

EXPERIMENTS ON HISTAMINE REFRACTORINESS

IV. HISTAMINE PRETREATMENT IN EGGWHITE-SENSITIZED GUINEA PIGS*†

LAURENCE FARMER, M.D., NEW YORK, N. Y.

IN EARLIER experiments Farmer^{1, 2} showed that uterine strips of serum-sensitized guinea pigs which had received histamine orally or by intraperitoneal injections were less sensitive to the antigen than were the control animals which had not been pretreated with histamine.

Karady³ confirmed these findings in the intact animal. Anaphylactic death in serum-sensitized guinea pigs which had been pretreated with histamine could be prevented in 75 to 90 per cent of the animals in contrast to only 15 to 25 per cent survival in the controls.

However, Karady found that if eggwhite was used as the sensitizing antigen pretreatment with histamine did not significantly protect the animals from fatal anaphylaxis. In contrast to this finding, Karády and Bentsáth⁴ have reported that in eggwhite-sensitized and histamine-pretreated guinea pigs a lesser drop in rectal temperature was observed on reinjection with the specific antigen than in the control animals. This observation was interpreted by Karády and Bentsáth as demonstrating that the histamine-pretreated animals showed a lesser degree of anaphylactic response.

It would seem that the different outcomes of the anaphylaxis experiments in serum- and eggwhite-sensitized guinea pigs after histamine pretreatment (Karady,³) are based on quantitative factors rather than on fundamental differences of mechanism. We subscribe to Karady's opinion that: "A possible explanation . . . lies in the fact that guinea pigs are very easily highly sensitized to eggwhite so that even minute amounts will release histamine in excess of the amount which treated animals will tolerate."

In view of the conflicting findings of Karady³ and of Karády and Bentsáth⁴ and because of the theoretic importance of the problem, the following experiments were undertaken.

EXPERIMENTAL

A. Technique.—In order to obviate the possibility of an interference with the immunologic process through histamine pretreatment, passive sensitization of the test animals was believed to be the method of choice.⁵ Anti-eggwhite rabbit serum, prepared according to the method of Grove,⁶ was used for passive sensitization. Two antiserums (III and V) were used. Serum V was divided into two parts (V_1 and V_2). The serums which varied in their sensitizing capacity (see Table II) were frozen, stored at -17°C ., and thawed out before use.⁷

*From the Allergy Clinic and Achelis Laboratory, Lenox Hill Hospital.

†Aided by a grant from Parke, Davis & Co., Detroit, Mich.

TABLE I. HISTAMINE PRETREATMENT EXPERIMENTS

GUINEA PIG	HORN	INITIAL EGGWHITE CONCENTRATION	EGGWHITE CONCENTRATION AT WHICH CONTRACTION OCCURRED	REACTION
<i>Rabbit Serum III</i>				
1882	1	1:10 ⁷	1:10 ⁴	++++
	2	1:10 ⁶	1:10 ²	++++
1883	1	1:10 ⁶	1:10 ⁶	++++
	2	1:10 ⁸	1:10 ²	+++
1885	1	1:10 ⁷	1:10 ²	++++
	2	—	1:5×10 ¹	Spontaneous
1886	1	1:10 ⁷	1:10 ⁴	++++
	2	1:10 ⁶	1:10 ⁶	++++
1887	1	1:10 ⁷	1:10 ⁶	++++
	2	—	—	Spontaneous
1876	1	—	—	Spontaneous
	2	1:10 ⁷	1:10 ⁷	+ (+)
1877	1	1:10 ⁷	1:10 ⁶	++
	2	—	—	Spontaneous
<i>Rabbit Serum V₁</i>				
1894	1	—	—	Spontaneous
	2	1:10 ⁷	1:10 ⁷	++
1895	1	1:10 ⁷	1:10 ⁷	+
	2	—	—	Spontaneous
1896	1	1:10 ⁷	1:10 ⁶	++
	2	1:10 ⁷	1:10 ⁷	++++
1934	1	—	—	Spontaneous
	2	1:10 ⁹	1:10 ⁸	++
1937	1	1:10 ⁹	1:10 ⁷	++ (+)
	2	1:10 ⁹	1:10 ⁹	++++
1938	1	1:10 ¹⁰	1:10 ⁹	++++
	2	1:10 ¹⁰	1:10 ⁸	+
1939	1	1:10 ¹⁰	1:10 ¹⁰	++++
	2	1:10 ¹⁰	1:10 ¹⁰	++
1941	1	1:10 ¹⁰	1:10 ¹⁰	++++
	2	1:10 ¹¹	1:10 ¹¹	++++
1942	1	1:10 ¹³	1:10 ¹²	++++
	2	—	—	Spontaneous

The test animals, young virgin guinea pigs, weighing approximately from 200 to 300 grams, were passively sensitized by an intraperitoneal injection of 1.0 c.c. of antiserum diluted 1:5. Twenty-four hours later the animals were killed by a blow on the head and their uterine strips suspended in a Schultz-Dale bath as described in earlier publications.^{1, 2} Frequently the horns showed spontaneous contractions on suspension in the bath; these horns were excluded from further examination.

If they remained relaxed the degree of their anaphylactic reactivity was determined by adding increasing amounts of fresh filtered eggwhite to the bath at intervals of one and one and one-half minutes until a contraction of the uterine strip was elicited. If no contraction occurred after addition of the eggwhite, 0.5 c.c. of a 1:1000 histamine phosphate solution was added to ascertain if the muscle was still reactive. In some of the histamine azoprotein experiments, 0.5 c.c. histamine azoprotein instead of histamine was added to the bath.

