

STUDIES IN BACTERIAL ALLERGY*†

IV. THE TRANSITORY NATURE OF DESENSITIZATION OF THE PASSIVELY SENSITIZED GUINEA PIG UTERUS WITH A BACTERIAL HAPTEN, WITH A NOTE ON 95 PER CENT O₂ - 5 PER CENT CO₂ AS AN AERATING MIXTURE

OSCAR SWINEFORD, JR., M.D.,‡ CHARLOTTESVILLE, VA., AND
MAJOR ROGER J. REYNOLDS, MEDICAL CORPS, UNITED STATES ARMY

D ESENSITIZATION of actively sensitized guinea pigs with whole antigens is transient,¹ probably because the desensitizing antigen stimulates further anaphylactic antibody formation.

The uterine horns of guinea pigs are reported to be desensitized by whole antigens.²⁻⁵ The duration of desensitization has not been studied.

The present experiments were planned to study the role of haptens in the desensitization of uterine horns of passively sensitized guinea pigs. Pneumococcal polysaccharides were selected as representative bacterial haptens because of their limited functions as antigens.^{6, 7}

Spontaneous resensitization of the desensitized uterine horn was observed, unexpectedly. This phenomenon, which has not been described before, is the main point of interest in this report. The relation between spontaneous resensitization and Dale's⁴ supposed in vitro passive sensitization of excised uterine horns will be discussed briefly.

METHODS

Small virgin guinea pigs were sensitized passively with 0.2 to 0.5 ml. of refined antipneumococcal rabbit serum,§ given intra-abdominally. They were killed by a blow on the head 24 to 72 hours later. The uterine horns were excised, suspended⁴ in 125 ml. of Tyrode's⁸ solution at 37° C., and aerated with a 95 per cent oxygen-5 per cent carbon dioxide mixture. When 0.2 mg. of type-specific polysaccharide|| was added to the Tyrode's bath, a marked contraction occurred in about two-thirds of the horns. Those that did not contract were discarded. Nonspecific polysaccharides were used as controls. Types I, II, and IV pneumococcal polysaccharides and antisera were used.

DESENSITIZATION

Two methods were used: (1) contracted horns were left in the hapten baths until they relaxed to their original lengths³; and (2) contracted horns were allowed to relax in fresh Tyrode baths. They were then re-exposed to

*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.

‡From the Allergy-Arthritis Division, Department of Internal Medicine, University of Virginia Medical School.

§Generously supplied by Dr. B. Scott Fritz of the Marietta Laboratories, Wyeth, Incorporated, Marietta, Pa. Types I and II antisera contained 4,000 units per milliliter. Type IV contained about 6,600 units per milliliter.

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

the hapten bath. This alternation between hapten and fresh bath was continued until there was no further reaction to the hapten. Horns desensitized by either method still gave strong reactions to histamine. Two of 3 horns passively sensitized to 2 types of pneumococci, when desensitized with one type-specific hapten, remained fully sensitive to the other.

Nonspecific polysaccharide control haptens did not elicit contractions at any time.

RETURN OF SENSITIVITY

After desensitization, the horns were flushed with Tyrode's solution and placed in fresh baths. The horns were rechallenged with 0.2 mg. polysaccharide after varying intervals. Table I shows that none of the horns reacted after 10 minutes in the fresh bath. Eighteen of 19 horns showed full contractions when tested after 4 or more hours in the baths. In sharp contrast, only one horn showed a full contraction after less than 4 hours in the bath.

TIME ELAPSED IN PARTIAL AND COMPLETE SPONTANEOUS RESENSITIZATION OF 26
DESENSITIZED UTERINE HORNS OF GUINEA PIGS

RETURN OF SENSITIZATION	INTERVAL BEFORE RETEST														
	MINUTES	HOURS													
	10	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Partial		1	1	2	1										
Complete				1	9	4		1					2		2
None	26	2													

The method of desensitization did not affect the return of sensitization.

When partially resensitized horns (less than 4 hours in the fresh bath, Table I) were again desensitized, they became fully resensitized after 4 or more hours in a second fresh bath.

Successive desensitization and resensitization was demonstrated 2 or more times in several horns.

Every horn which was desensitized exhibited the phenomenon of resensitization one or more times. The return of sensitivity was not impeded in the 2 instances in which the horns were left in the hapten bath for one hour after desensitization. The horns were challenged regularly with nonspecific polysaccharide controls. The return of sensitivity was always specific.

