

REACTIONS OF RABBITS TO INTRACUTANEOUS INJECTIONS OF PNEUMOCOCCI AND THEIR PRODUCTS

V. THE DEVELOPMENT OF EYE REACTIVITY TO DERIVATIVES OF PNEUMOCOCCI

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The present paper describes the development of eye reactivity to certain products of *Pneumococcus* as a result of repeated intracutaneous injections of the heat-killed bacteria. For the purpose of comparison, additional rabbits were given injections of the cells or their derivatives by other routes and the reactivity of the eyes in these animals was also studied.

EXPERIMENTAL

Methods.—The injection of rabbits with *Pneumococcus* or its derivatives was carried out as described in a previous communication (1).

Eye Test.—Eye tests were done according to the technique described by Derick and Swift (3, 4). The cornea was anesthetized with one drop of 10 per cent novocaine, and then it was lightly scarified with a cataract knife. Three scratches were made on one of the upper quadrants of the cornea beginning 1 to 2 mm. from the sclerocorneal junction and reaching to the center. One drop of the reagent was instilled into the conjunctival sac and the material was rubbed gently over the surface of the cornea by manipulation of the lid.

Rabbits received from 8 to 12 intracutaneous injections of bacterial suspension. About 20 days after the last injection eye tests were performed by dropping into the eye, prepared as previously described, one drop of a solution of "nucleoprotein" (2). For purpose of control, two drops of the same solution were dropped into one of the eyes of each of a series of normal rabbits.

In the normal controls no changes in the eyes were observed. In certain of the animals which had previously received intracutaneous injections definite reactions occurred. These reactions were of two kinds. In the less severe type of reaction there appeared, after

several hours, a more or less extensive injection of the vessels of the palpebral and scleral conjunctivae without any change in the cornea. The maximum reactivity was attained within 24 to 48 hours and the reaction usually lasted 3 to 5 days.

In the severe type of reaction, in addition to the conjunctival injection, there developed on the first or second day a turbidity over the scarified area on the cornea. On the third or fourth day, in certain cases, small loops of blood vessels, a pannus, began to appear near the sclerocorneal margin and the vessels grew downward toward the center. Occasionally, there appeared to be hemorrhage into the cornea surrounding the newly formed blood vessels. There was frequently a certain degree of edema of the conjunctivae, and in the more severe reactions, there was present in the conjunctival sac a mucopurulent

TABLE I
The Incidence of the Ophthalmic Reaction in Rabbits Following Repeated Intracutaneous Injections of Heat-Killed Pneumococci

Total number tested	Number giving eye reaction			Number giving no eye reaction
	Conjunctival	Corneal		
		With pannus	Without pannus	
60	8	15	32	20

discharge which was proved to be bacteria-free by smears and cultures. The general corneal turbidity did not last so long as the pannus, and the eye appeared normal again within 7 to 12 days, the duration depending upon the severity of the reaction. It will be noted that the corneal reaction described here resembles very closely the ophthalmic reaction reported by Derick and Swift (4) in their studies on hypersensitiveness of rabbits to non-hemolytic streptococci.

In Table I, a summary has been made of the occurrence of the eye reaction to nucleoprotein in rabbits which had received repeated injections of heat-killed pneumococci. It is seen that of 60 rabbits tested, in 40 an eye reaction occurred, in 20 there were no reactions. Of the 40 animals showing an eye reaction, in 8 the reaction was limited to the conjunctivae, in 32 the cornea was involved, and in 15 of these

a pannus developed. Experiments were then conducted to determine whether the eye reactions were elicited by a solution of pneumococci from which the acid and the heat coagulable proteins had been removed. As in the preceding experiments, a series of rabbits were given repeated intracutaneous injections of solutions of heat-killed pneumococci. These animals were then tested by placing in the eye one drop of the *Pneumococcus* solution. Two drops of the solution were also placed in the eye of each of a series of control rabbits. It was found that while the eyes of normal rabbits were unaffected by this material, eye reactions occurred in treated animals, but not with so great a regularity as with "nucleoprotein."

