

STUDIES IN BACTERIAL ALLERGY

V. PERSISTENCE OF DESENSITIZATION, WITH BACTERIAL HAPTENS, OF GUINEA PIGS SENSITIZED PASSIVELY WITH HETEROLOGOUS SERUM*†

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DESENSITIZATION of passively sensitized guinea pigs with bacterial haptens has been reported.¹ The duration of this desensitization has not been studied. Desensitization of actively sensitized guinea pigs with whole antigens² and desensitization of the passively sensitized guinea pig uterus with a bacterial hapten³ are transient. Passive sensitization with heterologous serum is transient.^{4, 5} Passive sensitization with homologous serum is lasting.⁶

These experiments were planned to study the duration of bacterial hapten desensitization of guinea pigs after passive sensitization with heterologous serum.

METHODS

Guinea pigs were used as experimental animals. Capsular pneumococcal polysaccharides,‡ Types I, II, and IV, were used and are referred to as haptens. Corresponding types of refined antipneumococcal rabbit serum§ were used as heterologous passively sensitizing antibodies. Types I and II antisera contained 4,000 units and type IV 6,000 units per milliliter.

Passive sensitization was produced by intra-abdominal injections of 0.2 to 0.5 ml. of the antiserum.

Desensitization was done by intra-abdominal injections of specific polysaccharides. Desensitization was proved by an intravenous challenging dose of specific polysaccharide in all but one experiment. Details are given in the tables. In preliminary experiments in 5 animals, subcutaneous injections of 5.5 mg. of polysaccharide in divided doses in 2 days did not desensitize. This method was not studied further.

Challenging doses of polysaccharide to test the presence of hypersensitivity were given intravenously. Preliminary experiments had shown that 1.0 mg. would elicit anaphylactic shocks quite regularly.

Experiment I shows the approximate duration of passive sensitization. Details are given in Table I. The data suggest that anaphylaxis can be produced regularly up to 9 days under the conditions of this experiment. Anaphylaxis was produced irregularly after 9 days and not at all after 13 days. A more accurate determination of the duration of passive sensitization would require standardization of the variables: types, dose, and strength of sensitizing antiserum; minimal shocking doses of polysaccharide for each type of antiserum; age, weight, and diet of the guinea pig. A more accurate determination was not thought to be germane to the current problem.

*These studies were made possible in part by a grant from Eli Lilly & Company, Indianapolis, Ind.

†Read at the Seventh Annual Meeting of the American Academy of Allergy, New York, Feb. 5-7, 1951.

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‡Generously supplied by Dr. H. D. Piersma of the Lederle Laboratories, Pearl River, N. Y.

§Generously supplied by Dr. B. Scott Fritz of the Marietta Laboratories, Wyeth, Incorporated, Marietta, Pa.

TABLE I. DURATION OF PASSIVE SENSITIZATION

GUINEA PIG NUMBER	SENSITIZING ANTISERUM (I.P.)		INTERVAL (DAYS)	CHALLENGE SPECIFIC CHO (I.V.) (MG.)	REACTION
	DOSE (ML.)	TYPE			
1	0.25	2	2	2.0	AD
2	0.25	4	2	2.0	AD
3	0.5	2	7	1.25	AD
4	0.5	2	7	1.25	AD
5	0.5	2	8	1.25	2+
6	0.5	2	9	1.25	2+
7	0.2	2	9	1.25	AD
8 & 9	0.25	2	10	2.0	O
10 & 11	0.25	4	10	2.0	O
12	0.5	2	11	1.25	O
13	0.5	2	11	1.25	2+
14	0.5	2	11	1.25	AD
15, 16	0.2	1	14	1.25	O
17, 18					
19, 20	0.2	1	16	1.25	O
21, 22					

I.P. = intra-abdominally.

CHO = pneumococcal polysaccharide.

2+ = marked respiratory difficulty, recovered.

AD = anaphylactic death.

Experiment II shows that bacterial haptens will desensitize specifically and that the desensitization is lasting. Details are given in Table II. Note that 1.25 mg. of specific polysaccharide intra-abdominally desensitized 2 guinea pigs to the extent that they did not react to an intravenous challenging dose the next day. On the other hand, 1.0 mg. of polysaccharide intra-abdominally desensitized only 6 of the remaining 16 animals.

Note, too, that the 2 animals desensitized by 1.25 mg. of hapten initially and 4 animals which were not desensitized by 1.0 mg. (but survived the reaction to the intravenous test of desensitization) remained desensitized when challenged with hapten 7 days later. The other animals were not challenged after the initial tests of desensitization.

Experiment III shows that nonspecific haptens do not prevent later specific anaphylactic reactions. Details are given in Table III. Three things should be noted. First, there were no reactions to the intra-abdominal nor to the intravenous injections of the nonspecific polysaccharide. Second, the nonspecific polysaccharide intra-abdominal and intravenous doses were the same as the specific doses which produced desensitization in Table II. Finally, anaphylaxis was not prevented when the animals were challenged with specific polysaccharide 7 days later. The intensity of the reactions was somewhat less than anticipated. No effort was made to explain this.