Fig. 1 illustrates a typical sequence of events.

DISCUSSION

The data presented confirm reports⁹ that specific pneumococcal polysaccharide will cause contraction and desensitization of uterine horns of passively sensitized guinea pigs. The data further show that this desensitization is transient. Resensitization is complete in 4 hours. It is demonstrable in lesser degree in from 1 to 3 hours. This is in contrast to implications²⁻⁵ that uterine horns are thus permanently desensitized to a given concentration of antigen.

Dale⁴ found that uterine horns specifically desensitized with horse serum became partially, but not completely, resensitized after 2½ hours in anti-horse guinea pig serum. His desensitized horns, after a similar interval in Ringer's

solution, showed no spontaneous resensitization. The only desensitized control horn tested for resensitization after more than $2\frac{1}{2}$ hours in Ringer's solution did not react. Dale interpreted these experiments to show passive sensitization *in vitro*. Inexplicably (to him), he found that normal uteri, after $2\frac{1}{2}$ hours in the anti-horse guinea pig serum, were not sensitive. The hypothesis is tenable, therefore, that Dale was dealing with this phenomenon of spontaneous resensitization instead of, as he supposed, with *in vitro* passive sensitization of the excised uterine horn of the guinea pig.

Experimental explanation of this spontaneous resensitization has not been attempted. Antigen-antibody combinations (precipitates, and so on) have been reversed by heat,¹⁰ alkali,¹¹ salts,¹⁰⁻¹² and excess of antigen.¹³ These conditions are not present in the Tyrode bath.

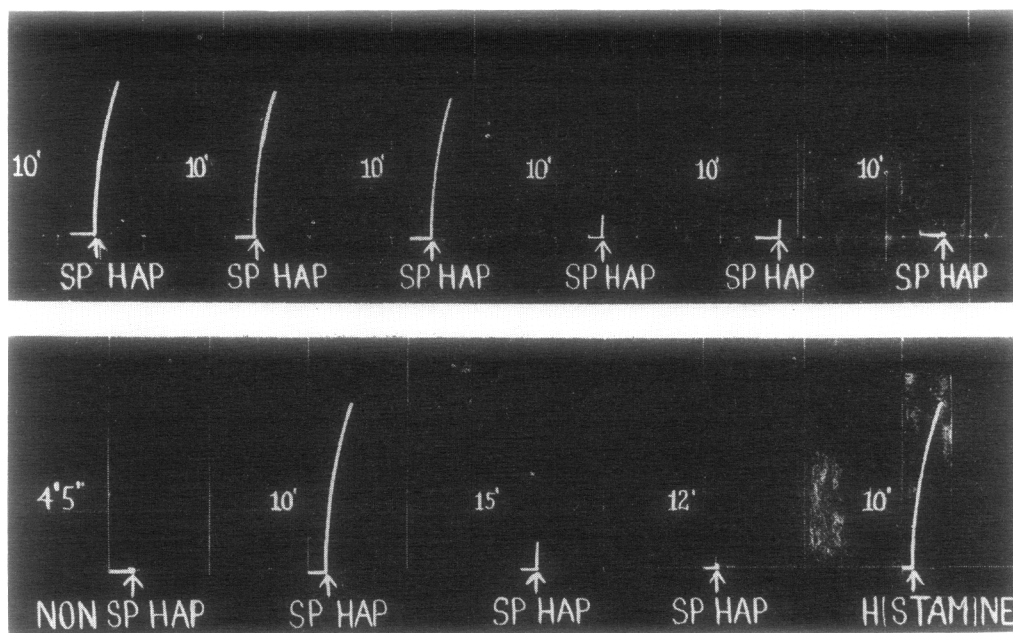


Fig. 1.—Illustration of specific desensitization (Method 2), spontaneous resensitization, and re-desensitization. Sp. Hap. = specific hapten; Non Sp. Hap. = nonspecific hapten.

Top row: 3 full and 2 slight specific contractions, at 10 minute intervals, followed by desensitization.

The horn relaxed in fresh Tyrode's bath after each reaction to hapten.

Bottom row, after 4 hours and 5 minutes in Tyrode's solution: no reaction to nonspecific hapten, full response to specific hapten, slight specific contraction, desensitization, full response to histamine.

