

# REACTIONS OF RABBITS TO INTRACUTANEOUS INJECTIONS OF PNEUMOCOCCI AND THEIR PRODUCTS

## III. REACTIONS AT THE SITE OF INJECTION

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It has been shown in a preceding paper (1) that the repeated intracutaneous injection of heat-killed pneumococci in rabbits stimulates the formation of species-specific, rather than type-specific, antibodies.

When Type I Pneumococcus was used as antigen, a small number (12 per cent) of the animals did develop a low serum titre of type-specific antibodies, but in all other instances, type-specific antibodies failed to appear in the serum. In a general way, moreover, the antibody response was the same when either S or R cells, or even soluble derivatives of Pneumococcus, were administered intracutaneously. Following the injections of S or R organisms, rabbits acquired a marked degree of resistance (2) which was effective against intravenous infection by any type of Pneumococcus. The injection of soluble derivatives, on the other hand, induced the development of no active immunity.

When rabbits received an intracutaneous injection of a suspension of heat-killed pneumococci a reaction always occurred at the site of injection, and as the inoculations were repeated week after week with a fixed quantity of bacteria, the local reactions following the injections manifested a change in size, character, intensity, and duration. The occurrence of reactions was in no way related to the type or variety of Pneumococcus employed in the injections nor was the intensity of the reaction dependent upon the culture employed. A study has been made of these local reactions.

### EXPERIMENTAL

Inoculations of 0.2 cc. of suspensions of heat-killed pneumococci<sup>1</sup> were made into the skin over the flanks. Repeated injections were made alternately on the two

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<sup>1</sup> In all cases, unless otherwise stated, the suspensions of heat-killed pneumococci were prepared in such manner that 1 cc. of the suspension contained the bacteria from 10 cc. of an 18 hour broth culture.

sides and a new location was chosen each time. Descriptions and measurements of the local reactions were made each day until a complete and final disappearance had taken place. The size of the area of skin involved in the reaction, as determined by actual measurement, has been held to be the only available method of quantitative estimation of the intensity of a given reaction. Usually the reactions reach their greatest size about 48 hours after the injection. Consequently, the sum of the two largest diameters, as measured at this time, has been considered to be an index of the intensity of any given reaction. This has permitted a comparison of the reactions occurring after the first and those after the subsequent injections.

#### *Skin Reactions Following Initial Injection*

The intracutaneous injection into a rabbit of 0.2 cc. of a suspension of heat-killed pneumococci is followed within a few hours by the appearance at the site of injection of a circumscribed, slightly raised and indurated nodule, measuring from 1 cm. to 1.5 cm. in diameter. The skin over this area is reddish in color. The reaction reaches its maximum size, of from 1 cm. to 1.5 cm. in diameter, in about 36 to 48 hours. It gradually becomes smaller and disappears within 4 to 5 days without the skin breaking down or becoming necrotic. There is some variation in the intensity of the reaction in individual animals but usually the course is about as stated above. Occasionally, regression occurs more rapidly, within two or three days, or, more rarely, complete disappearance is delayed, even for as long as two weeks or more.

#### *Skin Reactions Following Repeated Injections*

When after an interval of a week, a second injection is made into the skin the reaction which follows differs somewhat in intensity and character from that observed following the first injection. The modification of the reaction becomes more marked as the injections are repeated from week to week. Reactions of the greatest intensity occur usually after the fourth to the sixth injection.

In the more intense reactions the area of the skin involved reaches 4 cm. to 6 cm. in diameter. The skin is markedly elevated and of a deep red or purplish hue, and at times appears mottled with small yellow areas. Surrounding the raised area there is usually an areola of erythema varying from 0.2 cm. to 1.5 cm. in breadth. Outside of this the skin may be slightly edematous over a considerable area,

especially on the ventral margin. Not infrequently necrosis of the skin occurs, with discharge of purulent material. In case necrosis does not occur, the disappearance of the lesion is delayed. The time required for regression is apparently related directly to the intensity of the reaction.

To illustrate the change which gradually occurs in the intensity and character of the reactions following repeated injections, charts have been made which give the size of the area involved in each reaction, the length of time required for its disappearance, etc.

