

REACTIONS OF RABBITS TO INTRACUTANEOUS INJECTIONS OF PNEUMOCOCCI AND THEIR PRODUCTS

IV. THE DEVELOPMENT OF SKIN REACTIVITY TO DERIVATIVES OF PNEUMOCOCCUS

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Many reports have been made concerning the occurrence of reactions in the skin following the intracutaneous injection of Pneumococcus products in pneumonia patients at the time of crisis, or after the crisis, or into guinea pigs after they had previously received injections of the organism or its derivatives. These reports indicate that the reactivity of the skin to certain derivatives of Pneumococcus may become increased after the patient or animal has undergone infection, or after they have been injected with certain products of Pneumococcus parenterally. It has usually been assumed, therefore, that a state of increased reactivity of the skin to Pneumococcus protein may occur, probably analogous to the state of hypersensitiveness which arises following the administration of such a foreign protein as egg albumin. The exact conditions under which this state of altered reactivity of the skin may occur are not as yet well understood. Moreover, the relation of this condition of increased skin reactivity to the general state of altered reactivity or "allergy" is still as obscure in the case of pneumococci and their products as it is in the case of most other reacting agents. An understanding of the underlying principles can probably be arrived at only after the various phenomena have been carefully observed and recorded under a great variety of conditions and in various animals. Experience has shown that the various phenomena thought to be related to the general condition of hypersensitiveness, such as reactions occurring in the skin following the injection of foreign substances, the ophthalmic reaction, anaphylaxis in the guinea pig, increased reaction

of the musculature of the uterus, serum sickness, etc., usually cannot all be elicited in the same animal. It is also probable that they are not necessarily all manifestations of the same biological process.

In the present study an investigation has been made of the skin reactions in rabbits following the injection of certain derivatives of the pneumococcal cells.

This work is a part of the investigation of the changes which take place in rabbits following the repeated intracutaneous injections of suspensions of heat-killed pneumococci, some of the results of which have been reported in previous communications (1, 2, 3). Consequently most of the skin tests have been made in rabbits that have been previously treated in this way, but for the sake of comparison, skin tests have also been performed in normal rabbits and in rabbits treated in various other ways. The derivatives of pneumococci employed in the tests have been the so-called "nucleoprotein," the supernatant fluid after precipitation of the "nucleoprotein" from the bacterial extract and to a less extent the carbohydrate fraction, the specific polysaccharide. The method of preparing the first two derivatives was given in a previous communication (1). The specific polysaccharides were obtained from Dr. Avery and the method of their preparation has been fully described (3). The rabbits were prepared by depilation of the skin over the abdomen as previously reported (1).

The first skin tests were made in rabbits which had received repeated intracutaneous injections of suspensions of heat-killed pneumococci. About 3 weeks after the animal had received the last of 8 to 12 intracutaneous injections, tests were performed by injecting into the skin a solution of "nucleoprotein." The volume of fluid injected for each test was 0.2 cc. and the amount of the test substance was measured by the determination of the nitrogen present, considering this as all derived from protein. Control tests have been made repeatedly in normal animals that had received no preliminary treatment, and in none of these animals have reactions in the skin been observed following the injection of amounts of "nucleoprotein" two to four times larger than any doses employed in testing the previously treated animals.

It has been found that when 0.2 cc. of a solution of "nucleoprotein" is injected into the skin of a rabbit which has previously received intracutaneous injections of heat-killed pneumococci, within a few hours the skin about the site of injection becomes red, raised and edematous. The reactions reach a maximum in 24 to 48 hours, then begin to fade

and usually disappear within 3 to 4 days. The degree of reaction depends to a considerable extent on the amount of the protein contained in the solution. Reactions may occur with amounts of protein as small as 0.0025 mg. Usually larger amounts 0.2 to 0.6 mg. have been injected. Under the latter circumstances the area of skin involved in the reaction measures on an average 1.5 to 2 cm. in diameter. A certain area of erythema not infrequently is seen but a breaking down of the skin has never been observed.

The reactivity of the skin to the supernatant fluid after precipitation of the "nucleoprotein" from the bacterial extract was also studied in rabbits following repeated intracutaneous injections of heat-killed pneumococci. A solution of the concentrated bacteria was made by repeated freezing and thawing. The "nucleoprotein" was then precipitated out with acetic acid and the resulting supernatant fluid, adjusted to slightly acid to litmus, was boiled over a free flame for 10 minutes. This extract gave none of the usual qualitative tests for protein. In the doses employed in this study, it was found to possess a primary toxicity, so that in over half the normal rabbits, it caused a local reaction in the skin consisting of an erythematous blush with thickening. In these animals the reaction disappeared in 1 to 3 days. In rabbits which had previously received repeated intracutaneous injections, the skin reaction following the administration of this extract was more marked. The area of the skin involved was larger, the elevation was more marked, and a central area of ecchymosis, which was never seen in the untreated animals, was not infrequently observed. In some instances the skin over the lesion broke down. Moreover, in these animals the reactions lasted longer, disappearing in 3 to 5 days.

