

## STUDIES IN ALLERGY

### I. SEROTONIN, HISTAMINE, AND ANTIGEN-ANTIBODY COMPLEX ACTIVITY IN ISOLATED SENSITIZED GUINEA PIG UTERI

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DURING the second decade of the twentieth century, Schultz<sup>1</sup> and Dale<sup>2</sup> developed a method for assaying reactivity of smooth-muscle preparations to foreign proteins and antigens in vitro. Since that time the sensitized guinea pig uterus and ileum have been used extensively for the study of allergenic sensitization and the basic physiology of anaphylaxis and allergic reactions.<sup>3-6</sup>

The Schultz-Dale apparatus and technique were further developed by Coulson and co-workers,<sup>7</sup> who have shown the great specificity and sensitivity of this reaction in detecting antigens of the order of 0.05 mcg. of ovalbumin. This sensitivity is 20 to 200 times that of the precipitin test and much greater than gross anaphylactic shock and anaphylactic sensitization.

For these reasons, it was felt that the Schultz-Dale reaction would be useful in studying blocking antibodies to specific antigens which would prevent the anaphylactic reaction of sensitized guinea pig uterine muscle cells to specific antigens.

#### METHOD

Twenty-five virgin female guinea pigs, each weighing 300 grams, were sensitized by the injection of 1.0 mg. protein per 100 grams body weight of alum-precipitated protein antigen, egg albumin, ragweed, house dust, and feathers prepared by the method of Coulson and Stevens.<sup>8</sup> After a three-week incubation period the animals were sacrificed at weekly intervals by a blow on the head or by anaphylactic shock produced with antigen injected intracardially. This allowed ample time for full sensitization to develop and for adequate testing of the uterine muscles. The uteri were removed and tested in an oxygen-aerated muscle bath with a perfusion bath volume of 25 ml. at a standard room temperature of 25° C. The bath contained a low-calcium Tyrode solution recommended by Coulson,<sup>7</sup> the formula of which follows:

|                         |          |                                        |           |
|-------------------------|----------|----------------------------------------|-----------|
| NaCl -----              | 8.0 Gm.  | NaHCO <sub>3</sub> -----               | 1.0 Gm.   |
| KCl -----               | 0.2 Gm.  | NaH <sub>2</sub> PO <sub>4</sub> ----- | 0.05 Gm.  |
| CaCl <sub>2</sub> ----- | 0.05 Gm. | Glucose -----                          | 1.0 Gm.   |
| MgCl <sub>2</sub> ----- | 0.10 Gm. | Water to make                          | 1,000 ml. |

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The foregoing experimental conditions were used because of the recommendation by Dalglish and colleagues<sup>9</sup> that they reduce the spontaneous activity of smooth muscle.

The uterine muscle segments were suspended in the bath, allowed to come to a base line, and then subjected to various antigens, antigen-serum mixtures, and other test compounds, such as histamine, serotonin, and acetylcholine. The resulting contractions were recorded on a kymograph rotated at 0.492 cm. per minute.

The antigens were obtained from commercial sources (egg albumin)\* and the Aspenwall VA Hospital Allergy Service† at Pittsburgh, Pennsylvania. The sera for the antigen-sera mixtures were obtained from allergy patients seen in the outpatient service and in the wards of the medical service of the VA Hospital in Omaha.‡ Blood was drawn while patients were on hyposensitization treatment. Guinea pig serum was obtained by cardiac puncture from animals sensitized by subcutaneously injected alum-precipitated antigens.

Antigen-antibody complexes were formed by mixing an appropriate amount of antigenic protein (egg albumin, ragweed, house dust, and feathers) in a ratio of 1 to 100 with dialyzed human or guinea pig serum protein. These mixtures were incubated for thirty minutes at 37° C. and then for twenty-four hours at 4° C. Following this, the mixtures were centrifuged, and the supernatant was used for testing in the muscle bath.