TABLE I  
*The Duration of Successive Reactions Following Repeated Intracutaneous Injections of 0.2 Cc. Suspensions of Pneumococcus*

Rabbit No.	Number of the injection									
	1	2	3	4	5	6	7	8	9	10
12-56	9*	15	24	23	33	14	12			
12-57	17	16	24	22	37	15	21			
12-58	16	12	22	16	21	7	6			
12-59	22	20	59	28	44	10	10			
12-60	20	17	50	58	37	16	12			
13-89	7	62	55	50	40	31	21			
13-90	10	22	22	21	17	9	16			
13-69	5	21	25	36	32	22	13	9	5	5
13-70	4	18	34	36	31	12	5			
13-72	5	14	43	35	29					

\* The figures represent the number of days required for the disappearance of the reactions.

After reactions of maximum intensity occur, following the fourth or fifth injection, the reactions to subsequent injections vary considerably as regards size. At first, there occurs a gradual decrease but later marked variations occur. These variations are partly explained by the fact that the reactions to later injections are frequently less well circumscribed, and, therefore, less easily measured than are the reactions following the earlier injections. This variability in size is shown in Figures 1, 2, and 3.

In Table I is recorded the number of days required for complete disappearance of each reaction which follows each of ten consecutive

injections made at intervals of a week. It will be seen that the number of days required for the lesions to disappear becomes progressively greater up to about the fourth injection, when the duration of the lesion again becomes diminished. This is also shown in the curve in Figure 1. Any marked irregularities which occurred were usually due to necrosis of the lesions and discharge of pus, with consequent more rapid resolution. Although reactions to later injections became less intense, of smaller size and of less duration, they never completely fail to appear, even though many injections are made. The above observations indicate that the reactivity of the skin to injections of pneumococci becomes gradually increased at least, up to the time four or five weekly injections have been made.

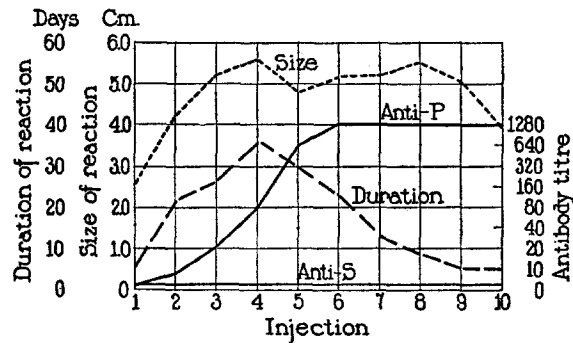


FIGURE 1. The size and duration of the skin reactions following each of a series of injections of heat-killed pneumococci, and the development of antibodies in the serum.

It seemed, however, important to attempt to demonstrate this in another manner, namely, by determining the minimum amount of bacterial suspension required to produce a visible reaction after the animal had received three previous injections, as contrasted with the minimal amount required to produce a reaction in the animal previously untreated.

Each of eight animals received intracutaneous injections of suspensions of heated pneumococci representing amounts of culture varying from 2 cc. to 0.0001 cc. In all cases the volume of fluid injected was 0.2 cc. It was found that reactions occurred when suspensions containing the bacteria from 1 cc. to 0.1 cc. of culture had been injected, but not when suspensions containing smaller numbers

of bacteria were employed. Each of the eight rabbits now received at the end of each of the two subsequent weeks the usual injections of 0.2 cc. of suspensions and the usual reactions were observed. At the end of the following week each of the rabbits received injections of varying amounts of suspension corresponding to those employed in the first test. It was now found that two of the animals showed reactions where amounts of suspension containing the bacteria from .001 cc. of culture were injected, one showed a reaction with an amount as small as that representing .01 cc. of culture, and five showed reactions with amounts of suspensions representing 0.1 cc. of culture. These results are shown in Table II.

After these animals had received injections for three weeks they reacted to amounts of culture smaller than those which were neces-

TABLE II  
*Minimal Doses of Suspensions of Pneumococcus Which Elicit Skin Reactions in Normal Animals and in Animals Previously Injected*

Rabbit No.	Minimal dose eliciting reaction at	
	First injection	Fourth injection
	cc.	cc.
8-47	0.01*	0.01
8-48	0.01	0.0001
8-49	0.2	0.01
8-50	0.01	0.0001
8-51	0.1	0.001
8-52	0.01	0.01
8-53	0.1	0.01
8-54	0.1	0.01

\* These quantities of bacterial suspension represent a tenfold concentration of culture.

sary to elicit a reaction originally. The previous observations of an increase of reactivity following successive injections were therefore confirmed by these tests.