In other rabbits the reactivity of the eye was tested by employing the soluble specific substances of *Pneumococcus*. The carbohydrates were used in dilutions of 1-1000, 1-10,000, and 1-25,000, but eye reactions were not observed in any of the animals tested with the homologous or heterologous carbohydrates.

Since Derick and Swift elicited the ophthalmic reaction described by them by the instillation into the eyes of living bacteria, experiments were conducted to determine whether the increased eye reactivity following the repeated intracutaneous injection of pneumococci could also be demonstrated by the instillation of living organisms. As the instillation of living S pneumococci incites an infection which may obscure the reaction, living R cells were employed. An R strain was selected which was derived from a type of *Pneumococcus* other than that used for the preparatory injections. After scarification of the cornea of each of a series of rabbits that had previously received repeated intracutaneous injections of a suspension of heat-killed pneumococci, one drop of the living culture, concentrated 25 to 30 times, was transferred into the conjunctival sac. In no instance, however, was an eye reaction observed following the instillation of living R pneumococci.

The Rate of Development and Duration of the Eye Reactivity

In the following experiment the rate of development of eye hypersensitiveness in rabbits previously injected intracutaneously with suspensions of heat-killed *Pneumococcus* was studied. Fourteen rabbits were given one or more injections each. The rabbits were divided

into seven groups of two each, and the animals of every pair received the same number of inoculations. The animals of the various pairs received a different number of inoculations, those of the first pair receiving 1 injection, those of the last pair 7 injections. Three weeks after the final injections, the rabbits were tested for eye reactivity to "nucleoprotein." The results of this experiment are given in Table II. It is apparent that there is no regularity between the development of the eye reactivity and the number of intracutaneous injections of bacterial suspension. Thus, both rabbits receiving only 1 inoculation of bacterial emulsion showed severe eye reactions, while the two

TABLE II

The Development of Eye Reactivity in Rabbits Injected Intracutaneously with Heat-Killed Pneumococcus

Number of rabbits	Number of injections	Results of eye reaction			
		Negative	Positive		
			Conjunctival	Corneal	
				With pannus	Without pannus
2	1	0	0	2	0
2	2	0	1	1	0
2	3	1	0	1	0
2	4	1	0	0	1
2	5	0	0	1	1
2	6	2	0	0	0
2	7	0	0	1	1

that received 6 injections showed no reactions; and again, eye reactions were obtained in both rabbits which had received 7 inoculations of pneumococci in the skin. This irregularity indicates that individual rabbits vary greatly in the manner in which they react to intracutaneous injections of pneumococci, especially as regards the development of eye hypersensitiveness.

In another experiment, the duration of the eye reactivity was studied. It was found that there is considerable variation in different animals. The reactivity of the eye may last as long as 4 months; in two rabbits, the eye reaction was elicited 6 months after the last intracutaneous injection of bacterial suspension.

During the course of this study, rabbits which had previously been shown to be eye sensitive but in which the eye sensitiveness had later disappeared were subsequently injected with "nucleoprotein" solution intravenously or by some other route. It was frequently noted under these conditions that although the cornea was not scarified and nothing was instilled into the eye, following the subsequent injection of the protein, the conjunctivae became congested and a typical eye reaction reappeared. The longer the interval between the disappearance of the ophthalmic reaction and the subsequent injection of protein, the less frequently did this reappearance of the reaction take place. However, in one rabbit, under these conditions an eye reaction reappeared after an interval of 6 months.

The Development of Eye Reactivity Following Administration of the Intact Cell by Different Routes

The following experiment was carried out to determine whether eye reactivity results not only when injections of heat-killed pneumococci are made into the skin but also when the injections are made by other routes. Accordingly, several rabbits of one group were given repeated intravenous injections and those of another group intraperitoneal injections. The results of this experiment are summarized in Table III. It will be seen that in rabbits injected intravenously eye reactions were never obtained. Only two rabbits were given intraperitoneal injections, and neither gave an eye reaction when tested. It is appreciated, however, that the number in this instance is too small to allow a definite conclusion. Swift and Derick (4) have also shown in the case of hypersensitiveness to nonhemolytic streptococci, that the increased eye reactivity does not result from intravenous injections.