An operation was devised whereby one uterine horn was removed from the sensitized guinea pig under ether anesthesia and the abdomen was sutured after hemostasis of the uterine stump. Two or three hours later the animal was anesthetized again and blood was drawn by cardiac puncture. Through the same intracardiac needle, 3 mg. of the appropriate antigen (egg albumin, house dust, ragweed, or feathers) was given. This was generally followed by an anaphylactic reaction. After death, the remaining uterus was removed and placed in the Schultz-Dale apparatus for testing. The uterine horn removed prior to antigenic shocking is called the preshock horn, and that removed after antigenic shocking is termed the postshock horn.

Routinely, each strip was tested for its reactivity to acetylcholine base§ (32 mcg. per milliliter), histamine base|| (0.2 mcg. per milliliter), and serotonin base¶ (7 mcg. per milliliter). Depending upon the antigen or antigens used in sensitization, the strips were next tested with saline solutions of ovalbumin (EA), 4 mcg. P per milliliter; ragweed protein (RW) 16 mcg. PN per milliliter; house dust protein (HD), 18 mcg. PN per milliliter; and feathers protein 7 mcg. P per milliliter. After the antigen testing the strips were again

\*Soluble egg albumin (Difco).

†Ragweed, house dust, and feather extract were kindly supplied by Dr. L. H. Crip, Aspenwall VA Hospital, Pittsburgh, Pennsylvania.

‡Sera supplied through the interest and kindness of Dr. Morris H. Brodsky, Omaha VA Hospital Outpatient Service.

§Acetylcholine chloride (Nutritional Biochemicals Corp.).

||Histamine diphosphate (Abbott).

¶Serotonin creatinine sulfate (California Research Foundation).

subjected to histamine, serotonin, and acetylcholine, respectively. Between each two test situations, the bath and strip were washed with several aliquots of 25 ml. of Tyrode solution.

A contraction was considered a significant response if it exceeded twice the height of the spontaneous contraction, if present, or was at least the same height as that produced by histamine base (0.2 mcg. per milliliter).

The use of a low-calcium Tyrode solution at 25° C. was found to be most satisfactory in producing a constant base line with a minimum of spontaneous contraction. We have found the uterine muscle of the guinea pig to be exceptionally responsive to acetylcholine and histamine but minimally responsive to serotonin in dosages approximating 300 to 400 times that needed for the rat and mouse uterus. With histamine, adequate responses are obtained with 5 mcg. per 25 ml. or 0.2 mcg. per milliliter. These are substantially less than those reported by Coulson (100 mcg. per 50 ml. or 2.0 mcg. per milliliter). Since we were not interested in the quantitative titration of the antigen reaction and since a preliminary pilot study showed that maximal consistent responses occurred with protein levels of the order of 4 mcg. egg albumin P per milliliter, 16 mcg. ragweed PN per milliliter, 18 mcg. house dust PN per milliliter, 7 mcg. feathers P per milliliter, these were used throughout the experiments in order to provide comparable contractions. All guinea pigs were sensitized with alum-precipitated proteins<sup>7, 8</sup> to ensure maximum sensitivity and to eliminate false negative reactions.

#### RESULTS

Fig. 1 shows the type of responses which were registered on the kymograph of the Schultz-Dale apparatus as a result of the contractions and relaxations of the guinea pig uterine strips. The upper half of the figure shows a typical pattern obtained from a normal untreated guinea pig uterine muscle strip. The normal strip responds repeatedly to acetylcholine and to histamine, but infrequently (30 per cent) to serotonin. The normal horn does not respond to such antigens as ragweed, house dust, feather extracts, and egg albumin. Fig. 1 (lower half) and Fig. 2 (upper experiment) show typical patterns obtained from sensitized horns. Here the responses are usually the same as those of the normal tissues, except that response is obtained from the antigen or antigens with which the animal had been sensitized.

In the case of the shocked pig the situation is quite different. As is shown in Fig. 2 (lower experiment), where the guinea pig had been sensitized and then shocked with feather extract, the strip responded to acetylcholine and histamine and also responded markedly to serotonin. The uterine horn segment did not respond to feathers because of previous desensitization by antigenic shocking.

