

STUDIES IN BACTERIAL ALLERGY*

VI. HAPTEN AEROSOL DESENSITIZATION OF GUINEA PIGS PASSIVELY SENSITIZED TO SPECIFIC PNEUMOCOCCAL POLYSACCHARIDES

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INVESTIGATION of the many problems of desensitization has been regrettably dormant. A good deal is known about desensitization with whole antigens.^{1, 8} Little is known about desensitization with haptens, although anaphylactic shock from bacterial polysaccharides was reported in 1926.² Theoretically, desensitization with haptens is of considerable importance, since haptens may neutralize antibodies but do not elicit them primarily or anamnesticly. Desensitization with whole antigens is transient.³ Theoretically, desensitization with haptens should be lasting, in the intact animal, after antibody production has ceased.

Previous studies in this laboratory have shown that desensitization of uterine strips of passively sensitized guinea pigs with pneumococcal polysaccharides was transient when brought about by additions of the hapten to the bath.⁴ On the other hand, in intact guinea pigs sensitized passively with heterologous serum, desensitization with the same polysaccharide, intra-abdominally, was apparently as lasting as passive sensitization.⁵

A good deal of attention has been given, recently, to aerosols in a variety of uses. This report deals with some qualitative observations on aerosol desensitization, using a pneumococcal polysaccharide as a hapten, in guinea pigs sensitized passively with specific heterologous antiserum.

METHODS

Guinea pigs were used as experimental animals. Pneumococcal polysaccharide type VI was used and is referred to as a hapten. Type VI refined antipneumococcal rabbit serum was used as the source of heterologous, passively sensitizing antibody.

Passive sensitization was produced, by intra-abdominal injections of 0.35 to 1.0 ml. of antiserum. Occasionally, more was used.

A DeVilbiss No. 40 nebulizer was connected with a source of compressed air, bubbled through water. The hapten was nebulized into a small glass box. This aerosol chamber was not quite airtight. The aerosol fog was kept dense enough to ooze visibly from the loose seams.

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Preliminary aerosol studies with acacia* and with the polysaccharide showed that reactions usually occurred, within two to five minutes, if they were going to. If no reaction occurred, in five to seven minutes, the challenge was considered negative.

Positive reactions were graded *slight dyspnea* when the respiratory volume per breath increased, definitely and unequivocally, usually with some slowing of respiration. *Moderate dyspnea* was used to describe dyspnea which was deep, slow, associated with definite use of the accessory muscles of respiration, and obviously distressing. Dyspnea was called *severe* when it looked as though asphyxia were imminent. *Anaphylactic death (A. D.)* described the severest reactions, in which the animal behaved, objectively, as though he had received a fatal intravenous challenging dose of antigen and had typical ballooned lungs at post mortem.

Slight-moderate and *moderately severe* were used, to describe intermediate degrees of reactions.

Local desensitization was assumed to be present when the guinea pigs were no longer affected by relatively prolonged exposures (ten minutes) to the fog, which, previously, had caused unequivocal dyspnea, promptly.

The first three experiments are not concerned with desensitization. They are presented as background for the desensitization studies.

TABLE I. INTRA-ABDOMINAL TYPE VI ANTIPNEUMOCOCCAL RABBIT SERUM SENSITIZES GUINEA PIGS TO INHALATION OF TYPE VI PNEUMOCOCCAL POLYSACCHARIDE AEROSOL

GP #	I.A.* SENS. DOSE TYPE VI ANTISERUM (ML.)	CONC. OF TYPE VI POLYSAC. AEROSOL (MG./ML.)	TIME OF EXPOSURE (MIN.)	REACTION†	RECOVERY TIME (MIN.)
1	0.5	3.0	1	A. D.‡	—
2	0.65	3.0	1	Severe	15
3	0.65	3.0	1½	Moderate	10
4	0.65	3.0	1	Severe	10
5	0.65	3.0	6	None	—
6	0.65	1.0	1	A. D.	—
7	0.65	1.0	4	None	—
8	0.65	1.0	1½	Mod.-severe	9
9	0.65	1.0	5	None	—
10	0.65	0.3	1¼	Moderate	10
11	0.5	0.6	4	Slt.-mod.§	4
12	0.5	0.2	4	Moderate	—
13	0.5	3.0	10	None	—
14	0.5	3.0	10	None	—
15	0.5	0.6	8	Slt.-mod.	4
16	0.5	0.2	4	Moderate	—
17	0.5	3.0	7	Slt.-mod.	3
18	0.5	3.0	10	None	—
19	0.35	3.0	1	A. D.	—
20	0.35	3.0	1	Mod.-severe	6
21	0.35	3.0	1	Moderate	6

*I.A.—Intra-abdominal.