The Development of Eye Reactivity Following Repeated Injections of Soluble Derivatives of Pneumococcus

A study was made of the incidence of the eye reactivity in rabbits that have received repeated injections of solutions of various cell constituents. Rabbits were given intracutaneous or intravenous injections of (1) a solution of the bacteria prepared by freezing and thawing, (2) a solution of the "nucleoprotein" obtained by precipita-

tion of the bacterial solution by weak acid, (3) the supernatant fluid after precipitation of the "nucleoprotein," and (4) a solution of the bacterial cells resulting from autolysis. In Table III a summary is given of the results of this experiment. It is seen that in none of a total of 41 rabbits have eye reactions been observed after the animals had received repeated intracutaneous or intravenous injections of solutions derived from pneumococci.

TABLE III
The Incidence of the Eye Reaction in Rabbits Repeatedly Injected with Pneumococci or Their Products by Different Routes

Material administered	Route of administration	Number of rabbits	Result of eye test	
			Positive	Negative
Heat-killed Pneumococcus (R or S)	Intracutaneous	60	40	20
	Intravenous	91	0	91
	Intraperitoneal	2	0	2
Solutions	Intravenous	17	0	17
Nucleoprotein	Intracutaneous	8	0	8
	Intravenous	8	0	8
Solution (nucleoprotein removed)	Intracutaneous	4	0	4
Autolysates	Intravenous	6	0	6

The Development of Eye Reactivity Following Infection by Pneumococcus

The question naturally arises whether eye reactivity accompanies or follows infections by *Pneumococcus*. Rabbits were infected by injections of live cultures of *Pneumococcus*. Infection was induced by three different routes.

1. *Infection by Skin*.—Each of 18 rabbits received an intracutaneous injection of 0.2 cc. of an 8 to 10 hour culture of Type III *Pneumococcus*. All the animals developed a septicemia lasting from 1 to 7 days, and all but 2 eventually recovered from the infection. Viable organisms were recovered from the skin lesion only

during the first 2 to 4 days. About 3 weeks after infection had been induced, the animals were tested by the instillation into the conjunctival sac of "nucleoprotein" derived from a strain of *Pneumococcus* Type II. Five of the 16 rabbits gave positive eye reactions.

2. *Infection by the Trachea.*—Twelve rabbits received varying amounts of young broth cultures introduced intratracheally. Temperature variation in these animals indicated that infection occurred. All recovered. Sixteen days later eye tests were conducted in the usual manner. Of the 12 rabbits, 4 gave the ophthalmic reaction when tested in the usual way.

3. *Infection by Spraying.*—Seventeen rabbits survived infection by spraying with pneumococci according to the technique described by Stillman (5). These animals were tested for the presence of eye reactivity 2 to 3 weeks after the spraying. Of the 17 rabbits, 5 gave positive eye reactions. The data of the experiment are given in Table IV.

TABLE IV

The Incidence of the Eye Reaction in Rabbits Following Artificial Infection with Pneumococcus

Method of infection	Number of rabbits	Eye reaction	
		Positive	Negative
Skin.....	16	5	11
Intratracheal.....	12	4	8
Spraying.....	17	5	12

It is evident that eye reactions may be elicited in rabbits following experimental infection by *Pneumococcus* in the skin or trachea.

Relation between Eye Reactivity and Active Resistance to Infection

It has been pointed out in an earlier communication (6) that rabbits acquire an active resistance to infection following repeated intracutaneous injections of heat-killed pneumococci. It is now seen that rabbits so injected also may develop an eye reactivity to derivatives of *Pneumococcus*. On the other hand, rabbits receiving intravenous injections also acquire a resistance to infection, but they do not become eye reactive. Moreover, rabbits which have been given repeated injections of solutions derived from *Pneumococcus* acquire neither a resistance to infection (6), nor eye reactivity. While it may be concluded, therefore, that eye reactions do not necessarily accompany

active resistance to infection it is important to note that eye reactions were never observed in animals without resistance to infection.

