EFFECT OF EXTRACTS OF *ANGELICA POLYMORPHA* ON REAGINIC ANTIBODY PRODUCTION

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ABSTRACT.—An aqueous extract of *Angelica polymorpha* was examined for its immunoregulating properties. Its effect on the production of antibodies was tested in (C57BL/6 x DBA/2)F_1 mice. When the animals were treated daily with the extract, the serum titers of reaginic antibodies normally observed after a single injection dinitrophenylovalbumin (DNP_3–OA) were significantly lower, and the higher and more sustained reaginic titers induced by booster injections of DNP_3–OA were also inhibited. The immunosuppressive activity was observed both by oral and intraperitoneal administration of the extract, and it was not removed by dialysis. In contrast, the serum titers of IgG were not significantly altered by the administration of the extract. The extract had little or no effect on the passive cutaneous anaphylaxis reaction or the release of histamine from sensitized rat lung fragments.

Those conditions in which individuals are sensitive to a variety of environmental substances are called atopy and include hayfever, asthma and atopic dermatitis. These conditions are associated with antibodies of the IgE class, and sera of atopic patients contain IgE levels that are often 3 to 10 times greater than the upper limit found in normal human sera, which is in the range of 0.1–0.4 μg/ml. These antibodies have a high affinity for receptors on mast cells and basophilic leukocytes. The affinity of IgE for these cells apparently derives from sites in the Fc domain, leaving the two antigen binding sites at the Fab terminal free to react with the antigen. When antigen molecules combine with the IgE antibodies on these target cells, a cascade of intracellular metabolic processes is triggered, resulting in the synthesis and release of mediators which include histamine, slow-reacting substance, eosinophil chemotactic factor, and platelet-activating factor. When these mediators are released in sufficient quantities the clinical syndromes of atopy, consisting of bronchoconstriction, edema, increased secretion of mucus and vascular collapse, are manifested (1,2).

In view of the crucial role which IgE plays in atopic diseases, it seems likely that prevention or suppression of IgE antibody formation would be beneficial for atopic patients. In a search for this pharmacological action, we included the evaluation of extracts of natural products. Roots of *Angelica polymorpha* var. *sinensis diels* (known as "Tang-Kuei" in Chinese), an aromatic herb belonging to the Umbelliferae family, have long been used in Chinese medicine. The material is taken in the form of a decoction for a variety of disorders such as dysmenorrhea, metrorrhagia, rheumatism, ulcers, and anemia. It is said to be antispasmodic, analgesic, sedative, anti-inflammatory and a remedy for disorders of the respiratory system and blood circulation (3,4). Although it has not been clearly reported, "Tang-Kuei" has been used for the prevention and relief of allergic symptoms and, in some cases, asthmatic attacks. The aqueous extract of *Angelica polymorpha* roots was therefore studied for its effect on IgE antibody production and anaphylactic reactions.

RESULTS AND DISCUSSION

The ability of the aqueous extract of *Angelica polymorpha* to affect reaginic antibody production was determined by administration of the extract to (C57BL/6 X DBA/2)F_1 mice appropriately immunized with DNP_3–OA. Initially, it was tested for its potential to alter the primary reaginic response. In this system

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the mice were injected intraperitoneally with 1 μg of DNP_3–OA in the presence of 0.05 ml alhydrogel in 0.5 ml saline. The immunological response was followed by measuring serum reaginic titers and the immunocompetence of the splenocytes. Figure 1 shows that serum reaginic titers and the ability of the splenocytes to produce reaginic antibodies were maximal on Day 11 as measured by heterologous passive cutaneous anaphylaxis and adoptive cutaneous and anaphylaxis, respectively. The results presented in figure 1 are for a 1/80 dilution of serum and a concentration of $2x10^6$ cells per site of injection. At these concentrations no detectable wheals were observed on Day 21 or thereafter. However, positive reactions were obtained for the later days if the sera were not diluted as much and greater numbers of cells were injected per site.

