STUDIES ON THE BIOLOGY OF STREPTOCOCCUS.

VII. Allergic Reactions with Strains from Erysipelas.

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Dochez and Sherman (1) have recently described sensitization reactions which could be neutralized by immune serum. These reactions were obtained in guinea pigs and rabbits which had been previously inoculated with living cultures of *Streptococcus scarlatinæ* or with filtrates of this streptococcus. About 10 days after the inoculation, intracutaneous injections of filtrate of cultures of scarlatinal streptococcus caused local edema and erythema. The reaction was not obtained with filtrate which had been heated or with mixtures of filtrate and scarlatinal immune serum.

Some strains of hemolytic streptococcus recovered from erysipelas produce toxic substances similar in certain respects to the substances produced by strains from scarlet fever. Cutaneous reactions may be obtained in man with the filtrates of the strains from erysipelas. The reactions obtained with a majority of the filtrates from these strains are not neutralized by scarlatinal sera but may be neutralized with sera prepared with erysipelas strains. Having this similarity in mind, we have studied the cutaneous reactions in rabbits during immunization with filtrates of strains from erysipelas.

Methods and Experimental Data.

Two strains of hemolytic streptococcus, EA and EM, were selected for the immunization of the rabbits employed in these experiments. One strain was obtained from facial erysipelas, and the second from a blood culture on a patient with phlebitis. Filtrates from cultures of both strains caused local erythema when injected intracutaneously in children and adults. The strongest reactions were obtained in children with filtrates from 48 hour cultures in tryptic digest broth.

Reactions were not obtained with filtrate which had been heated 2 hours in the Arnold sterilizer. Scarlatinal antitoxin added to the filtrates before injection did not modify the size or intensity of the erythema following intracutaneous inoculation, but the serum from rabbits immunized with erysipelas filtrates completely neutralized some of the reactions occurring with a 1/250 dilution of the filtrate.

A number of normal rabbits were tested with 0.1 cc. of undiluted filtrate before sensitization. About 20 per cent showed an indefinite erythema at the site of the intracutaneous inoculation, but none of the reactions observed at this time was as intense as the mildest reactions occurring later during immunization. Following these preliminary tests, six of the rabbits were inoculated intracutaneously with 1.0 cc. of undiluted filtrate at intervals of about 12 days. On the day preceding each inoculation skin tests were done with 0.1 cc. of the undiluted filtrates of cultures of the two strains EA and EM. Control tests were done with filtrate heated 2 hours at 98°C.

After the third inoculation edematous reactions were observed in four of the six rabbits at the site of the cutaneous injection of the filtrate. Edema and erythema occurred about 24 hours after 0.1 cc. of the undiluted filtrate was injected into the skin. This erythema lasted approximately 48 hours. The first positive reactions were observed about the 30th day after immunization was begun and occurred only with unheated filtrate. At this time the heated filtrates did not cause reactions. After the fourth inoculation erythema and some edema occurred with the heated as well as the unheated filtrate. After the fifth inoculation all the reactions were less intense than those previously observed and after the 57th day strongly positive reactions were no longer obtained. Two rabbits showed no sensitization during this period of observation.

Intracutaneous inoculations of 1.0 cc. of filtrate were continued until seven or eight injections had been given. During the interval between the time that the animals last showed cutaneous sensitization (approximately the 57th day) and the final intracutaneous inoculation two of the rabbits showed a transient recrudescence of activity. During this recrudescence the reactions obtained were exceedingly mild. After the seventh or eighth intracutaneous inoculations which were given between the 80th and the 100th days, filtrates were injected intravenously. 5 cc. and later 10 cc. were given at intervals of 2 weeks over a period of 3 months.

On the 187th day after the first intracutaneous inoculations, when the animals had been immunized for 3 months intracutaneously and 3 months intravenously, cutaneous reactions were again done. At this time all the rabbits, including the two which had previously shown no cutaneous hypersusceptibility reacted strongly with the unheated and heated erysipelas filtrates and with a filtrate of a scarlatinal streptococcus. Tests were also done at this time with filtrates of a strain of hemolytic streptococcus from a normal throat and with broth. Only one of five rabbits reacted with the throat strain. Two of the five gave questionable reactions with broth. None of the reactions was neutralized by the addition of scarlatinal or erysipelas immune sera to the filtrates previous to the injection. Serum was

TABLE I.

Cutaneous Allergic Reactions with Filtrates of Strains of Hemolytic Streptococcus from Erysipelas during the Immunization of Rabbits with Filtrates of Erysipelas Strains.

