

REPEATED AEROSOL DESENSITIZATION AND SPONTANEOUS RESENSITIZATION OF NEWBORN GUINEA PIGS, PASSIVELY SENSITIZED IN UTERO

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INTEREST in specific clinical and experimental responses to aerosols of antigens and haptens is increasing.¹⁻¹³ The earlier lively interest in congenital sensitization of infants and animals¹⁴⁻²³ has shifted to the question of inheritance of clinical allergy.^{14, 24-29}

During earlier studies of aerosol desensitization,³⁰ it was noted that a few pigs just born of actively sensitized mothers were exquisitely sensitive to aerosols of the antigen to which the mothers were sensitive. Several violent reactions began a few seconds after exposure to the aerosols. In these pigs, anaphylactic death occurred in spite of prompt removal from the aerosols.

This is the report of some observations on repeated aerosol desensitization and spontaneous resensitization of congenitally sensitized newborn guinea pigs.

MATERIALS AND METHODS

The aerosols were administered in a rectangular box, 5 by 8 by 10 inches, with glass top and sides, loosely fitted to a grooved wooden base to permit free escape of air and aerosol fog. A DeVilbiss No. 40 nebulizer was inserted into the aerosol chamber through a small hole in the base. Compressed air, filtered through water, was used to nebulize the various dilutions of egg white (EW) used for desensitization and challenge. A dense aerosol fog was maintained by a continuous flow from the nebulizer.

Egg white (EW) was used instead of CEA (crystallized egg albumin) because of the large quantities employed, the ease of preparation, and because it was known to be an effective challenging and desensitizing agent. It was assumed, for purposes of these experiments, that the minor antigens in egg white would not play important roles.

In previous experiments,^{7, 30} hyperventilation from excitement and from what seemed to be a conditioned reflex had made it difficult to interpret minor

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reactions to aerosols of specific antigens. This was effectively avoided by subjecting the pigs to an aerosol of normal saline for 15 minutes before each exposure to the specific (EW) fog.

Two pigs were challenged at the same time usually. They were put into the fog-filled chamber quickly. The onset of initial symptoms was timed, beginning with the insertion of the pigs. The total time of exposure to the aerosol was also measured.

Three female guinea pigs were actively sensitized by intraperitoneal injections of 1.0 mg., 2.0 mg., and 5.0 mg. of CEA on 3 successive days for use in another experiment.³⁰ They received no more injections of CEA. Three litters, a total of 8 guinea pigs, were born 121 ± 5 days after sensitization of the mothers. Allowing 70 days for gestation,¹⁵ the mothers became pregnant 51 ± 5 days after the sensitizing injections of CEA.

The newborn pigs were 7 days old when they were first challenged by the specific aerosols. One of the 8 newborn pigs reacted weakly (1+) during the exposure to the 1:100,000 dilution of the egg white (EW). Two had weak reactions to the 1:10,000 EW dilution in 5 minutes. All of them reacted promptly to the 1:1,000 EW dilution, 6 severely (3+) and 2 moderately (2+). Two of the 8 newborn pigs died in acute or protracted anaphylactic shock during brief preliminary exposures to 1:1,000, 1:100, or 1:20 EW.

After those unplanned fatal reactions, care was taken to remove the animals immediately upon the appearance of dyspnea, cough, or scratching of the nose. After several exposures, when the time of onset was prolonged to more than 5 minutes, they were allowed to remain in the aerosol until the dyspnea became moderate (2+). Even then, they were removed promptly whenever the dyspnea developed quickly or became severe.

Two of the remaining 6 pigs were selected for repeated desensitization and spontaneous resensitization.^{7, 11} The other 4 pigs were saved for controls.

Details of the desensitization procedures and the intervals between completion of desensitization and the tests of resensitization are shown in Table I.

The 2 pigs, A and B, chosen for desensitization were selected because they had had moderate reactions (2+) to 1:1,000 EW within 5 to 10 minutes when 7 days old and severe, near-fatal reactions (3+) to 1:20 EW 2 days later. The 4 controls had had near-fatal reactions (3+) to a 3 minute exposure to the 1:1,000 EW aerosol.

The pigs were considered suitable for re-challenge after recovery from reactions to the specific aerosols, usually within 10 minutes. Actually, however, the interval between the repeated desensitizing aerosol challenges was quite variable.

EXPERIMENTAL RESULTS

Desensitization was started when the pigs were 18 days old (see Table I).