†Reactions—Grades of dyspnea; see text.

‡A. D.—Anaphylactic death.

§Slt.-mod.—Slight to moderate dyspnea.

*Acacia was used, here and elsewhere in this report, because its immunologic properties are being studied in this laboratory. Thus, a handy anaphylactic system is provided for comparison. The acacia studies will be reported separately.

Experiment I (Table I).—Guinea pigs are sensitized to inhalation of type-specific polysaccharide aerosol, by intra-abdominal injections of type VI anti-pneumococcal rabbit serum.

Note that there were differences in the time of onset and in the intensity of the reactions and that several animals did not react at all.

Experiment II (Table II).—Illustration of effective thresholds for aerosols of polysaccharide and of histamine. Note that a concentration greater than 0.03 mg./ml. of polysaccharide was necessary, to elicit a reaction. Only one reaction has been elicited by an aerosol concentration of 0.05 mg./ml., so far. There have been several reactions to concentrations of 0.1 and 0.2 mg./ml. Note too that there was a sharp difference in the intensity and in the reaction time between histamine 1:15,000 and 1:3,000.

TABLE II. ILLUSTRATION OF EFFECTIVE THRESHOLDS FOR AEROSOL CONCENTRATIONS OF POLYSACCHARIDE AND OF HISTAMINE

PASSIVELY SENSITIZED GUINEA PIG					
GP #	I.A.* SENS. DOSE TYPE VI ANTISERUM	CONC. OF TYPE VI POLYSAC. AEROSOL (MG./ML.)	TIME OF EXPOSURE (MIN.)	REACTION*	RECOVERY TIME (MIN.)
1	0.5 ml.	0.003	8	None	—
		0.03	10	None	—
		0.3	1¼	Moderate	10
2	0.5 ml.	0.001	7	None	—
		0.01	10	None	—
		0.1	6	Slt.-mod.	10
		0.2	4	Mod.-severe	—
HISTAMINE ALONE					
3	None	1:15,000	6	Slt.-mod.	2
	None	1:3,000	1	Severe	5
4	None	1:15,000	4	Slt.-mod.	1
	None	1:3,000	1	Severe	—

*See legend for Table I.

Experiment III (Table III).—Reactions to polysaccharide haptens differ with the route of administration. Negative reactions to aerosol may be followed by anaphylactic death from intravenous polysaccharides.

TABLE III. REACTIONS TO CHALLENGING DOSAGES OF POLYSACCHARIDE DIFFER WITH ROUTE OF ADMINISTRATION: NEGATIVE REACTIONS TO AEROSOL FOLLOWED BY ANAPHYLACTIC SHOCK FROM INTRAVENOUS POLYSACCHARIDE

GP #	I.A. SENS. DOSE ANTI- SERUM (ML.)	CONC. OF POLYSAC. AEROSOL (MG./ML.)	TIME OF EXPOSURE (MIN.)	REACTION	INTERVAL (HR.)	I.V. POLY- SAC. (MG.)	REACTION
Type VI		Type VI				Type VI	
1	1.0	4.0	14	0	20	4.0	A. D.
2	0.5	3.0	7	0	1	1.5	A. D.
Type II		Type II				Type II	
3	0.65	5.0	12	0	20	2.0	A. D.
4	0.65	1.0	13	0	20	2.0	2+
5	0.65	5.0	13	0	20	2.0	A. D.

Failure to elicit aerosol reactions with the use of type II antiserum and polysaccharide is discussed later.