*Relation of the Increasing Skin Reactivity to the Development of Circulating Antibodies*

An attempt was made to correlate if possible the increasing skin reactivity described above with the appearance of circulating antibodies. As has already been pointed out in previous communications (1, 2) the appearance of type-specific and protective antibodies following intracutaneous injections of suspensions of heat-killed pneu-

mococci occurs only occasionally and then only when pneumococci of Type I have been employed in the injections. On the other hand, the present study shows that all rabbits develop an exalted skin reactivity following successive intracutaneous injections of heat-killed pneumococci, no matter what type of *Pneumococcus* is employed. Consequently, skin reactivity cannot be related directly to the appearance of type-specific antibodies in the blood serum.

On the other hand, as has previously been shown, following repeated intracutaneous injections of heat-killed pneumococci all rabbits develop in their serum a high titre of species-specific antibodies. A study of increased skin reactivity and the presence of species-specific anti-

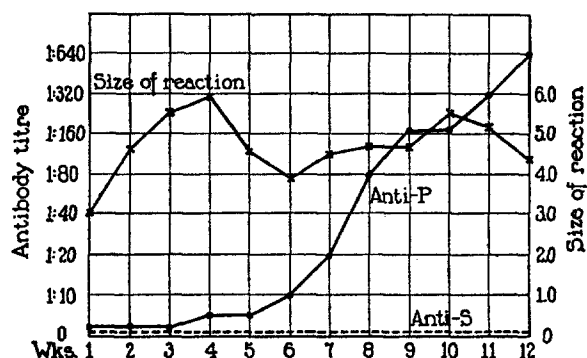


FIGURE 2. The development of antibodies and the size of skin reactions following each of a series of injections of *Pneumococcus*, Type I.

bodies in the serum was made in a large number of animals, but for the sake of brevity, the observations made on 8 rabbits only will be given in detail as typical. Four rabbits received intracutaneous injections of suspensions of heat-killed *Pneumococcus* Type I, and four received similar injections of suspensions of *Pneumococcus* Type III. The size of the reaction at the site of each of the injections was recorded, and the type-specific and species-specific titre of the blood was determined in each animal each week. In Figure 2 (Type I *Pneumococcus*) and Figure 3 (Type III *Pneumococcus*) composite curves indicating the increase of skin reactivity and the development of each kind of antibody during the period of the experiment are given. In each instance, it is seen that during the early injections, the skin reactivity

increased rapidly, while species-specific antibodies appeared in the blood only slowly. With the later injections and the approach of the height of the skin reactivity, the content of the serum in species-specific antibodies increased; later although the skin reactivity remained constant or decreased, nevertheless, the species-specific antibodies increased rapidly. It has not been possible, therefore, to demonstrate any direct relationship between the appearance of species-specific antibodies in the blood and the development of heightened skin reactivity.

Previous experiments have indicated that the degree of active resistance which develops following intracutaneous injections also increases

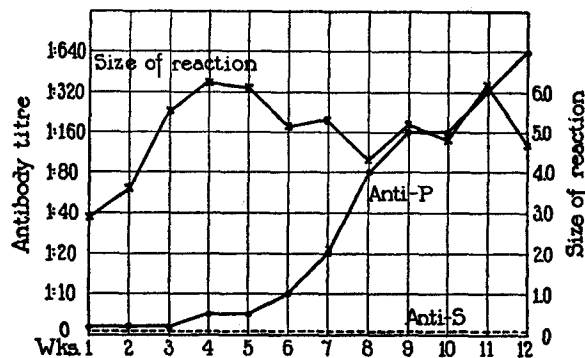


FIGURE 3. The development of antibodies and the size of skin reactions following each of a series of injections of Pneumococcus, Type III.

with the number of injections. It seems possible, therefore, that although a relationship may exist between these two phenomena, the exact nature of this relationship is, at the present time, obscure.

#### *Passive Transfer of Increased Skin Reactivity*

Serum obtained from rabbits at the height of skin reactivity was injected intravenously into normal rabbits in quantities varying from 10 to 30 cc. Other normal rabbits were given serum from rabbits which had received repeated intracutaneous injections of egg albumin or Pneumococcus "nucleoprotein." Twenty to twenty-four hours following the transfers, injections of suspensions of heat-killed pneumococcus were made into the skin of all the animals which had re-

ceived the serum. Control normal rabbits which had received no serum were also given similar intracutaneous injections. It was found that the rabbits which had received serum from highly reactive animals, those which had received serum from the animals immunized to proteins, and the normal rabbits all exhibited skin reactions of the same character and the same degree of intensity. It seems, therefore, that the property of increased skin reactivity cannot be transferred to the normal rabbits by intravenous injection of serum from the highly reactive animals.