Both pigs, A and B, had uniformly severe, near fatal (3+), prompt reactions to the first 10 exposures to 1:100 EW during the first 2.5 days. Each reaction began in approximately 5 minutes. They were removed promptly at the beginning of the reaction to prevent anaphylactic death. Reactions to the

TABLE I. REPEATED AEROSOL DESENSITIZATION AND SPONTANEOUS RESENSITIZATION OF CONGENITALLY SENSITIZED GUINEA PIGS

AGE (DAYS)	GUINEA PIG	CONCEN- TRATION OF EW	NO. CHALLENGES OF EACH PIG	EXPOSURE TIME (MIN.)		REAC- TION*	
				TO ONSET	TOTAL		
18	A and B	1:100	4	5	5	3+	
19			4				
20			2				
21			2	5-10	10	2+	
22		1:20	2	5-10	45		
			1	5	30	1+	
			1	5	5	3+	
			1	5	15	2+	
23			1	5	60	1+	
1			7	7	2+		
1:100			1	5	30	1+	
25			1:20	1	5	5	2+
1:100			1	5	5	2+	
1:1000			1	0	30	0	
1:100			1	5	30	1+	
26			A	1:20	1	0	60
	1:20	1		0	30	0	
	1:20	1		3	5	1+	
	1:20	1		5	5	3+	
	A and B	1:100	1	5	30	2+	
		1:100	1	5	30	1+	
		1:20	2	0	60	0	
		1:20	1	0	60	0	
27	A and B	1:100	1	0	30	0	
		1:20	1	15	15	3+	
		1:100	1	0	60	0	
		1:20	1	0	60	0	
28		1:100	1	0	15	0	
		1:20	1	5	20	1+	
		1:20	2	5	10	2+	
		1:20	1	0	60	0	
29	A and B	1:20	1	5	60	1+	
		1:20	2	10	15	2+	
		1:20	1	0	30	0	
		1:20	1	0	30	0	
30	A and B	1:100	1	0	30	0	
		1:20	4	0	180†	0	
32			1	0	60	0	
First Resensitization							
50	A and B	1:20	1	2	2	2+	
	1 Control	1:100	1	1	1	3+	
	3 Control	1:100	1	1	1	AD	
Second Desensitization							
54	A and B	1:1000	1	0	60	0	
		1:100	1	0	60	0	
		1:20	1	1	60	0	
55		1:20	1	0	180	0	
Second Resensitization							
76	A	1:20	1	10	10	2+	
	B		1	1	10	2+	
Third Desensitization							
82	A and B	1:1000	1	0	30	0	
	A	1:100	1	5	15	1+	
	B	1:100	1	5	10	2+	
	A and B	1:1000	1	0	45	0	
83		1:100	1	0	30	0	
		1:100	1	0	30	0	
		1:20	1	0	90	0	

TABLE I, CONT'D

AGE (DAYS)	GUINEA PIG	CONCEN- TRATION OF EW	NO. CHALLENGES OF EACH PIG	EXPOSURE TIME (MIN.)		REAC- TION*
				TO ONSET	TOTAL	
Third Resensitization						
98	A and B	1:20	1	0	60	0
	Control	1:1000	1	15	15	2+
128	A	1:20	1	5	30	1+
	B		1	5	5	3+
	Control	1:1000	1	10	30	2+
Spontaneous Desensitization						
209	A	1:1000	1	10	30	±
	Control				30	±
	A	1:100	1		30	±
	Control		1		30	±
	A	1:20	1		30	±
	Control		1		30	±
Final Intravenous Challenge						
235	A		1 mg. CEA IV			0
	Control		1 mg. CEA IV			0

Pig A was exposed 47 times and Pig B 49 times.

*0 = No reaction. ± = Slight hyperventilation. 1+ = Definite dyspnea with heaving flanks. 2+ = Troublesome dyspnea with marked heaving of flanks. 3+ = Severe dyspnea with convulsions. Recovery. AD = Anaphylactic death.

†Desensitization completed.

next 6 exposures (at the age of 21 and 22 days) were also uniform in severity (2+), time of onset, and total exposure. The seventeenth exposure (age, 22 days) to 1:100 EW resulted in a weak, borderline (1+) reaction which was tolerated for 30 minutes. The next 3 exposures, the same day, showed a progressive increase in tolerance to 1:20 EW, ranging from a prompt, near-fatal (3+) anaphylaxis to a weak (1+) reaction which did not threaten life or increase during a 60 minute exposure.

This apparent marked increase in tolerance for the aerosol did not last overnight. Note the widely variable responses (at age of 23-29 days) when the pigs were exposed to 1:1,000 EW once, 1:100 EW 8 times, and to 1:20 EW 10 times.

Finally, when 30 days old, they failed to react during one exposure to 1:100 EW and 4 exposures to 1:20 EW for a total of 3.5 hours. Two days later they were still desensitized, as shown by failure to react to a 60 minute exposure to 1:20 EW.

