms mentioned after receiving intracutaneous injections of heat-killed indifferent streptococci.
3. Injections of pneumococci into the skin are followed in some rabbits by an eye sensitivity to pneumococcus protein but not to streptococcus protein nor to suspensions of either living organism.
4. Injections of indifferent streptococci into the skin are followed, in some rabbits, by an eye sensitivity to suspensions of live indifferent *Streptococcus*, but not to R *Pneumococcus* or to the nucleoprotein of either organism. Measured in terms of bacterial specificity therefore, the eye sensitivity possesses a specificity which is not shared by the skin sensitivity.
5. The sera of rabbits injected intracutaneously with pneumococci

or indifferent streptococci contain agglutinins for both R Pneumococcus and indifferent Streptococcus.

6. Only those animals injected with pneumococci acquire resistance to pneumococcal infection.

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