In vitro Anaphylaxis in the Sensitized Mouse Uterus. (22071)

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Anaphylaxis in the mouse has been studied by many workers, and most of their findings indicate that histamine plays little or no role in anaphylactic shock in this species. Using the Schultz-Dale technic of *in vitro* anaphylaxis, this paper is concerned with an attempt to rule histamine in or out as being part of the basic mechanism in the reaction and to study the effect of pertussis vaccine on *in vitro* anaphylaxis.

Materials and methods. Schultz-Dale technic in mouse anaphylaxis. Fourteen virgin female Swiss mice over 3 months old were injected subcutaneously with 0.6 ml of alum precipitated egg albumin† prepared by the method of Proom(1). This amount contained 15 mg of the antigen. The mice were used 2 to 4 weeks later, at a time when, as judged by gaping of the vaginal orifice, they were in estrus, and the uterus consequently larger.‡ After sacrificing the animal by stretching its neck, the uterus was immediately removed and placed in a modified Ringer's solution(2). It was kept at 0°-4°C until used, usually within 2 hours. Two aerated tissue baths, each containing 45 ml of the Ringer's solution maintained at 38°-39°C were set up. One-half of one uterine horn was suspended in one bath; one-half of the other horn in the other bath.§ After 30 minutes, the lever on each of the strips was connected to a kymograph, and the pattern of normal contractions obtained. Crystalline egg albumin‖ was then added to each bath to a concentration of 15 μg per ml of bath. All additions were made with a 3-inch needle attached to a tuberculin syringe, with the tip of the needle bent in a manner to insure spraying of the liquid. After contraction of the strip, which invariably occurred within 30 seconds after the first addition of antigen, the tissues were washed, the normal contractions recorded, and a second addition of the same amount of egg albumin made to test for desensitization. When desensitization had been effected, to test the reactivity of the tissue, acetylcholine¶ was added to a concentration of 10 μg per ml, and the contraction recorded. At least one tissue strip from each of the 14 mice was found to react to egg albumin. In 13 of the mice, at least 2, and sometimes 3 or 4 reacted; in only one mouse was just one piece reactive. Duplicate contractions of tissues from the same uterus were usually but not always of a similar magnitude. The occasional dissimilarity in reactivity of different portions of the uterus is in line with similar observations on the guinea pig ileum(3). A typical contraction is shown in Fig. 1.

Tissues from 12 of the mice did not react on the second addition of antigen; but those from the other 2 mice were not desensitized until 3 additions of antigen had been made. Similar observations have been reported for the guinea pig(4). After desensitization, all of the tissues reacted to acetylcholine in a concentration of 10 μg per ml of bath. The uteri from 10 normal mice were similarly tested. In no case was there a reaction to egg albumin; but in every case a reaction occurred in response to the 10 μg per ml of acetylcholine.

Role of histamine and acetylcholine in mouse anaphylaxis. This experiment was de-

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† Albumin, Egg Impalpable Powder, J. T. Baker Chem. Co.

‡ After this first experiment was completed, it was found that administration of an estrogen 4 to 5 days prior to testing resulted in uniformly large uteri. In all other experiments to be reported mice were injected intramuscularly with 8.8 γ of *Progynon-B* (Schering Corp.) in mineral oil prior to testing.

§ In some instances, the other 2 pieces were tested in a similar manner.

‖ Armour and Co. preparation.

¶ Acetylcholine chloride, Matheson Co.

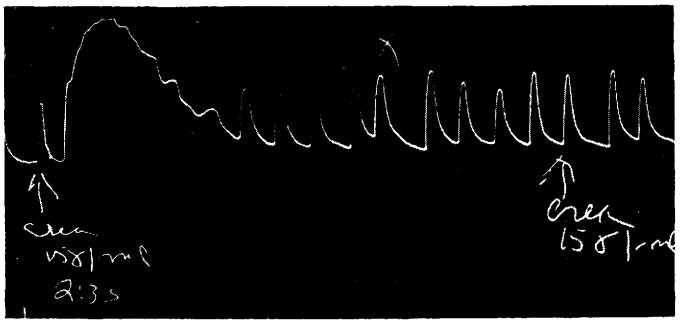

FIG. 1. Typical anaphylactic contraction of actively sensitized mouse uterus. Arrow = crystalline egg albumin added 15 μg/ml. × = contraction following wash.

signed to determine the role of histamine and acetylcholine by blocking reactions due to these drugs with Benadryl and atropine, respectively. It was first demonstrated that these drugs could block the reactions when histamine or acetylcholine were added to the sensitized mouse uterus; but that they could not block the reactions when antigen was added to the sensitized uterus. Ten mice actively sensitized as before and 3 mice passively sensitized by the intra-abdominal injection 4 hours prior to testing of 0.4 ml of pooled serum from actively sensitized mice were used in this study. This antiserum contained sufficient antibody for a positive precipitin reaction when antigen in a concentration of 0.01 mg per ml was added.