First Spontaneous Resensitization.^{7, 11}—The desensitized pigs, A and B, had no more contact with antigen until they were challenged again (age, 50 days) 18 days after the last desensitizing exposure. They reacted moderately (2+) but promptly to the 1:20 EW aerosol. They were removed after 2 minutes in that aerosol to prevent more severe reactions. The 4 control pigs reacted violently after one minute in the 1:100 EW aerosol. Three died quickly in anaphylactic shock, the other one recovered.

Second Desensitization.—The test pigs, A and B, were re-desensitized easily. Apparently the 2+ reaction to the post-resensitization challenge (when 50 days old) plus an exposure of 1 hour to 1:1,000 EW (age, 54 days) was sufficient to

re-desensitize them, as shown by failure to react to one exposure to 1:100 EW and to 2 exposures to 1:20 EW for a total of 5 hours on that and the next day.

Second Spontaneous Resensitization.^{7, 11}—The pigs became sensitive again, spontaneously, as shown by moderate reactions (2+), when challenged with 1:20 EW aerosol 21 days later (age, 76 days).

Third Desensitization.—The test pigs were easily desensitized a third time, 6 days after the last positive test for resensitization. On that day (age, 82 days) they did not react to 1:1,000 EW in 30 minutes, but did react somewhat slowly to the 1:100 EW aerosol. They failed to react to 4 subsequent exposures to 1:1,000, 1:100, and 1:20 EW, a total of 3.25 hours, on that and the next day.

Third Spontaneous Resensitization.^{7, 11}—The test pigs, A and B, did not react to the 1:20 EW aerosol when challenged at the age of 98 days, 15 days after the third desensitization, although the control reacted promptly (2+) to 1:1,000 EW. But when challenged 30 days later (age, 128 days), 45 days after the third desensitization, both pigs reacted promptly to 1:20 EW. The control pig reacted moderately (2+) to a 10 minute exposure to 1:1,000 EW.

*Spontaneous Desensitization.*⁷—The test animals and the remaining control pig had equivocal reactions to 30 minute exposures to aerosols of 1:1,000, 1:100, and 1:20 EW at the age of 209 days. At this point it was assumed that the surviving test animal and the control had become spontaneously desensitized. Neither pig reacted when challenged with CEA intravenously when 235 days old.

DISCUSSION

Ratner, Jackson, and Gruehl¹⁵ reviewed the problem of congenital sensitization of guinea pigs in 1927. Two forms, active and passive, were described. One, passive sensitization in utero by mothers which had been *actively sensitized before conception*, was remarkably uniform and profound. The sensitized mothers continued to sensitize litter after litter. The sensitization lasted 78 to 120 days in their pigs, whereas Cohen and Woodruff's¹⁷ remained sensitive for 199 to 286 days. This type of sensitization was not transferred to the second generation. The other form, active sensitization in utero, was induced by giving the mothers sensitizing doses of antigen shortly *before birth* of the young pigs. Active sensitization in utero was unpredictable. A majority of the young pigs did not become sensitive. One group became mildly to moderately sensitive and a third group had severe anaphylactic reactions to intravenous injections of antigen. Those newborn pigs were not sensitive at birth, in contrast to those sensitized passively in utero. When the mothers were given injections of antigen shortly before birth, Ratner, Jackson, and Gruehl¹⁵ thought it took longer for the offspring to become sensitive than the 21 days which young pigs required for active sensitization.

The pigs reported in these experiments were obviously sensitized passively in utero since the mothers had been sensitized actively 46 to 56 days before conception. They reacted violently to the aerosols of antigen but not to saline when they were 7 days old. Violent reactions had been observed previously in 2-day-old pigs.³⁰ Anaphylactic reactions to aerosols of antigens have been shown to be specific.^{10, 11}

A number of observations on these few animals are worthy of note. All 8 of the guinea pigs, which had been passively sensitized in utero by their actively sensitized mothers, were exquisitely sensitive to aerosols of the specific antigen. Two of them died in acute or prolonged anaphylactic reactions before it was realized that they should have been removed promptly at the *very first sign* of a reaction to the aerosol. Three controls died in spite of this precaution.

The pigs became sensitive again spontaneously^{7, 11} after each desensitization. They had moderate reactions when challenged at intervals of 18 to 45 days after desensitization. The test pigs did not react to any of the 3 aerosol challenges when 54 days old, 4 days after they had reacted moderately (2+) to the test for spontaneous resensitization. This is the only example of desensitization following a single reaction to an aerosol which has been observed in this laboratory. Later, the test pigs did react to the 1:100 EW aerosol when 82 days old, 6 days after they had reacted to the test for resensitization. It is uncertain whether this reaction at the age of 82 days represents failure to be desensitized by the reaction 6 days earlier, or whether the reaction at the age of 82 days indicated resensitization within 6 days. It seems obvious from these observations that spontaneous resensitization in congenitally sensitized guinea pigs is predictable, but varies in time and severity.