In some of the rabbits the reactivity of the skin to the specific soluble substances, the polysaccharides, was also studied. Dilutions of 1-1000, 1-10,000 and 1-25,000 of the carbohydrates were injected in the skin about 3 weeks after the last intracutaneous injection of heat-killed pneumococci. No reactions were seen following the intracutaneous introduction of the carbohydrate derived from homologous or heterologous types of *Pneumococcus*.

In terms of bacterial specificity, then, the skin reactions observed in rabbits after repeated intracutaneous injections of heat-killed

pneumococci are not type-specific, but species-specific. The skin reactions to the derivatives of *Pneumococcus* are elicited by materials which are not associated with type-specificity, and in all instances, the test reagents were derived from a type other than that employed during the repeated injections.

The Occurrence of the Skin Reaction Following Repeated Injections, by Different Routes, of Pneumococci or Their Products

Experiments were next undertaken to determine whether rabbits which have received repeated intravenous or intraperitoneal injections of dead pneumococci exhibit an increased reactivity of the skin, just as do animals which have received repeated intracutaneous injections.

TABLE I

The Incidence of the Skin Reaction to Pneumococcus Protein in Rabbits Following Repeated Injections of Pneumococcus or Its Derivatives

Material injected	Route of injection	Number of rabbits	Skin test	
			Positive	Negative
Heat-killed <i>Pneumococcus</i>	Intracutaneous	60	60	—
Heat-killed <i>Pneumococcus</i>	Intravenous	40	40	—
Heat-killed <i>Pneumococcus</i>	Intraperitoneal	2	2	—
Nucleoprotein.....	Intracutaneous	8	8	—
Nucleoprotein.....	Intravenous	8	8	—
Supernatant fluid from bacterial extract.....	Intracutaneous	4	4	—

It was found that in the former animals, skin reactions of the same nature might follow the injections of the derivatives of pneumococci. The data are presented in Table I. It is seen that 40 rabbits received intravenous administration, and two, intraperitoneal injections of heat-killed bacteria. All the rabbits were found to be skin reactive.

While skin reactions to the nucleoprotein were obtained with regularity in this group of rabbits following intravenous injections of pneumococci, it has been found that when skin tests are undertaken, as here, 3 weeks after the last injection of organisms, the skin reaction may not be of great severity or in some cases it may be absent.

In another experiment, the skin reactivity was studied in rabbits which had previously received repeated injections of the "nucleoprotein," also in rabbit which had received repeated injections of the supernatant fluid after precipitation of the "nucleoprotein" from a bacterial extract. The data of this experiment are given in Table I. It is seen that 8 animals received the "nucleoprotein" intracutaneously and 8, intravenously, while 4 rabbits received the supernatant fluid from the bacterial extract intracutaneously. The animals were tested for skin reactivity 19 days after the last injection of these materials. Skin reactions, as described above were obtained in all the animals.

It may be concluded, therefore, that regardless of the material employed for the injections and irrespective of the route of administration of the material, skin reactivity resulted in all the rabbits.

Relation of the Skin Reaction to Resistance to Infection

In a previous publication (3), it was shown that following repeated intracutaneous injections of heat-killed pneumococci, rabbits acquire a marked degree of resistance to infection by *Pneumococcus* of homologous or heterologous types. It was also shown that following similar injections with the "nucleoprotein" and supernatant fraction of the bacterial extract of *Pneumococcus*, rabbits do not become resistant to infection. In the present study it has already been pointed out that skin reactivity to derivatives of *Pneumococcus* is found to occur in rabbits which have been injected by any route with the intact cell or its protein derivatives. It is obvious, therefore, that skin sensitivity occurs with equal frequency in animals which are resistant to infection (*i.e.*, following injections with the intact cell) and in non-resistant animals, as for example, following repeated injections with derivatives of *Pneumococcus*.