In preliminary studies it was found that histamine in a concentration of 50 μg of histamine** base per ml of bath was necessary to produce a uniform uterine contraction of the sensitized uterus. Benadryl†† in a concentration of 0.4 to 0.8 μg per ml of bath was always sufficient to prevent the reaction to histamine. Strips not responding to histamine in the presence of Benadryl would, after washing, respond to a corresponding dose of histamine.

It was also shown that atropine‡‡ in a concentration of 2 μg per ml of bath was invariably sufficient to prevent the reaction to 10 μg or more of acetylcholine per ml of bath.

** Crystalline histamine diphosphate, Eimer and Amend, dissolved in phosphate buffered saline, pH 7.2.

†† Parke, Davis and Co. preparation.

‡‡ Obtained from local pharmacy.

The technic of testing was as described previously. It was first established that a positive reaction to egg albumin could be obtained on one-half of one uterine horn. Then 2 fresh pieces of the same uterus were set up in the 2 water baths. To one of these Benadryl was added to a concentration of usually 0.4 μg per ml of bath; to the other, atropine was added to a concentration of 2 μg per ml of bath. After about one minute, the antigen was added as before. In some instances, the fourth piece of tissue was tested in the presence of both atropine and Benadryl.

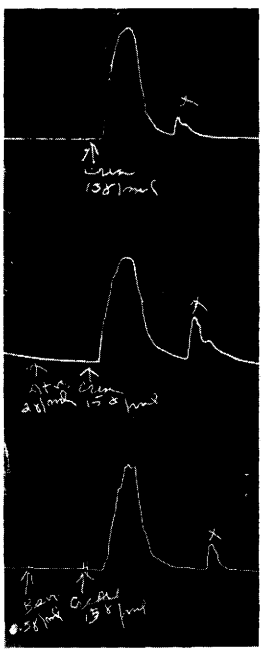

FIG. 2. Persistence of anaphylactic contraction in presence of atropine and Benadryl. × = contraction following wash.

In all 13 mice—10 actively and 3 passively sensitized—neither Benadryl nor atropine inhibited or diminished the anaphylactic contraction (Fig. 2).

Uterine mast cells. In view of reports indicating that histamine may be released from tissue mast cells(5,6), an experiment was done to determine whether there were any changes in the uterine mast cells subsequent to *in vitro* anaphylaxis. Two strips of uterus from an actively sensitized mouse were placed in Ringer's solution as usual. After shocking a strip *in vitro,* it was placed in 10% formalin; the second, non-shocked strip served as a control. The tissues were prepared by a standard histological technic. In the 3 times the experiment was done, the mast cells were abundant and easily identified, but there were never any discernible changes in their morphology after shock.

Effect of pertussis vaccine on anaphylactic and histamine sensitivity in vitro. It has been shown that an injection of pertussis vaccine increases both histamine and anaphylactic sensitivity in the intact mouse. This experiment was done to determine whether similar findings could be observed when the tests were done *in vitro.* Five groups of female Swiss mice over 3 months old were used in this experiment. The first group of 18 were not injected, and served as controls. Mice in the other 4 groups were all injected subcutaneously with the 0.6 ml of alum precipitated egg white as before. One of these 4 groups received no further treatment. The other 3 groups received pertussis vaccine as follows: 9 mice were injected intra-abdominally with 0.1 ml of pertussis vaccine§§ immediately after the egg white injection; a group of 12 received 0.1 ml of pertussis vaccine 4 to 5 days prior to testing for sensitivity; and the last group of 8 received 0.2 ml of pertussis vaccine 4 to 5 days prior to testing. On the 10th, 11th, 12th, and 13th day following the egg white injection, proportionate numbers from each of the 5 groups were tested for anaphylactic sensitivity as previously described until all had been tested. They were tested for histamine sensitivity by recording the reaction to 50 μg of histamine base per ml of bath.