Intervals	Rabbit I					Rabbit II					Rabbit VI					Rabbit X					Rabbit XIV					Rabbit XVI				
	Cutaneous reactions					Cutaneous reactions					Cutaneous reactions					Cutaneous reactions					Cutaneous reactions					Cutaneous reactions				
	Method of inoculation	Filtrate A unheated	Filtrate A heated	Filtrate M unheated	Filtrate M heated	Method of inoculation	Filtrate A unheated	Filtrate A heated	Filtrate M unheated	Filtrate M heated	Method of inoculation	Filtrate A unheated	Filtrate A heated	Filtrate M unheated	Filtrate M heated	Method of inoculation	Filtrate A unheated	Filtrate A heated	Filtrate M unheated	Filtrate M heated	Method of inoculation	Filtrate A unheated	Filtrate A heated	Filtrate M unheated	Filtrate M heated	Method of inoculation	Filtrate A unheated	Filtrate A heated	Filtrate M unheated	Filtrate M heated
days																														
1	m	−	−	−	−	m	±	±	±	±	m	+	−	+	−	m	−	−	−	−	a	±	−	±	−	a	−	−	−	−
12	m	−	−	−	−	m	±	−	±	−	m	+	−	±	−	m	−	−	−	−	a*	±	−	−	−	a*	−	−	−	−
24	m	±	−	±	−	m	±	−	−	−	m	−	−	−	−	m	−	−	−	−	a*	+	−	−	−	a*	−	−	−	−
34	m	++	+	−	−	m	+++	−	++	−	m	+++	±	++	−	m	−	−	−	−	a*	±	−	−	−	a*	−	−	−	−
46	m	+	−	−	−	m	++++	++	++++	+	m	++	+	+	+	m	−	−	−	−	a	++	+	+	−	a	−	−	−	−
57	m	±	−	−	−	m	++	+	−	−	m	+	−	+	−	m	−	−	−	−	a	±	−	±	−	a	−	−	−	−
68	m	−	−	−	−	m	−	−	±	−	m	±	−	±	−	m	−	−	−	−	a	−	−	−	−	a	−	−	−	−
78	m	−	−	−	−	m	+	−	±	±	m	+	+	+	±	m	−	−	−	−	A	−	−	−	−	A	−	−	−	−
92		−	−	−	−		−	−	−	−		±	−	−	−		−	−	−	−	A					A				
115	M	−				M	−				M	−				M	−				A*	−				A*	−			
123	M					M					M					M					A					A				
136	M					M					M					M														
153	M					M					M					M					A*					A*				
176	M					M					Died of respiratory disease					M					A*					A*				
187		++	+++				+++	++									+++	++												
210		+++	+++				+++	++									++++	++++				++++	++++				++++	++++		

Six rabbits were immunized with filtrates of two strains of hemolytic streptococcus from erysipelas. Filtrates of these strains have been designated by the letters A and M corresponding to the strain with which the filtrates were prepared. The rabbits were first inoculated intracutaneously and later intravenously at the intervals indicated in the first column on the left. Intracutaneous filtrate and intravenous filtrate are indicated by small and capital letters respectively in this table. Inoculations followed by an asterisk were given only approximately at the interval indicated in the first column. Preceding each inoculation cutaneous reactions were done with heated and unheated filtrates A and M. Each rabbit was tested with these four preparations. The reactions resulting from these intracutaneous tests are indicated by plus signs. Plus signs in heavy type have been used for edematous reactions.

obtained from each animal at this time and later tested for neutralizing substances in a second series of hypersusceptible animals. After the serum was obtained, desensitization was attempted with filtrates of scarlatinal and erysipelas strains, and with broth. 10 cc. of filtrate or broth were given intravenously 24 hours previous to intracutaneous tests. Desensitization occurred with the streptococcal filtrates but not with broth.

TABLE II.

The Neutralization of Cutaneous Allergic Reactions in Rabbits Sensitized with Filtrates of Strains of Hemolytic Streptococcus from Erysipelas.

Sensitized rabbit tested	Reaction with 0.1 cc. of Filtrate M	Filtrate M and Serum I		Filtrate M and Serum II		Filtrate M and Serum X		Filtrate M and Serum XIV		Filtrate M and Serum XVI	
		Mixture a	Mixture b	Mixture a	Mixture b	Mixture a	Mixture b	Mixture a	Mixture b	Mixture a	Mixture b
28	**++++**	**+++**	+	**+++**	−	**++++**	+	**++++**	−	**+++**	±
27	**+++**	**++**	±	−	−	**++++**	±	**+++**	−	**+++**	−
26	**++**	**+**	−	−	−	**++**	−	**++**	−	**++**	−
25	**+**	+	±	−	±	**+**	−	**+**	−		±
29	**+**	±	−	−	−	±	−	±	−	−	−
24	+	+	−	−	−	+	−	±	−	−	−

Six rabbits were sensitized with filtrates of strains of hemolytic streptococcus from erysipelas. The numbers of these rabbits are given in the first column. When the rabbits first showed cutaneous reactions with 0.1 cc. of erysipelas filtrate, neutralization of these reactions was attempted by the addition of immune erysipelas sera obtained from rabbits immunized by intracutaneous and intravenous injections of filtrate from erysipelas strains to the filtrate previous to injection. The sera of five immunized rabbits (Table I) were tested for neutralizing qualities. Each serum was added to filtrate of Strain M. Two proportions of serum and filtrate were employed, Mixture a (serum one part and filtrate four parts) and Mixture b (serum and filtrate equal parts). The rabbits showed no reaction with heated filtrate at the time the neutralization tests were done. Edematous reactions have been indicated in heavy type.