TABLE IV. DESENSITIZATION FOLLOWS REACTIONS TO AEROSOL: 2 PATTERNS OF DESENSITIZATION

A) DESENSITIZATION MAY FOLLOW RECOVERY FROM DYSPNEA WHILE STILL IN AEROSOL											
GP #	I.A. SENS. DOSE TYPE VI ANTI-SERUM (ML.)	CONC. OF TYPE VI POLYSAC. AEROSOL (MG./ML.)	FIRST EXPOSURE TIME (MIN.)	REACTION	INTERVAL	SECOND EXPOSURE TIME (MIN.)	REACTION	INTERVAL (MIN.)	THIRD EXPOSURE TIME (MIN.)	REACTION	FOURTH EXPOSURE TIME (MIN.)
1	0.65	3.0	12	Severe	21 min.	8	None				
2	0.5	0.2	27	Mod.-severe	22 hr.	12	None				
B) DESENSITIZATION FOLLOWS SEVERAL DECREASINGLY INTENSE REACTIONS TO AEROSOL											
3	0.5	0.3	2½	Moderate	12 min.	2	Slt.-mod.	5	13	Slt. dysp. 5 min.	13
4	0.65	1.0	6	Mod.-severe	24 hr.	5	Very slt.	(OK in fog in 5 min.)			
5	0.65	3.0	3	Severe	24 min.	9	Slt. dysp.	71	7	None	
6	0.65	3.0	3	Moderate	14 min.	7	Slt. dysp.	19	7	None	

TABLE V. LOCAL DESENSITIZATION BY AEROSOL WITH ONE CONCENTRATION OF POLYSACCHARIDE MAY DESENSITIZE AGAINST SEVERAL MULTIPLES OF THAT CONCENTRATION

GP #	I.A. SENS. DOSE TYPE VI ANTI-SERUM (ML.)	CONC. OF TYPE VI POLYSAC. AEROSOL USED TO DESENSITIZE (MG./ML.)	NUMBER EXPOSURES	TOTAL EXPOSURE TIME (MIN.)	REACTION		INTERVAL	CONC. OF TYPE VI POLYSAC. AEROSOL (MG./ML.)	EXPOSURE TIME (MIN.)	REACTION
					FIRST EXPOSURE	LAST EXPOSURE				
1	0.5	0.3	4	37	Moderate	None	15 min.	3.0*	9	Very slt. dysp.
2	0.5	0.2	3	47	Mod.-severe	None	16 min.	3.0†	10	None
3	0.5	0.6	1	8	Slt.-mod.	None	17 min.	3.0†	14	None

*10, †15, ‡5 multiples of original reacting and desensitizing concentrations of polysaccharide.

Experiment IV (Table IV).—Desensitization follows reactions to aerosol. Two patterns of desensitization were observed. One pattern was the desensitization which followed recovery from dyspnea while still in the aerosol. Guinea pigs Nos. 1 and 2 are examples of desensitization after recovery from single severe reactions. Desensitization of pigs Nos. 3 and 4 was completed while in the aerosol fog after their reactions had become very slight. Repeated examples of desensitization, after recovery from moderately severe dyspnea, while still in the fog have been observed in animals sensitized to and reacting to acacia aerosol.

The other pattern is the desensitization which follows two or more decreasingly intense reactions to aerosol. Guinea pigs Nos. 3, 4, 5, and 6 illustrate typical variations. Note that three were desensitized after two reactions while one required four reactions.

Experiment V (Table V).—Local desensitization with one concentration of polysaccharide may desensitize against several multiples of that concentration of hapten.

Table V illustrates this point. Note that it took a total of thirty-seven minutes in four separate exposures to desensitize one animal completely against an aerosol of a solution of 0.3 mg./ml. of type VI polysaccharide. Fifteen minutes after the last nonreacting exposure, there was a barely perceptible reaction to a polysaccharide aerosol of 3.0 mg./ml., a tenfold increase in tolerance approximately. The tolerance was increased fifteen fold by three exposures in animal No. 2 and fivefold by one exposure in the third one.