In several experiments we were able to show that a soluble antigen-antibody complex produces not only a higher percentage of responses in sensitized guinea-pig uteri but also a greater contraction of uterine strips than does the antigen alone. Fig. 3 shows two strips taken from the same sensitized

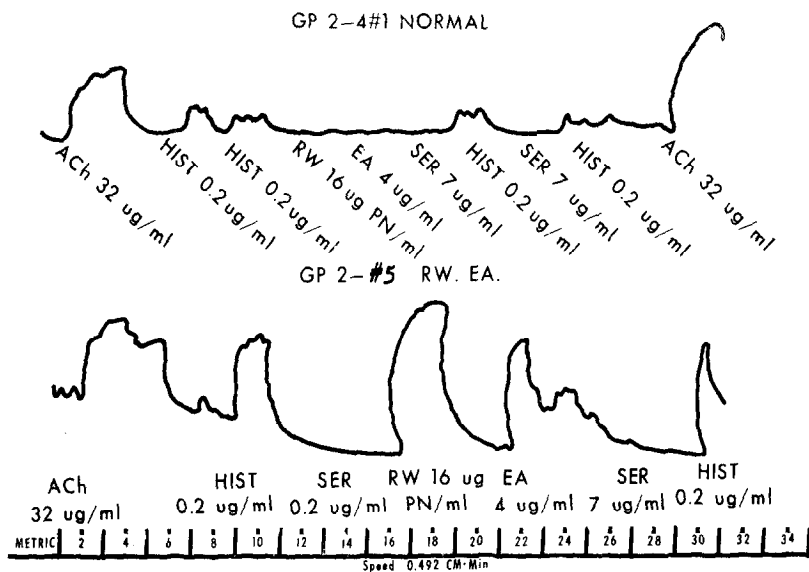


Fig. 1.—Upper half: Responses of a normal guinea pig uterine strip to various test substances.

Lower half: Responses of a guinea pig uterine strip sensitized to ragweed and egg albumin antigens.

Metric scale in centimeters. One minute equivalent to 0.492 cm.

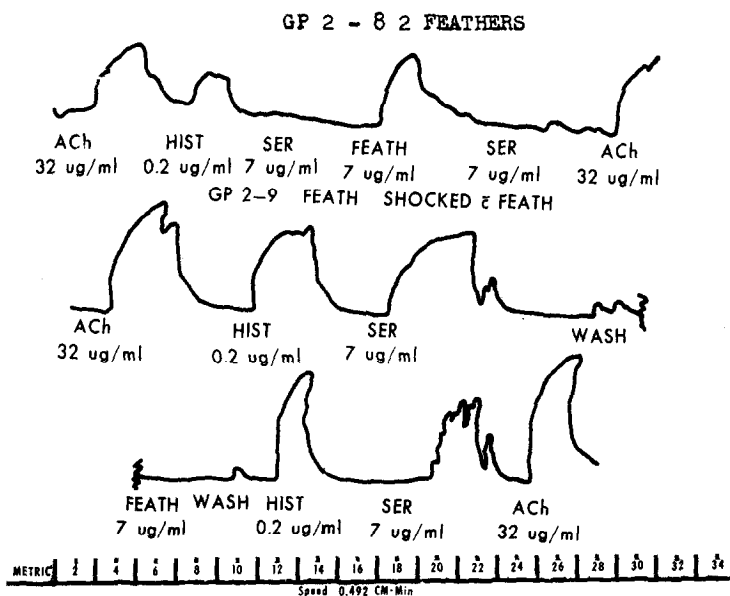


Fig. 2.—Upper experiment: Responses of a guinea pig uterine strip sensitized to feather antigen.

Lower experiment: Responses of a uterine strip taken from a guinea pig sensitized with feather antigen and shocked with feathers.

animal (HD). Both muscles responded equally to the same amount of histamine. When house dust (450 meg. PN) was mixed with saline-dialyzed serum protein (in a ratio of 1:100) from a patient who had been on a hypersensitization regimen and this mixture was added to the 25 ml. bath, the response obtained was much greater than that obtained from the other strip treated with the antigen alone.