The sera obtained from five of these immunized rabbits were tested for neutralizing substances in a second series of sensitized rabbits. Twenty rabbits were given intracutaneous injections of erysipelas filtrate at weekly intervals until they were sensitive to cutaneous inoculations with 0.1 cc. of the filtrate. Only eight of the twenty rabbits became allergic. The neutralization tests were carried out as soon as allergy developed. Two mixtures of each serum and the filtrate—one part of serum and four of filtrate, and equal parts of serum and filtrate—were pre-

pared and incubated 3 hours. Each of the allergic rabbits was then tested with the two proportionate mixtures of each of the five sera and the filtrate. The amounts of the mixtures used were so graduated that 0.1 cc. of filtrate was injected. Although neutralization occurred with both proportions of sera and filtrate, it was most complete and uniform with the mixture containing equal parts. The neutralizing properties of the immune sera were not uniform, but no relationship was discerned between the strength of the serum and the degree of hypersusceptibility to filtrate shown by the rabbit from which the serum was obtained.

When these neutralization experiments were carried out, these rabbits reacted with unheated erysipelas filtrate but not with heated. 2 weeks later positive reactions were obtained with heated erysipelas filtrate and with filtrates of scarlatinal strains. At this time neutralization of the reaction by the addition of immune serum to the filtrate was questionable. These animals were desensitized with scarlatinal and erysipelas filtrates intravenously. 10 cc. of broth did not have this desensitizing effect.

The results of these experiments have been tabulated. Table I gives the intervals between the injections of filtrate, the method of inoculation, whether intracutaneous or intravenous, the filtrate employed, and the results of the cutaneous tests. Edematous reactions are indicated by heavy type. The neutralization tests with immune erysipelas sera and filtrate have been arranged in Table II.

DISCUSSION.

Allergic Phases during Immunization.—The rabbits which we have immunized apparently passed through consecutive periods of sensitization—a negative period from the first inoculation to the appearance of the first positive reactions, a positive allergic phase of approximately 4 weeks' duration, next a prolonged period of inactivity, and finally a second phase during which they showed cutaneous sensitization. The first period of negative reactions need not be discussed; it is observed previous to all hypersusceptible reactions before the development of sensitivity. In the animals studied this period varied from 2 to 4 weeks. The second, third, and fourth periods require discussion.

The second period was one of positive hypersusceptible reactions. This period is divided into an early phase of short duration, during which the reactions were neutralized by the addition of immune serum to the filtrate, and a later phase when the reactions were not neutralized. During the first part of this phase when the reactions were

neutralized reactions occurred only with unboiled erysipelas filtrate. The reactions observed later when they were not neutralized by immune serum were less specific, and could be obtained with heated erysipelas filtrate and with scarlatinal filtrate. The most intense reactions were obtained with the erysipelas filtrates.

The third period was a phase of inactivity of uncertain duration. This third phase began about the 68th day when hypersusceptible reactions were no longer obtained. The transition from the second period to this non-allergic state was gradual. The intensity of the reactions gradually diminished from about the 40th day of immunization until the 68th day when not even the slightest erythema occurred after the intracutaneous injection of filtrate. This gradual fading of hypersusceptible reactions has been observed by Zinsser and Grinnell (2) with streptococcus and by Mackenzie and Woo (3) with pneumococcus. Except for the positive reactions occurring later, this negative phase might be considered a return to the normal non-allergic state, or as Zinsser suggests, a state of immunity to the allergin due to the presence of antibodies.

The fourth phase was a positive phase in which the reactions with heated and unheated erysipelas filtrate and with scarlatinal filtrate were equally intense. This phase differed from the first positive phase in three respects; the reactions could not be neutralized, unheated and heated filtrate gave reactions of equal intensity, and all animals, some of which did not become hypersusceptible during the first allergic period were found hypersusceptible at this later time. During this period some of the animals were sensitive to horse serum.

Possible Factors Responsible for Allergic Reactions.—The simplest explanation of the various phases of allergy observed in these experiments is the assumption that the phases are the result of hypersusceptibility to different allergins. Dale and Hartley (4) have shown that when serum albumen and globulin are employed to sensitize animals, the intervals required for sensitization are not identical. This would appear to be a satisfactory explanation for the early and late periods of allergy. The somewhat different character of the reactions observed during the two periods supports this hypothesis.