Relation of Eye Reactivity to the Secondary Reaction

It has been previously reported (7) that the reaction at the site of injection following the first intracutaneous inoculation of heat-killed pneumococci in a rabbit usually disappears within 4 or 5 days. At about the tenth day, however, in certain animals there occurs a recrudescence. It seemed of interest to determine if possible whether the animals showing a secondary reaction were especially prone to develop eye hypersensitiveness. The data bearing on this point are summarized in Table V. It will be seen that a total of 23 rabbits were observed. Of 15 animals developing a secondary reaction, 12

TABLE V

Relation of the Ophthalmic Reaction to Secondary Skin Reaction

Total number of rabbits	Rabbits giving eye reaction	Rabbits not giving eye reaction
With secondary skin reaction, 15.....	12	3
Without secondary skin reaction, 8.....	3	5

gave a positive ophthalmic reaction, while 3 did not. Of 8 rabbits showing no secondary reaction, only 3 gave positive eye reactions. Although there is no absolute correlation between the development of eye sensitivity and the secondary reaction, nevertheless, eye reactions appear to occur with greater frequency in the rabbits which show the secondary reaction. Derick and Swift (4) also report the existence of a "rough parallelism" between the occurrence of eye reactions and the secondary reactions in rabbits injected with *Streptococcus viridans*.

DISCUSSION

The present communication records the development of an increased eye reactivity in certain rabbits which have previously received repeated intracutaneous injections of heat-killed pneumococci. The eye reactivity is manifested by a reaction, after scarification of the

cornea, to either the "nucleoprotein" or a solution of *Pneumococcus* from which the acid precipitable and heat coagulable proteins have been removed. The reaction, in the less severe form, consists of the injection of the conjunctival blood vessels; in the more severe forms, the cornea is also involved and exhibits a turbidity with frequently the formation of a pannus. Living R cells or the specific soluble substances of *Pneumococcus* do not elicit the eye reaction.

Unlike the skin reactivity reported previously, the eye reactivity does not occur in rabbits which have been given repeated intravenous injections of the heat-killed bacteria. Eye reactivity does not follow repeated intravenous or intracutaneous injections of rabbits with various solutions derived from the organism.

Increased eye reactivity was also found to be present in animals which had recovered from infection induced by injection of pneumococci into the skin or trachea, or by spraying. The eye reaction was elicited only in animals resistant to infection, but the relationship of the eye reactivity to active resistance still remains obscure. The eye reaction occurred more frequently in rabbits with a secondary reaction following the primary skin reaction to the first intracutaneous injection of bacteria than it did in animals not exhibiting a secondary reaction.

It may be of interest to point out that Clough (9) called attention to the "ophthalmo-reaction" in pneumonia patients. Without scarifying the cornea, he observed in 6 of 15 patients an eye reaction to one drop of 1 per cent *Pneumococcus* protein, while only 1 of 20 normal individuals gave a similar reaction.

SUMMARY AND CONCLUSIONS

1. About two-thirds of the rabbits injected intracutaneously with suspensions of heat-killed S or R pneumococci develop an increased eye reactivity.

2. Eye reactions in these animals may be elicited by the instillation of "nucleoprotein," or of a solution of *Pneumococcus* from which the acid precipitable and heat coagulable proteins have been removed. The eye reactions are not elicited, under the conditions described, by living R cells or the protein-free, type-specific, polysaccharides.

3. Rabbits do not develop an increased eye reactivity following intravenous injections of the intact cell.
4. Rabbits do not develop an increased eye reactivity following injections of soluble derivatives of *Pneumococcus*.
5. Experimental infection by *Pneumococcus* may stimulate eye reactivity in rabbits.
6. Eye reactivity occurs in animals actively resistant to infection.
7. Eye reactions are observed more frequently in rabbits which show the secondary reaction.

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