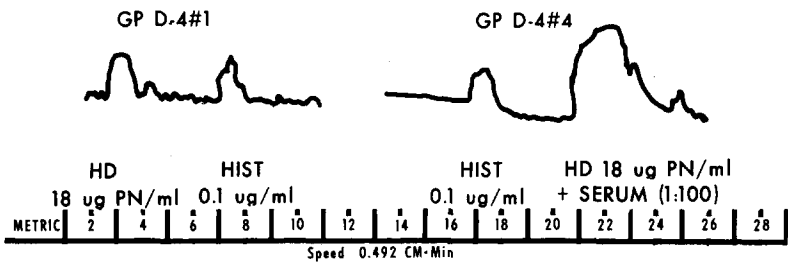


Fig. 3.—Responses of uterine strips taken from the same guinea pig sensitized with house dust antigen. First pattern shows response to antigen alone. Second pattern shows increased response to antigen-antibody complex.

Table I summarizes the results of the present study. Eighty per cent of normal, nonsensitized uterine horns responded to histamine. Following allergenic sensitization, 66 per cent of the sensitized uterine horn strips responded to histamine. The observed decrease in the number of horns reacting to histamine after sensitization was significant at the 0.05 level of probability. Histamine reactivity of the preshock sensitized uterine horn was not different from the histamine reactivity of the postshock sensitized horn after in vivo antigen shocking.

TABLE I. RESPONSE OF VIRGIN GUINEA PIG UTERI TO ANTIGENIC SHOCKING

| NO. SEGMENTS TESTED                   | RESPONSE ANTIGEN                                                                          | RESPONSE TO ANTIGEN AND SERUM COMPLEX | SEROTONIN RESPONSE                                 | HISTAMINE RESPONSE                                 |
|---------------------------------------|-------------------------------------------------------------------------------------------|---------------------------------------|----------------------------------------------------|----------------------------------------------------|
| 13 normal nonsensitized horn segments | 0%                                                                                        | 0%                                    | 30% <sup>(a)</sup>                                 | 80% <sup>(a)</sup>                                 |
| 108 sensitized preshock horn segments | (a) RW = 52%<br>(b) EA = 59%<br>(c) HD = 29%<br>(d) F. = 60%                              | RW = 87%<br>EA = 78%<br>HD = 65%      | 41% <sup>(b)</sup><br>21 of 51                     | 66% <sup>(b)</sup><br>72 of 108                    |
| 69 sensitized postshock horn segments |                                                                                           |                                       | 61% <sup>(c)</sup><br>25 of 41                     | 66% <sup>(c)</sup><br>46 of 69                     |
| Chi-square tests                      | (a) RW - RW + serum P > .01<br>(b) EA - EA + serum P > .01<br>(c) HD - HD + serum P > .01 |                                       | (a-b) P > 0.10<br>(a-c) P > 0.01<br>(b-c) P > 0.01 | (a-b) P > 0.05<br>(a-c) P > 0.20<br>(b-c) P > 1.00 |

Thirty per cent of normal uterine horns were sensitive to serotonin. After allergenic sensitization, 41 per cent of the sensitized uterine horns were sensitive to serotonin. This difference is not significant at the 0.10 level. When

the uterine muscle strips were subjected to *in vivo* anaphylaxis, the sensitivity to serotonin increased to 61 per cent positive responses, which was significant at the 0.01 level. Normal control uteri showed unchanged responses to serotonin in all cases when treated with antigen in the Coulson low-calcium Tyrode bath. Sensitized uterine muscles responded to ragweed protein 52 per cent, egg albumin 59 per cent, house dust protein 29 per cent, and feathers 60 per cent, demonstrating the relative activity of these proteins and protein mixtures as antigens and anaphylactogens. When ragweed protein was incubated with the dialyzed sera of patients undergoing hyposensitization to ragweed and this complex was added to the solution bathing the sensitized muscle, the positive response increased to 87 per cent. This increase was significant at the 0.01 level. Egg albumin mixed with sensitized guinea pig sera showed response increased to 78 per cent. This was significant at the 0.01 level. Similarly, when house dust protein was incubated with dialyzed sera of patients and added to the sensitized uterine muscle bath, the percentage of positive responses rose to 65 per cent. This rise was significant at the 0.01 level. Sera of patients under hyposensitization regimens alone showed only slight reactions on sensitized or unsensitized guinea pig uteri, and these were diminished or removed by dialysis of the sera.