§§ Pertussis vaccine, Cutter lot No. E5452; 0.1 ml contains 6 x 10^9 organisms.

TABLE I. Effect of Pertussis Vaccine on Anaphylaxis and Histamine Sensitivity *in Vitro.**

Exp.	Normal	Ea	Ea + .1 pertussis 4-5 days earlier	Ea + .2 pertussis 4-5 days earlier	Ea and pertussis injected together
A+† H+		8/12‡	3/12	2/8	3/9
A+ H−		1/12	2/12	2/8	0/9
A− H+		2/12	6/12	3/8	3/9
A− H−		1/12	1/12	1/8	3/9
Total H+	3/18	10/12	9/12	5/8	6/9
Total A+		8/12	5/12	4/8	3/9

* All mice received estrogen intramuscularly 4-5 days prior to testing.

† A = anaphylaxis; H = histamine—50 γ/ml of bath.

‡ No. positive/No. tested.

In these, and in all following experiments, only one strip was tested if the reaction were positive; 2 strips if negative. The results of these experiments are recorded in Table I.

The results of the experiment on the effect of pertussis vaccine on anaphylactic and on histamine sensitivity are expressed in Table I. To determine any difference in sensitivity to histamine and to anaphylaxis, the groups were compared statistically using the method of χ^2. Each group was compared with every other group in the 2 categories.

Analysis of the data reveals that pertussis vaccine had no effect on anaphylactic sensitivity as tested *in vitro* when given 4 days prior to testing at 2 dosage levels; or when administered with the sensitizing dose of antigen. Nor does pertussis vaccine have any more effect on histamine sensitivity as measured *in vitro* than does the injection of the sensitizing antigen alone. None of the treated groups were statistically different from each other, but all were statistically different from the normals.

Discussion. Numerous publications have questioned the role of histamine in mouse anaphylaxis. The relative insensitivity of the

intact mouse to histamine(7-9), the suggestion that the body of the mouse does not contain sufficient histamine to produce a reaction (7), the observation that antihistaminic drugs have little if any effect on anaphylaxis (10), and the inability to establish a correlation between histamine and anaphylactic sensitivity in this species(10,11), all indirectly suggest that histamine is not concerned in anaphylaxis in the mouse.

By using the Schultz-Dale technic of *in vitro* anaphylaxis, it has been shown in this study that the isolated tissue, like the intact animal, is very resistant to histamine; and that the sensitized mouse uterus reacts to the specific antigen to the same extent in the presence of a demonstrably anti-histaminic drug as it does in the absence of that drug. Furthermore, no change in the tissue mast cells, believed to be involved in the release of histamine(5,6), was manifested after the *in vitro* anaphylactic reaction. These findings strengthen the hypothesis that histamine is not concerned in mouse anaphylaxis.

The reaction also proceeds to its full extent in the presence of atropine, a finding which would argue against an important role for acetylcholine.

The fact that pertussis vaccine increases both histamine and anaphylactic sensitivity in the intact mouse has been amply confirmed (8,9,12). Studies on the isolated tissues, however, reveal that anaphylactic sensitivity to the egg white antigen was not increased; and that the increase in the histamine sensitivity was of the same order if the animal received the antigen alone, or the antigen plus pertussis organisms. In light of the finding that the tissues were all similarly sensitive, this suggests that the effect of pertussis vaccine in increasing anaphylactic sensitivity in the intact animal must be through alteration of some mechanism other than an immunological one. The finding that the egg white injection increases histamine sensitivity to the same extent that pertussis vaccine does (Table I) is difficult to explain. Many bacterial antigens have been tested(10,13) to determine whether or not they increased sensitivity to histamine, but only those prepared from *Brucella abortus* were found to have any effect. Parventjev(14), however, has shown that injection of a mouse with Sarcoma 180 tends to increase the histamine sensitivity.

Summary. 1. The Schultz-Dale technic for demonstrating anaphylaxis *in vitro* was used successfully on uteri from egg white sensitized mice. 2. The anaphylactic reaction persisted in the presence of either atropine or Benadryl, thus ruling out any major role for histamine or acetylcholine in mouse anaphylaxis. 3. The uterine mast cells were found to be morphologically unaffected by *in vitro* anaphylaxis. 4. Pertussis vaccine administered under varying conditions did not increase *in vitro* sensitivity to anaphylaxis. Immunization with egg white alone increased histamine sensitivity *in vitro* as much as did the pertussis vaccine.

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