Experiment IV shows 2 things in guinea pigs passively sensitized with 2 types of antiserum: first, that hapten desensitization is lasting and specific. Second, desensitization with one specific hapten does not prevent anaphylactic reaction to intravenous injection of the other specific hapten. Details are given in Table IV. Note that 3 intra-abdominal desensitizing doses of specific polysaccharide were used instead of the intra-abdominal and intravenous desensitizing doses shown in Table II. Other experiments not given in detail had shown

TABLE II. DESENSITIZATION STUDIES

GUINEA PIG NUMBER	SENSITIZING ANTISERUM (I.P.)		INTERVAL (DAYS)	DESENSITI- ZATION SPECIFIC CHO (I.P.) (MG.)	INTERVAL (DAYS)	I.V. TEST OF DESENSITI- ZATION WITH SPE- CIFIC CHO (I.V.) (MG.)	REACTION	INTERVAL (DAYS)	CHALLENGE SPECIFIC CHO (I.V.) (MG.)	REACTION
	DOSE (ML.)	TYPE								
1	0.2	2	1	1.25	1	1.25	0	7	1.25	0
2	0.2	2	1	1.25	1	1.25	0	7	1.25	0
3	0.2	2	1	1.0	1	1.25	AD	-	-	-
4	0.4	2	1	1.0	1	1.25	3+	7	1.25	0
5	0.4	2	1	1.0	1	1.25	3+	7	1.25	0
6	0.4	2	1	1.0	1	1.25	AD	-	-	-
7	0.4	2	1	1.0	1	1.25	AD	-	-	-
8	0.2	2	1	1.0	1	1.25	0	7	1.25	0
9	0.2	2	1	1.0	1	1.25	0	7	1.25	0
10	0.2	1	1	1.0	1	1.25	0	-	-	-
11	0.2	1	1	1.0	1	1.25	0	-	-	-
12	0.2	1	1	1.0	1	1.25	2+	-	-	-
13	0.2	1	1	1.0	1	1.25	AD	-	-	-
14	0.2	1	1	1.0	1	1.25	AD	-	-	-
15	0.2	1	1	1.0	1	1.25	AD	-	-	-
16	0.2	1	1	1.0	1	1.25	AD	-	-	-
17	0.2	2	1	1.0	1	1.25	0	-	-	-
18	0.2	2	1	1.0	1	1.25	0	-	-	-

3+ = Severe dyspnea, convulsions, recovered.

Other abbreviations same as Table I.

TABLE III. NONSPECIFIC HAPTENS (I.P. AND I.V.) DID NOT PREVENT ANAPHYLAXIS FROM SPECIFIC HAPTEN 7 DAYS LATER

GUINEA PIG NUMBER	SENSITIZING ANTISERUM, TYPE II (I.P.) (ML.)	INTERVAL (DAYS)	DESENSITIZATION, NONSPECIFIC CHO, TYPE IV (I.P.) (MG.)	INTERVAL (DAYS)	DESENSITIZATION, NONSPECIFIC CHO, TYPE IV (I.V.) (MG.)	INTERVAL (DAYS)	CHALLENGE, SPECIFIC CHO, TYPE II (I.V.) (MG.)	REACTION
1	0.2	1	1.25*	1	1.25*	7	1.25	2+
2	0.2	1	1.25	1	1.25	7	1.25	2+
3	0.4	1	1.0	1	1.25	7	1.25	AD
4	0.4	1	1.0	1	1.25	7	1.25	3+
5	0.4	1	1.0	1	1.25	7	1.25	1+
6	0.4	1	1.0	1	1.25	7	1.25	AD
7	0.4	1	1.0	1	1.25	7	1.25	3+

*No reaction to I.P. nor to I.V. nonspecific polysaccharide.

1+ = Ruffled hair, slight to moderate dyspnea, huddled, sick-looking, recovered.

Other abbreviations same as Table I.

TABLE IV. SPECIFIC I.P. DESENSITIZATION LASTS AT LEAST 6 DAYS, I.P. DESENSITIZING AND I.V. CHALLENGING DOSES OF HAPTEN OF ONE TYPE DO NOT PREVENT SPECIFIC ANAPHYLAXIS FROM HAPTEN OF THE OTHER TYPE (1 EXCEPTION)

GUINEA PIG NUMBER	SENSITIZING ANTISERUM, TYPE II (I.P.) (ML.)	INTERVAL (DAYS)	DESENSITIZATION, SPECIFIC CHO TYPE I (I.P.)			INTERVAL (DAYS)	CHALLENGE, SPECIFIC CHO, TYPE I (I.V.) (MG.)	REACTION	INTERVAL (HR.)	CHALLENGE, SPECIFIC CHO, TYPE II (I.V.) (MG.)	REACTION
			A.M. (MG.)	NOON (MG.)	P.M. (MG.)						
1	0.25	1	0.5	1.0*	1.0*	6	1.5	0	1	1.5	AD
2	0.25	1	0.5	1.0	1.0	6	1.5	0	1	1.5	AD
3	0.25	1	0.5	1.0	1.0	6	1.5	0	1	1.5	3+
TYPE II											
4	0.25	1	0.5	1.0	1.0	6	1.5	0	1	1.5	3+
5	0.25	1	0.5	1.0	1.0	6	1.5	0	1	2.0	O
6	0.25	1	0.5	1.0	1.0	6	1.5	0	1	2.0	AD

*Ruffled hair, huddled, sick, recovered slowly.