Anaphylactic shocking by ragweed, egg albumin, feather, and house dust antigens resulted in increased serotonin sensitivity in guinea pig uteri. A significant reduction was noted in histamine sensitivity. This finding suggested that anaphylaxis may be a more complex phenomenon than previously postulated. Increased serotonin sensitivity at the shock-organ cell level may play a role in the production of anaphylaxis.

Antigen-antibody complexes formed by admixture of hyposensitized sera and antigen produce a greater incidence of positive anaphylactic reactions in sensitized guinea pig uteri than antigens alone. This may confirm published works of other authors concerning antigen-antibody complexes.<sup>6, 10, 11</sup>

No evidence was found in this study for the presence of blocking antibodies which protect sensitized uteri against antigens, either in human hyposensitized blood or in the blood of guinea pigs sensitized by alum-precipitated antigens.

#### DISCUSSION

We were puzzled by the lack of blocking activity exhibited by hyposensitized sera. Instead, we noted that anaphylactic reactions in sensitized uteri were markedly enhanced by addition to the muscle bath of mixtures of hyposensitized human sera and specific antigen. The human sera had been dialyzed against normal saline solution to remove histamine, serotonin, and other uterine muscle stimulants. Our conclusion is that the antigen-antibody complex acted directly upon the mediating system in the uterine muscle or nerve plexus to produce the anaphylactic contraction. The lack of species specificity for this reaction was demonstrated by the finding of a similar reaction produced by guinea pig sera and egg albumin against uteri sensitized to egg albumin. No evidence of antigenic blocking was found.

Can anaphylaxis be a result of increased sensitivity of the uterus to serotonin or histamine? Our study of histamine reactions showed that histamine response was significantly decreased by sensitization, and therefore anaphylaxis cannot be considered to be mediated by histamine alone. Uterine sensitivity to serotonin is not changed significantly by sensitization of the uteri, and an increased level of serotonin in the tissue is not presumed to be the factor responsible for this increased contraction rate and intensity. Fink and Gardner<sup>4</sup> were unable to demonstrate mediation of anaphylactic contractions by serotonin alone in guinea pig uteri. At this time the best explanation is that advanced by Germuth and McKinnon,<sup>6</sup> namely, that the shocking property of antigen-antibody mixtures is due to the presence of soluble antigen-antibody complexes. They suggest that anaphylactic shock does not require actual interaction of antigen-antibody complexes in the tissues but may be produced by preformed antigen-antibody complexes acting on susceptible cells.

Serotonin sensitivity is markedly and significantly increased by *in vivo* shocking with antigen. This is true for any antigen used in this series. Weissbach and associates<sup>12</sup> and Fink and Gardner<sup>4</sup> demonstrated decreased serotonin levels in guinea pig lungs and lung perfusates after shocking by antigen. These findings have led us to consider that anaphylactic responses in the guinea pig uterus at least may be produced by a more complex reaction than previously hypothesized. Anaphylaxis may involve the action of higher concentrations of serotonin released from platelets<sup>13, 14</sup> upon a tissue whose sensitivity to serotonin has been greatly altered by the *in vivo* anaphylactic reaction due to soluble antigen-antibody complex. Histamine sensitivity, though not significantly changed by anaphylaxis, may act to enhance or perhaps synergize serotonin reaction at the tissue level. One anaphylactic reaction to antigen or antigen-antibody complex blocks further response to the same antigen.

#### SUMMARY

The uteri of twenty-five virgin allergen-sensitized guinea pigs were studied in the Schultz-Dale muscle bath apparatus before and after *in vitro* and *in vivo* shocking. All uterine horns from guinea pigs responded to acetylcholine. Eighty-two per cent of normal nonsensitized horns contracted on addition of histamine. After sensitization, 61 per cent of the sensitized horns responded to histamine. Thirty to forty per cent of the uterine horns responded to serotonin before and after sensitization. After shocking by *in vivo* antigenic exposure, 61 per cent of the uteri responded maximally to serotonin. A significant increase in response of sensitized uteri was obtained through the use of soluble antigen-antibody complexes over the use of antigen alone.

Illustrations were prepared by Frank A. Roberts and John E. Friel, Medical Illustration Service, VA Hospital, Omaha, Nebraska.

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