Experiment VI (Table VI).—Desensitization by aerosol is local. It does not prevent anaphylactic shock from intravenous hapten. Each of the animals was completely desensitized to the stated hapten concentration. In some acacia experiments,⁶ definite systemic desensitization followed aerosol exposures of ten hours, but not four or six hours. Note that the anaphylactic reaction was weak in the last animal. Presumably, passive sensitization was fading.⁷

TABLE VI. DESENSITIZATION BY AEROSOL IS LOCAL; IT DOES NOT PREVENT SUBSEQUENT ANAPHYLACTIC SHOCK FROM INTRAVENOUS POLYSACCHARIDE

GP #	I.A. SENS. DOSE TYPE VI ANTI- SERUM (ML.)	INITIAL REACTION TO AEROSOL	CONC. OF TYPE VI POLYSAC. AEROSOL USED TO DESENSITIZE (MG./ML.)	INTERVAL (DAYS)	CONC. OF TYPE VI POLYSAC. AEROSOL (MG./ML.*)	REACTION	AMOUNT I.V. TYPE VI POLYSAC. (MG.)	REACTION
1	0.5	Moderate	3.0	5	3.0	None	3.0	A. D.
2	0.65	Mod.-severe	1.0	9	3.0	Not Done	1.5	A. D.
3	0.65	Severe	3.0	1	3.0	None	1.0	2+
4	0.5	Slt.-mod.	3.0	10	3.0	None	2.0	A. D.
5	0.5	Mod.-severe	3.0	11	3.0	None	1.0	1+

*Exposure time = 10 minutes.

Experiment VII (Table VII).—Local desensitization by aerosol may last at least nine days.

In each instance, local desensitization was still complete 5 to 9 days after the last desensitizing challenge with the concentrations of hapten indicated. In

two animals in Table VI, local desensitization lasted 10 and 11 days. Table VI and previous experiments⁵ show that systemic passive sensitization lasts 9 days or longer.

TABLE VII. LOCAL DESENSITIZATION BY AEROSOL MAY LAST AT LEAST 9 DAYS

GP #	I.A. SENS. DOSE TYPE VI ANTI- SERUM	INITIAL REACTION TO AEROSOL	CONC. OF TYPE VI POLYSAC. AEROSOL USED TO DESENSITIZE (MG./ML.)	INTERVAL (DAYS)	CONC. OF TYPE VI POLYSAC. AEROSOL (MG./ML.)	EXPOSURE TIME (MIN.)	REACTION
1	0.5	Moderate	3.0	5	3.0	5	None
2	0.65	Severe	3.0	7	1.0	6	None
3	0.65	Moderate	3.0	7	1.0	6	None
4	0.5	Mod.-severe	3.0	9	3.0	8	None
5	0.5	Slt.-mod.	3.0	9	3.0	8	None
6	0.5	Slt.-mod.	3.0	9	3.0	8	None

POSITIVE CONTROLS

GUINEA PIG	I.A. SENS. DOSE TYPE VI ANTI- SERUM (ML.)	INTERVAL (DAYS)	CONC. OF TYPE VI POLYSAC. AEROSOL (MG./ML.)	REACTION	DESENS. TIME IN 3.0 MG./ML. AEROSOL (MIN.)	INTERVAL (MIN.)	AMOUNT I.V. TYPE VI POLYSAC. (MG.)	REACTION
1	0.5	5	3.0	Severe	50	5	3.0	A. D.
2	0.5	5	3.0	Moderate	60	10	3.0	A. D.
3	0.5	5	3.0	Slight	8	5	3.0	None
4	0.5	5	3.0	Slight	8	10	3.0	Slight
5	0.5	9	3.0	Slight	20	30	3.0	Severe
6	0.5	9	3.0	Moderate	35	15	3.0	A. D.
7	0.5	9	3.0	Moderate	35	20	3.0	A. D.
8	0.5	9	3.0	Slight	20	40	3.0	Severe

Experiment VIII (Table VIII).—Prior aerosol treatment with acacia and/or with type II polysaccharide do not prevent specific reactions to type VI polysaccharide, i.e., aerosol reactions are specific. Note that, in three instances, there were prior negative aerosol challenges, with type II polysaccharide, and that, in two animals, there had been negative prior challenges, with aerosols of both acacia and type II polysaccharide. The specific reactions to the type VI polysaccharide aerosol seemed to be unimpaired.