In numerous instances greatly varying amounts of eggwhite were needed to effect anaphylactic contraction of the individual uterine horns of the same animal. Results were therefore recorded and evaluated on the basis of the anaphylactic response of the individual uterine horn. The results of all experiments are given in Table II.

No attempt was made to express the results of these experiments in strictly quantitative terms as each subliminal dose of antigen will cause some degree of desensitization without eliciting a contraction of the uterine strip. In evaluating the experiments only the ranges in which contractions in the control and histamine pretreatment series occurred were compared.

B. Results.—

Control Experiments: Seventy-nine guinea pigs were passively sensitized and their anaphylactic reactivity determined as already described. (See Table II.)

TABLE II

EGGWHITE CONCENTRATION	CONTROL EXPERIMENTS		HISTAMINE PHOSPHATE PRETREATMENT		HISTAMINE AZOPROTEIN PRETREATMENT	
1×10 ⁻¹⁸	×					
1×10 ⁻¹⁷	×					
1×10 ⁻¹⁶	×					
1×10 ⁻¹⁴	xxx					
1×10 ⁻¹³	xxxx					
1×10 ⁻¹²	×		×			
1×10 ⁻¹¹			×			
1×10 ⁻¹⁰			xxx			
1×10 ⁻⁹	×		xxx			
1×10 ⁻⁸	xxxx	+	xx		×	
1×10 ⁻⁷	xxxx	0	xx	0	×	
1×10 ⁻⁶	xx	000000000	×	0000	×	
1×10 ⁻⁵	xx	00000+++++	×		×	
1×10 ⁻⁴	xxxxx	0000++		00	×	++++
1×10 ⁻³		0			×	++
1×10 ⁻²		00		00	xxx	++

Eggwhite concentration is the amount of eggwhite needed to elicit contraction of the uterine strip.

0, Contraction of a uterine strip of an animal sensitized with serum III.

x, Contraction of a uterine strip of an animal sensitized with serum V₁.

+, Contraction of a uterine strip of an animal sensitized with serum V₂.

Histamine Phosphate Pretreatment Experiments: Sixty-six guinea pigs were used. They were given thirteen intraperitoneal injections of histamine phosphate in increasing amounts over a period of two weeks. The highest average dose given was 100 γ per 100 Gm. of body weight. In a few instances up to 150 γ per 100 Gm. was used. The total amount of histamine phosphate given was 1,085 γ per 100 Gm. of body weight. This amount was seemingly not well tolerated as 30 per cent of the animals died before they could be used for passive sensitization. Two or three days after the last histamine injection the animals were passively sensitized and twenty-four hours later their uterine strips were tested as has been described. (See Table II.)

Histamine Azoprotein Pretreatment Experiments: Forty guinea pigs were used in this group. They received thirteen intraperitoneal injections of histamine azoprotein in increasing amounts over a period of two weeks. Varying

amounts of histamine azoprotein were used for pretreatment. First, a total of 3.26 c.c., then, 0.61 c.c., and finally, 0.20 c.c. per 100 Gm. of body weight were given. The larger amounts of undiluted histamine azoprotein were very poorly tolerated so that recourse was taken to a 1:10 and a 1:100 dilution. The percentage of fatalities in the histamine azoprotein group was 62 per cent so that only fifteen pretreated animals survived. Two or three days after the last histamine azoprotein injection the animals were passively sensitized and their uterine strips were tested twenty-four hours later. (See Table II.)

DISCUSSION

As appears from the table there is no appreciable difference in the amounts of eggwhite needed to effect contraction of the uterine strips of the control animals and of the animals pretreated with histamine phosphate or histamine azoprotein. This finding is in accordance with Karady's experiments with egg-white sensitization. It is in contrast to our findings in guinea pigs sensitized with horse serum.

SUMMARY

Histamine pretreatment of guinea pigs (passively) sensitized to eggwhite failed to decrease their anaphylactic response to the specific antigen. This result is in contrast to our findings in serum-sensitized guinea pigs in which pretreatment with histamine decreases the anaphylactic response to serum.

REFERENCES

1. Farmer, L.: Nonspecific "Desensitization" through Histamine, *J. Immunol.* **36**: 37, 1939.
2. Farmer, L.: Experiments on Histamine-Refractoriness. II. Non-Specific "Desensitization" Through Oral Application of Histamine, *J. Immunol.* **37**: 321, 1939.
3. Karady, E. S.: Histamine Tolerance and Anaphylactic Death in Sensitized Guinea-Pigs, *J. Immunol.* **41**: 1, 1941.
4. Karády, S., and Bentsáth, A.: Histamingewöhnung und experimentelle Anaphylaxie, *Ztschr. f. d. ges. exper. Med.* **100**: 48, 1936.
5. Farmer, L.: Experiments on Histamine Refractoriness. III. The Effect of Histamine Pretreatment on the Establishment of Active Sensitization, *J. ALLERGY* **19**: 353, 1948.
6. Grove, Ella F.: Studies in Anaphylaxis in the Rabbit. I. A Study of the Factors Concerned in the Establishment of Maximal Hypersensitiveness in Rabbits to Egg White and Horse Serum, *J. Immunol.* **23**: 101, 1932.
7. Ehrlich, P., Neisser, M., and Sachs, H.: Untersuchungen über das Verfahren von M. Neisser und H. Sachs zur forensischen Unterscheidung von Menschen- und Tierblut, *Klin. Jahrb.* **19**: 85 (footnote), 1908.