Relation of the Skin Reaction to Circulating Antibodies

It has already been stated above that the skin reaction described in this communication cannot be related to type-specificity. The skin reactions are elicited by a species-specific reagent in the absence of demonstrable type-specific antibodies, as is seen, for example, in the rabbits which receive repeated intracutaneous injections of heat-killed pneumococci (1). On the other hand, the skin reaction to the "nucleo-

protein" always occurs in the presence of circulating species-specific antibodies, and in a general way the intensity of the reaction to a given quantity of protein varies with the titre of species-specific antibodies in the sera of the animals.

DISCUSSION

That increased reactivity of the skin to *Pneumococcus* or its products may be induced artificially in animals or may accompany natural infection by *Pneumococcus* has been pointed out by a number of authors.

Mackenzie and Woo (1) described the development of a skin reaction to bacterial protein following intracutaneous injections of an alkaline extract of *Pneumococcus*. Zinsser and Tamiya (12) reported that guinea pigs sensitized by injections of pneumococci or the bacteria protein gave skin reactions to autolysates and they concluded that this reaction was not related to type-specificity. Later Zinsser and Grinnell (13) induced skin sensitization in guinea pigs to autolysates of *Pneumococcus* by previous inoculations of the same material. More recently Bull and McKee (14) demonstrated that after recovery from infection induced by intranasal instillation of live pneumococci, rabbits become highly skin sensitive to an autolysate of pneumococci. These authors also reported that such rabbits die of shock following intravenous injection of the same material.

Skin reactions in patients suffering with pneumonia have also been reported. Clough (6) showed that pneumonia patients reacted locally when a protein extract of the organism was injected into the skin, but this reaction did not vary materially from the one occurring in normal individuals. Weil (15) described a skin reaction in patients to an autolysate of *Pneumococcus* which begins to appear at about the time of crisis. Steinfeld and Kolmer (16) reported the occurrence of a skin reaction following intracutaneous injections of heat-killed bacteria in 30 per cent of pneumonia patients after crisis. Later Weiss and Kolmer (17) showed that patients with pneumonia gave skin reactions to pneumotoxin (a sodium choleate solution of the cell) from the fifth to the thirteenth day of the disease. Bigelow (18) also obtained skin reactions in pneumonia patients with heat-killed pneumococci and several soluble derivatives. Herrold and Traut (19) found that 73 per cent of pneumonia patients failed to react to injections into the skin of a filtrate of a 5-day culture of *Pneumococcus*, while only 15 per cent of normal persons failed to react. Poole, Bumstead and Blake (21) obtained skin reactions with a protein extract of *Pneumococcus* in patients after crisis. Tillett and Francis (20) have recently demonstrated that patients with pneumonia react locally to injections of the protein-free carbohydrate of *Pneumococcus*. The reaction occurs at about the time of crisis, is type-specific, immediate, and is of the wheal and erythema variety. They also obtained delayed skin reactions in patients with the

nucleoprotein of *Pneumococcus*. Except for immediate reactions to the carbohydrate described by Tillett and Francis the skin reactions described by all these observers followed injections of whole bacteria or protein derivatives and the reactions were of the "delayed" type.

In the present paper is reported the occurrence of skin reactions in rabbits to derivatives of *Pneumococcus*. It has been found that following repeated intracutaneous injections either of heat-killed *Pneumococcus*, or the "nucleoprotein," or of the supernatant liquid after the "nucleoprotein" has been precipitated from a bacterial extract, rabbits become skin reactive. The skin reactions are of the delayed type and may be elicited by injections of "nucleoprotein," or of a solution of the cells from which both acid precipitable and heat coagulable proteins have been removed. This skin reactivity, however, is not elicited by the specific carbohydrate of *Pneumococcus*.

The skin reactivity was found to occur in rabbits following repeated injections by the intravenous or intracutaneous route of the bacteria, or the protein derivatives of *Pneumococcus*. The skin reactivity to *Pneumococcus* protein appears to be unrelated to type-specificity, or to resistance of the animal to infection. On the other hand, the occurrence of the skin reactivity appears to be associated with the presence of circulating species-specific antibodies.

SUMMARY AND CONCLUSIONS

1. A skin reaction elicited by the injection of the *Pneumococcus* "nucleoprotein," or of a solution of the cells from which the acid and heat-coagulable proteins have been removed is described in rabbits which have previously received repeated intracutaneous injections of heat-killed pneumococci.
2. In terms of bacterial specificity, the skin reaction is considered to be not type-specific, but species-specific.
3. A similar skin reaction to the proteins of *Pneumococcus* occurs in rabbits following the repeated administration by the intravenous or intracutaneous route of the heat-killed organisms or their protein derivatives.
4. The skin reaction may occur independently of resistance to infection.
5. The skin reaction appears to be related to the presence of circulating species-specific antibodies.

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