TABLE VIII. PRIOR AEROSOL INHALATIONS OF ACACIA AND/OR TYPE II POLYSACCHARIDE DO NOT PREVENT SPECIFIC REACTIONS TO TYPE VI POLYSACCHARIDE; AEROSOL REACTIONS ARE SPECIFIC

GP #	I.A. SENS. DOSE TYPE VI ANTI- SERUM (ML.)	CONC. NON- SPECIFIC AEROSOL (MG./ML.)	REACTION	CONC. NON- SPECIFIC AEROSOL (MG./ML.)	REACTION	CONC. SPECIFIC AEROSOL (MG./ML.)	REACTION
<i>Type II</i>				<i>Type VI</i>			
1	0.65	1.0	None	—	—	3.0	Severe
2	0.65	5.0	None	—	—	3.0	Moderate
3	0.5	5.0	None	—	—	0.6	Slt.-mod.
<i>Acacia</i>				<i>Type II</i>			
4	0.65	30.0	None	1.0	None	1.0	A. D.
<i>Type II</i>				<i>Acacia</i>			
5	0.65	1.0	None	30.0	None	1.0	Mod.-severe

Experiment IX.—Repeated efforts were made to sensitize against type II polysaccharides alone or in combination with acacia or with type VI polysaccharide. In no instance, was the slightest suggestion of local sensitization to type II polysaccharide aerosol produced. Four different lots of type II rabbit antiserum and three different lots of type II polysaccharide were used, in varying doses of the sensitizing antiserum and concentrations of shocking type II polysaccharide aerosol. The animals which failed to react to type II aerosol invariably reacted with anaphylactic death to intravenous challenge with even weaker concentrations of polysaccharide than were used in the aerosol. The precipitin titers of the different type II antisera and polysaccharides were 1:100,000 or stronger. No explanation of the failure to sensitize to type II, in the face of ready sensitization to type VI pneumococcal polysaccharide, has been forthcoming.

DISCUSSION

The experiments reported here emphasize three aspects of desensitization which are not new; namely, the use of haptens, the use of the airway as a portal of entry for the desensitizing hapten or antigen,⁷ and local versus systemic desensitization. This report does, however, present several new facets to these three broad aspects of desensitization.

These experiments show, for example, that there was no spontaneous resensitization in passively sensitized guinea pigs, after desensitization with hapten aerosol, using these relatively small doses of sensitizing antiserum. The potential advantage of desensitizing with a nonsensitizing hapten aerosol may be less obvious when excess antibody is available for resensitization of the bronchi. This is suggested by the report that actively sensitized guinea pigs desensitized by an aerosol of a whole antigen become resensitized, spontaneously, in a few days.⁷ Studies of the duration of desensitization with hapten aerosols in active sensitization and in passive sensitization with homologous antiserum must wait until more suitable haptens are available. These are being sought. This delay is due to the difficulty in sensitizing guinea pigs actively with whole bacteria and immunizing them with vaccines sufficiently to obtain sera suitable for homologous serum passive sensitization.

These observations on the use of the airway as a portal of entry for a desensitizing hapten supplement those of Herxheimer,⁷ who used whole antigen aerosols to desensitize. It is hoped that they will restimulate the long-dormant interest in the problems of desensitization. It is surprising that this method of local desensitization has not been exploited long before now. It has been forty years since Sewell¹² reported (on shaky grounds) that guinea pigs could be sensitized by nasal instillation and shocked by inhalations of atomized horse serum and, further, that they could be desensitized simply by living in a stable. There have been numerous other references to sensitization and shock by the inhalation of antigens.

The experiments in this report show quite clearly that the desensitization produced by the hapten aerosol is local, and not systemic. The supply of these polysaccharide haptens was too limited to attempt to measure the time required

in polysaccharide fog to absorb enough hapten to produce systemic desensitization. Feinberg¹¹ estimated that guinea pigs inhale about 13 gamma of 1:2,000 histamine, in 3 minutes, and about 30 gamma of Pyribenzamine while in a fog of 1 per cent Pyribenzamine, for 5 minutes. At that rate, it would take about 27 hours to inhale 1 mg. of the hapten, using a 1 mg./c.c. concentration for the aerosol. This is a borderline desensitizing amount if we assume that all of the inspired hapten was absorbed.