We have already observed that allergic reactions occurring between the 30th and the 60th days of immunization could be neutralized with immune erysipelas serum up to about the 15th day after positive

cutaneous reactions first occurred. Except for the report of Dochez and Sherman we know of no authenticated instance where cutaneous allergic reactions with bacteria have been neutralized with immune serum. Reactions occurring in animals sensitized with the bodies of tubercle bacillus, staphylococcus, streptococcus, pneumococcus, and the Gram-negative pathogens or with extracts of these bacteria have never been satisfactorily neutralized. The filtrates of streptococci from scarlet fever and erysipelas contain toxic substances which are easily separated from the bacterial cells. In one respect these toxic substances resemble the exotoxins of the *diphtheriæ* and tetanus bacilli in that a satisfactory antitoxic serum can be developed by the immunization of animals. Von Behring observed sensitization to diphtheria toxin in 1893. Since this observation allergy to other bacterial exotoxins has been produced experimentally in animals. In view of these previous instances of allergy to exotoxins, and the neutralization of the cutaneous allergic reactions with immune serum in our experiments it seems possible that these cutaneous reactions are sensitization reactions to the toxic substances in the erysipelas filtrate. It also seems probable that the toxicity of these streptococcus products for man is due largely to a state of hypersensitiveness. The less specific reactions occurring later during this first period of sensitization could not be neutralized. These late reactions appear analogous to the sensitization occurring with the bodies and cell extracts of bacteria.

Experimental evidence at the present time indicates that bacterial antigens are of a composite nature and that two substances at least are linked together in the bacterial cell. Zinsser has found that precipitates from extracts of tubercle bacillus contain a non-protein substance designated the "residue antigen," and a second fraction containing nucleoproteins. These fractions are comparable to the S and P substances which Avery and Heidelberger (5) have obtained from the pneumococcus. The S substance is non-antigenic and incapable of inducing allergy. The P substance is antigenic. Animals immunized with this fraction become allergic, and from past experimental work it is probable that this fraction or some substance intimately associated with it is responsible for the allergic reactions ordinarily observed in animals sensitized with bacteria, or to the protein fractions of bacterial extracts.

Rather than assume that two substances were concerned in the first allergic phase, the reactions might also be explained by assuming that the allergin were a complex substance similar to the SP antigen in pneumococcus. The toxic fractions of streptococcus filtrates would be analogous to the SP combination. Allergic reactions to this toxic antigen would be neutralized by immune sera, while reactions due to the P analog from which the specific toxic S element had been separated would be non-specific and not subject to neutralization. As with pneumococcus antigens, reactions would occur with the SP combination and with the less specific P antigen during the immunization of animals.

Possible Relationship of Allergic Reaction to the Symptoms of Disease Due to Hemolytic Streptococcus Producing Toxic Substances.—Bristol (6) has recently drawn attention to the hypothesis that the rash and clinical symptoms of scarlet fever are allergic reactions to the products of hemolytic streptococcus. He has carefully reviewed the literature and summarized the evidence presented previously by other authors having a similar opinion. Our sensitization experiments with streptococcal filtrates afford a possible explanation of the rash of scarlatina on this allergic basis.

Infants apparently do not react to intracutaneous injections of scarlatinal filtrate. In adults negative cutaneous tests have been explained by the presence of circulating antitoxin, but Cooke (7) has found that negative reactions occur in infants whose sera have no neutralizing properties. In view of the allergic reactions which Dochez and Sherman have observed in animals hypersusceptible to *Streptococcus scarlatinæ* and the reactions which we have obtained with strains from erysipelas, we suggest that the positive cutaneous reactions with filtrates of streptococcus containing toxic fractions as well as the rash in scarlet fever are the result of previous sensitization. The development of a natural immunity to toxic fractions of scarlatinal filtrates might be explained satisfactorily by this hypothesis. Infants previous to sensitization would react negatively to the toxic substance in the absence of circulating antibody. After sensitization, positive cutaneous reactions would occur accompanied by a susceptibility to rash in event of infection with *Streptococcus scarlatinæ*. With the development of sufficient circulating antibody the

positive reactions would become negative and the tendency to develop the rash would disappear. In this immune state scarlatina *sine* exanthem might occur.

CONCLUSIONS.

Rabbits immunized with filtrates of cultures of hemolytic streptococcus from erysipelas show cutaneous allergy. Two periods of allergy have been observed, an early and a late phase. The earliest reactions occurring in the first period of allergy can be neutralized with erysipelas immune sera.

The rash of scarlet fever and the Dick reaction are apparently allergic reactions to products of *Streptococcus scarlatinæ*.

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