#### *The Secondary Reaction*

Andrewes, Derick, and Swift (3) have shown that when rabbits are injected in the skin with live or dead bacteria, particularly with certain strains of *Streptococcus viridans*, a local reaction occurs. This begins to retrogress in about 48 hours, but in over half of the animals, about the eighth or ninth day, a recrudescence of the reaction takes place. Among other observations, they showed that when living R pneumococci, derived from a Type I strain, were injected, secondary reactions occurred in 2 of 13 rabbits tested. When the same organisms killed by heat were injected, or when heated S pneumococci of Type I were injected, a secondary reaction occurred in 2 of 15 rabbits.

In the present study secondary reactions have been observed in 31 out of 54 rabbits which received intracutaneous injections of suspensions of heat-killed pneumococci.

The secondary reaction occurred only after the first injections, never following subsequent ones. The secondary reaction after injections of pneumococci usually appears 7 to 12 days after the injection, and usually after the primary reaction has entirely disappeared. The secondary reaction persists from a minimum of 3 days to a maximum of 25 days, the average duration being 12 days. In appearance it usually resembles the primary reaction, being circumscribed, slightly elevated, pink to reddish in color, with little or no edema, and the lesion has never been observed to break down. Secondary reactions have not been observed following the intracutaneous injections of soluble derivatives of pneumococcus cells.

No relationship could be established between the appearance of secondary reactions and the appearance of type-specific or species-specific antibodies in the blood, nor was there any apparent relationship between the appearance of secondary reactions and the development of active immunity.



In the rabbits mentioned in the previous experiment which had received serum from animals highly skin reactive to pneumococci or from animals which had previously received injections of solutions of egg albumin or "nucleoprotein," secondary reactions following the intracutaneous administration of pneumococci were observed in the same frequency as those occurring in untreated animals.

#### DISCUSSION

The repeated injections of suspensions of heat-killed pneumococci into the skin of rabbits stimulate the development of an increased reactivity of the skin as is shown by the greater size and intensity of the reactions to subsequent injections, and by the fact that doses of heat-killed pneumococci, which in the normal animal are too small to produce any reaction, in the animals which have been previously injected produce marked reactions. The height of increased skin reactivity is reached when 4 to 6 intracutaneous injections have been made, after which the reactivity diminishes. This increased skin reactivity develops after the intracutaneous injection of pneumococci of any type or form, and the reactions are similar, no matter what type or form of *Pneumococcus* has been employed. There is apparently no relationship between the development of an increased skin reactivity and the appearance of type-specific antibodies in the blood, nor does the development of heightened skin reactivity seem to bear any definite relationship to the appearance of species-specific antibodies. While increased skin reactivity appears at a time when increased resistance to infection is present, the relationship between these two phenomena is still obscure.

The heightened skin reactivity is probably dependent upon some alteration in the tissues themselves, since transfers of the blood serum of highly reactive animals to normal rabbits does not endow the latter with the property of reacting with increased intensity.

Finally, it has been found that a secondary reaction may occur following the primary reaction. This is similar to the reactions following the intracutaneous injections of dead or living cells of *Streptococcus viridans* as studied by Swift and his associates (3, 4, 5). With pneumococci this secondary reaction only occurs following the first injection, never following the subsequent ones.

## SUMMARY AND CONCLUSIONS

1. Following repeated intracutaneous injections of heat-killed pneumococci rabbits acquire an increased skin reactivity.
2. The increased skin reactivity reaches a maximum after 4 to 6 injections have been made, after which it becomes greatly diminished.
3. The relationship of increased skin reactivity to active resistance to infection by *Pneumococcus*, and to the presence of species-specific antibodies in the blood, is still obscure.
4. The increased skin reactivity is not transferable by serum from a highly reactive to a normal rabbit.
5. After regression of the reaction to the first injection of *Pneumococcus* into the skin, there frequently follows a recrudescence, or exacerbation, of the reaction.
6. The increased skin reactivity and secondary reactions are incited alike by all types and all forms of *Pneumococcus*.

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