One can postulate that the hapten-antibody combination is less firm than the antibody-tissue union. Sufficient hapten could thus be released to the bath in 4 hours to free enough antibody-tissue combinations to react fully with new hapten. This should be susceptible to proof.

The experiments were handicapped initially by the development of hyper-reactivity of the excised horns, followed by their death. This was traced to a relatively rapid increase in the pH of the Tyrode solution when compressed air

was used to aerate the bath. When a 95 per cent O_2 - 5 per cent CO_2 mixture was substituted, the approximate pH 7.4 was maintained for hours. Thereafter, all initially sensitive horns maintained an almost uniform reactivity for the duration of the individual experiments (Table I). No effort was made to see how much longer the horns would remain reactive. Sustained allergic reactivity, plus the phenomenon of resensitization, should increase the usefulness of excised uterine horns as experimental tools.

CONCLUSIONS

1. Pneumococcal polysaccharides, specifically, elicit contractions of, and desensitize, excised uterine horns of passively sensitized guinea pigs.

2. After 4 or more hours in Tyrode's solution the uterine horns become fully resensitized and react fully to the readdition of the original concentration of the specific pneumococcal polysaccharide. In other words, desensitization of uterine horns, as described, is transient.

3. This sequence of desensitization and spontaneous resensitization was repeated 2 or more times in the same horn.

4. It is believed that Dale's observation of the reappearance of sensitivity in uterine horns in immune serum baths may have been due, in part, to spontaneous resensitization.

5. Sustained specific allergic reactivity for 12 hours or longer in Tyrode's bath aerated with 95 per cent O_2 - 5 per cent CO_2 , plus the phenomenon of spontaneous resensitization after desensitization should increase the usefulness of excised uterine horns as experimental tools.

REFERENCES

1. Wilson, G. S., and Miles, A. A.: *Topley and Wilson's Principles of Bacteriology and Immunity*, Vol. II, Baltimore, Md., 1946, The Williams and Wilkins Company.
2. Smith, D. T., and Martin, D. S.: *Zinsser's Textbook of Bacteriology*, New York, 1948, Appleton-Century-Crofts, Inc.
3. Walzer, M., and Grove, E. F.: Studies in Specific Hypersensitivity: XVI. On Antigens. A Comparative Study of the Antigenic Properties of Pollens, Egg White, and Glue in Guinea Pigs, as Determined by the Intravenous and Dale Methods of Testing in Anaphylaxis, *J. Immunol.* **10**: 483, 1925.
4. Dale, H. H.: The Anaphylactic Reaction of Plain Muscle in the Guinea Pig, *J. Pharmacol. and Exper. Therap.* **4**: 167, 1912-13.
5. Coca, A. F., Walzer, M., and Thommen, A. A.: *Asthma and Hay Fever in Theory and Practice*, Springfield, Ill., 1931, Charles C Thomas, Publisher.
6. Avery, O. T., and Heidelberger, M.: Immunological Reactions of the Isolated Carbohydrate and Protein of the Pneumococcus, *J. Exper. Med.* **42**: 347, 1925.
7. Avery, O. T., and Neill, J. M.: The Antigenic Properties of Solutions of Pneumococcus, *J. Exper. Med.* **42**: 355, 1925.
8. Best, C. H., and Taylor, N. B.: *The Physiological Basis of Medical Practice*, Baltimore, Md., 1950, The Williams and Wilkins Company.
9. Mehlman, J., and Seegal, B. C.: Passive Sensitization of the Guinea Pig With Rabbit and Horse Anti-Pneumococcus Type I Serums, *J. Immunol.* **26**: 1, 1934.
10. Huntoon, F. M., and Etris, S.: Antibody Studies. II. The Recovery of Antibody From Sensitized Antigens: Technique, *J. Immunol.* **6**: 123, 1921.
11. Heidelberger, M., and Kendall, F. E.: Quantitative Studies on Antibody Purification. I. The Dissociation of Precipitates Formed by Pneumococcus Polysaccharides and Homologous Antibodies, *J. Exper. Med.* **64**: 161, 1936.
12. Felton, L. D.: Dissociation of Specific Protein Precipitate of Antipneumococcus Horse Serum, and a Comparison With a Protein Isolated by Chemical Means From This Immune Serum, *J. Immunol.* **22**: 453, 1932.
13. Boyd, W. C.: The Time Factor in Solubility of Precipitates in Excess Antigen, *J. Immunol.* **38**: 143, 1940.