Congenitally sensitized pigs were not considered fully desensitized until they failed to react to concentrated aerosols on 2 or 3 successive days. For example, the 25-day-old test pigs, A and B, seemed to be completely desensitized to 1:20 EW, but reacted promptly when challenged the next day. The sequence was observed again when they were 26-27, 27-28, and 28-29 days old. Apparent desensitization to one dilution does not imply desensitization to a stronger one, as shown when the pigs were 27-28 days old.

One pig (B) seemed more resistant to desensitization than the other (A) and required 2 additional desensitization exposures (age, 28-29 days).

The quicker the onset the more severe the reaction was apt to be.¹⁰ Most reactions began within 10 minutes of the aerosol exposure. Fifty-nine of the 78 reactions to 130 aerosol exposures occurred between 3 and 5 minutes, 5 in less than 3 minutes, and 12 between 5 and 10 minutes. Only 2 reactions began after 15 minutes in the aerosol. This is in agreement with Feinberg and Malkiel³¹ who considered their pigs insensitive to the aerosol if there was no reaction within 10 minutes.

Both similarities and differences were noted when the behavior of these congenitally sensitized pigs during aerosol desensitization was compared with that of actively or passively sensitized adult pigs. The similarities were (1) the reaction of guinea pigs to specific aerosols which varied in intensity and in time of onset^{7, 11}; and the fact that (2) repeated aerosol exposures delayed the onset of reactions.⁷

The differences were: (1) The initial desensitization of the congenitally sensitized pigs was much more difficult than in passively sensitized adult pigs.^{10, 11} The more than 40 aerosol exposures required to desensitize these test pigs contrasts sharply with the quick desensitization of passively sensitized

adult pigs.^{10, 11} The ease with which the pigs were desensitized a second and a third time was comparable to the desensitization of the passively sensitized adult pigs.^{10, 11} (2) Spontaneous resensitization *did not return* within 15 days on at least one occasion.^{10, 11} (3) Spontaneous resensitization was observed 3 times, 18, 20, and 25 days after each desensitization. In spite of these rather long periods of resensitization, the reactions were less intense than in the initial pre-desensitization responses. Spontaneous resensitization has been observed within 2 to 8 days in passively¹¹ and in actively sensitized adult pigs.⁷ (4) Spontaneous desensitization occurs within 2 to 3 weeks in guinea pigs passively sensitized with heterologous serum.³² Spontaneous desensitization occurred in these pigs between the ages of 128 and 209 days. This was between the duration (199+ days) of sensitization in the pigs studied by Cohen and Woodruff¹⁷ and those (78+ days) reported by Ratner, Jackson, and Gruhl.¹⁵

Congenitally sensitized pigs did not become actively sensitized by the aerosols in spite of inhalation of the antigen for over 34 hours.¹ Had they become actively sensitized by the aerosol, spontaneous desensitization would have been postponed several months beyond the date of the last aerosol reactions (age, 128 days) and they would have reacted to the intravenous CEA challenge at the age of 235 days.

A detailed comparison of the several methods^{7, 10, 11} of aerosol desensitization is needed. Aerosol desensitization has been observed following recovery from one or more anaphylactic reactions while still in the aerosol,¹⁰ after prolonged exposures to sub-reacting concentrations of aerosol,¹¹ after several decreasingly intense reactions to aerosols,^{10, 11} and, now, after many exposures to concentrations of antigen which caused repeated severe reactions. The advantages and disadvantages of the several methods of aerosol desensitization should be compared in actively and passively sensitized adult and newborn guinea pigs.

There is a growing literature on the clinical use of aerosols of allergens in the diagnosis and treatment of hay fever and asthma.^{4, 5, 9, 12, 13} This should be encouraged by providing detailed assessments of a variety of techniques to be perfected in experimental animals.

The demonstration of reactions to inhaled allergens in congenitally sensitized guinea pigs revives the unsettled question of congenital sensitization of infants.^{16, 20-23, 25, 29, 33} A carefully planned immunological study of the first few post-natal days and of the first few weeks of infants born of allergic and nonallergic mothers should lead to the solution of this long-disputed problem.

SUMMARY

Newborn guinea pigs, sensitized in utero, were highly sensitive to aerosols of specific antigens.

They were desensitized by aerosols of the antigen. This required an average of 48 exposures to increasing concentrations of the antigen aerosols for a total of 34 + hours in 14 days.

They became sensitive again spontaneously, but less intensely within 18 days.

This sequence of aerosol desensitization and spontaneous resensitization was repeated 3 times in 128 days. Desensitization was obtained much more easily the second and third times.

The pigs were no longer sensitive when challenged with the specific aerosol at the age of 209 days.

The significance of these observations is discussed.

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