Other abbreviations same as other tables.

that 2 intra-abdominal injections, 1.0 mg. in the morning and 1.0 mg. in the afternoon, desensitized 12 successive animals. It is interesting that these (Table III) were the only animals in any of these experiments which showed obvious reactions to the intra-abdominal injections of polysaccharide.

Type I polysaccharide desensitized 3 animals completely against a large challenging dose of type I polysaccharide 6 days later. One hour later all 3 animals had typical violent reactions to type II polysaccharide. The reverse was true in the other 3 animals. Namely, type II polysaccharide desensitized completely against challenging doses of type II polysaccharide 6 days later. Two of the 3 animals reacted violently to the challenging dose of type I polysaccharide 1 hour later. No effort was made to explain the failure of the third animal to react.

This same experiment was repeated in its essential details, with one exception. The interval between the desensitizing doses and the challenging specific doses was 9 days instead of 6. In these animals intravenous tests of desensitization were done on the second day. All 12 animals were desensitized. However, 11 days after sensitization and 9 days after the second desensitizing dose, none of the animals reacted to challenging doses of either type of polysaccharide.

Experiment V consisted simply of rechallenging 4 animals (which had been desensitized and had received at least 2 intravenous injections of specific polysaccharide) 25, 30, 30, and 31 days, respectively, after the last dose of polysaccharide. There was no reaction in any of the animals.

DISCUSSION

The data presented show that passive sensitization with a heterologous antibody is transient. This is not new.^{4,5} The variable number of days sensitization persists (Table I) is consistent with previous reports.

Pneumococcal polysaccharides, bacterial haptens, can desensitize passively sensitized animals specifically. This is not new.¹ However, rather large divided doses of polysaccharide subcutaneously failed to desensitize 5 animals. Brown¹ reported desensitization with 1.0 mg. subcutaneously in a single dose. One milligram intra-abdominally desensitized less than one-half of the animals in Table II. Eighteen animals in Experiment IV were completely desensitized by 2 or 3 intra-abdominal injections totaling 2 to 2.5 mg. of polysaccharide.

Experiments and Tables II and IV show that desensitization lasts, definitely, from 6 to 7 days. Experiment IV (text) shows that sensitization does not last, reliably, as long as 9 days. From this, the assumption seems reasonable that the desensitization lasts as long as the passive sensitization lasts.

The desensitization is highly specific. Table IV shows this in 5 of 6 animals sensitized with 2 types of antiserum. They had violent reactions when challenged with one specific polysaccharide 6 days after complete desensitization to the other type and one hour after a negative challenge with the desensitizing polysaccharide.

Tomezik⁷ showed that the uterine horns of passively sensitized guinea pigs remained sensitive from 2 hours to 12 days. He did not test the intact guinea pig for duration of sensitization. He showed also that polysaccharide could be

used to produce anaphylactic shock. Avery and Tillett⁸ showed that polysaccharides will not sensitize anaphylactically, nor produce precipitins. Experiment V confirms this lack of sensitizing capacity.

The impressions are gained that nonspecific polysaccharides tend to lessen the intensity of reactions to specific challenging doses toward the end of the period of sensitization, and that they tend to shorten the duration of sensitization. These impressions need experimental confirmation. No effort was made to confirm them.

It is proper to point out that desensitization with haptens does not imply desensitization to other antigenic components of the pneumococcus. Furthermore, similar experiments may yield different results in animals actively sensitized or sensitized passively with homologous antiserum.

SUMMARY AND CONCLUSIONS

1. In guinea pigs sensitized passively with antipneumococcal rabbit serum, the type specific capsular polysaccharide hapten of the pneumococcus will: (a) elicit anaphylactic shock when given intravenously; (b) desensitize when given intra-abdominally.

2. Passive sensitization with heterologous antiserum is transient. In these experiments sensitization lasted, variably, between 8 and 12 days.

3. Pneumococcal polysaccharides, used as in these experiments, do not produce active sensitization.

4. Desensitization by specific polysaccharide hapten intra-abdominally will: (a) last, apparently, as long as passive sensitization with heterologous antiserum; (b) not prevent anaphylactic reactions to polysaccharide of another type in guinea pigs sensitized to 2 types simultaneously.

5. The roles of active sensitization, passive sensitization with homologous serum, and other haptens and antigenic fractions of the pneumococcus have not been studied.

6. Lasting desensitization of the intact animals is in striking contrast to the transient desensitization of the excised uterine horn reported earlier.³

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