This concept of local desensitization is not a new one. In 1922, MacKenzie¹³ and Caulfield¹⁴ reported the successful treatment of pollen hay fever by topical nasal application of pollen extract. There are many other examples of local desensitization, which need not be reviewed. It is interesting that 30 years elapsed between MacKenzie's¹³ local, specific, topical desensitization of the nose in man and Herxheimer's⁷ local, specific, topical desensitization of the bronchi in guinea pigs. Perhaps, the deterrent was MacKenzie's demonstration, that local desensitization of the skin with an antigen was transient. The need to investigate the whole problem of local versus systemic sensitization and desensitization is emphasized, by these and other¹⁰ experiments.

The results recorded in the tables seem clear cut.

It is of some interest that the duration of the desensitization was about as long as the duration of passive sensitization with heterologous serum: about ten to thirteen days.

The intensity of reactions to the same sensitizing doses of antiserum and challenging concentrations of polysaccharide varied widely.

The most violent reactions occurred most quickly, after 45 to 90 seconds in aerosol. Conversely, the mildest reactions were the slowest to appear. This conforms to the common clinical observation, that the severity of reactions to allergen injections tends to vary inversely with the delay in onset.

Since these are qualitative experiments, no effort was made to measure the amount of polysaccharide inhaled by the animals.

It would have emphasized the specificity of the desensitization if some guinea pigs had been sensitized to two antigens or haptens and had, then, been desensitized, specifically against one, without prevention of the aerosol reaction to the other. Attempts were made to sensitize against (a) type VI and type II pneumococcal polysaccharides, (b) type VI and type II polysaccharides and acacia, and (c) type VI polysaccharide and acacia. In no instance, was there evidence of multiple sensitization, with these, the only antigens tried so far. Sensitization to acacia alone is produced, with about the same frequency as to type VI polysaccharide alone. This inability to sensitize one animal to two aerosol antigens has not been explained, but should be. Experiments with different antibody-hapten systems and sensitizing schedules are planned.

A sufficient number of desensitized animals was challenged with and reacted to histamine 1:3,000 aerosol to show that the reacting mechanism was still intact.

Adequate controls seem to be found in the animals in Table I which did not react plus those in Table VIII which reacted to type VI, after failing to react to type II polysaccharide and acacia, plus those in Table II showing the threshold of concentrations reacting to type VI.

The general principles established by these experiments should be just as applicable to immunologic systems using polysaccharides from sources other than bacteria.

The pneumococcal polysaccharides are called haptens, here, with full knowledge that they may, under proper conditions, be antigenic in man and in rabbits. They are haptens because, in this type of experiment, they do not sensitize guinea pigs.

SUMMARY AND CONCLUSIONS

1. The following observations are thought to be new. They are based on studies of the effects of aerosols of pneumococcal polysaccharides, as haptens, in guinea pigs sensitized passively with specific heterologous (rabbit) antiserum.

- a. Aerosols of specific haptens elicit respiratory distress of varying intensity, from slight dyspnea to typical anaphylactic death.
- b. Reactions to inhaled hapten may differ from intravenous hapten: i.e., negative reactions to aerosol may be followed by anaphylactic death from intravenous hapten.
- c. There are definite thresholds of effective concentrations of hapten aerosols.
- d. Desensitization may be readily produced by specific hapten aerosol, in minutes.
- e. Aerosol desensitization is local: i.e., subsequent intravenous hapten will cause prompt anaphylaxis. Quantitative factors have not been studied.
- f. Aerosol desensitization may last at least nine days.
- g. Aerosol desensitization follows at least two patterns: (1) after several successive decreasingly intense respiratory reactions; and (2) recovery from respiratory distress while still in the aerosol.
- h. Local desensitization by a weak, but effective concentration of aerosol may desensitize against five to fifteen multiples of that concentration.

2. This is the third type of desensitization with bacterial polysaccharides as haptens reported from this laboratory.

3. These qualitative observations were selected for this report and are not intended to represent a comprehensive study of the problem. Others will be reported as they mature.

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