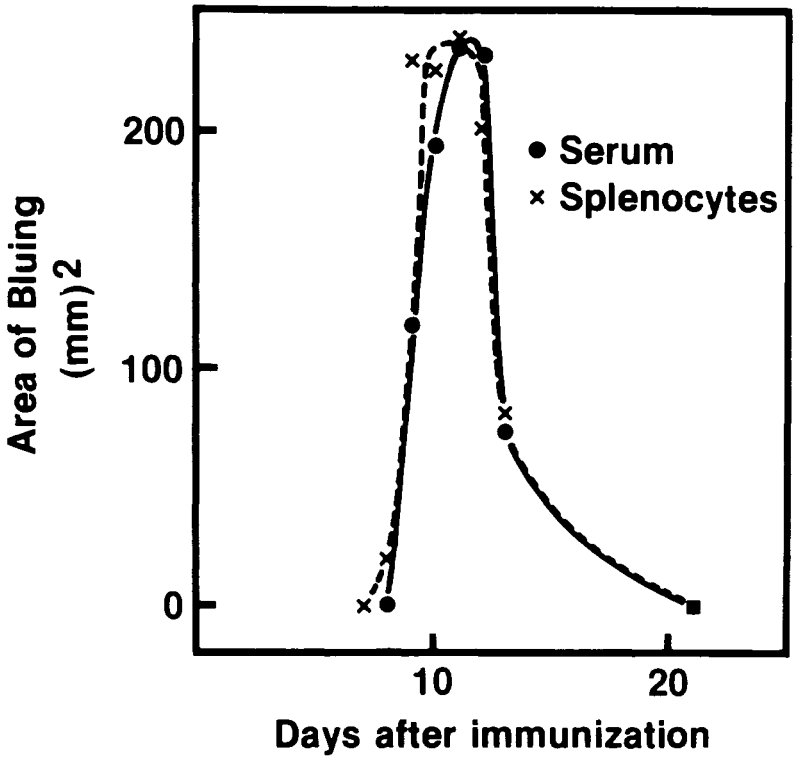

FIG. 1. The induction of reaginic antibodies in mice by a single intraperitoneal injection of 1 μg DNP_3–OA. Serum reaginic antibodies were measured by heterologous passive cutaneous anaphylaxis; the sera were diluted 1/80. The immunocompetence of the splenocytes was determined by heterologous adoptive cutaneous anaphylaxis: $2x\ 10^6$ cells were injected per site.

To test the effect of extracts of *Angelica* on this primary response, the mice were given the extract prior to immunizing on Day 0 with DNP_3–OA. Dosing was continued through Day 10 excluding the weekend (Days 5 and 6). A control group received the vehicle on days of scheduled test dosing. On Day 11, the mice were exsanguinated, and serum and/or splenic cells were evaluated in the rat for their IgE levels and IgE-synthetic capability, respectively. Donor mouse sera or spleens were pooled within each test/control group for the evaluation. The results in table 1 show that the intraperitoneal administration of dilutions of the original extract up to 1:160 completely or nearly completely suppressed the serum titer of IgE.

Immunosuppressive activity was also observed when the extract was orally administered (table 2). In these studies, the serum titer of IgE was monitored,

TABLE 1. The effect of intraperitoneal administration of an extract of *Angelica polymorpha* on the production of reaginic antibody formation in the mouse.

Dilution of Extract	HPCA Reaction			
	Titer^{-1a}	Comparison at 1/20 dilution of serum		
		Wheal Size (mm)[2]	Percentage Inhibition	P[b]
Saline Control.....	80	137±28	—	—
1/3.....	<20	0	100	—
1/5.....	<20	0	100	—
1/10.....	<20	0	100	—
1/20.....	20	37±27	73	0.01
1/40.....	<20	0	100	—
1/80.....	20	43±31	69	0.01
1/160.....	20	30±28	78	0.01
1/320.....	80	142±11	(4)	NS
1/640.....	80	111±25	19	NS

[a]Maximal dilution of serum which gave a positive passive cutaneous anaphylaxis reaction (wheal size >5 mm x 5 mm).
[b]n=4.

and the spleens were removed and examined for their immunocompetence by heterologous adoptive cutaneous anaphylaxis. When the extract was administered undiluted, the serum titers of IgE were significantly lower than those of the controls, and the immunocompetence of the splenocytes was correspondingly suppressed. When the mice were orally dosed with diluted preparations of the extract, inhibition, although not always statistically significant, was observed. When the response as measured by HPCA was compared to the response as measured by HACA, fairly good parallelism was seen (62 vs 67, 43 vs 58, and 44 vs 21). The differences are most probably due to differences in the experimental protocols of the two assay systems.

TABLE 2. The effect of oral administration of an extract of *Angelica polymorpha on the* production of reaginic antibody formation in the mouse.

Dilution of Extract	HPCA Reaction				HACA Reaction[b]		
	Titer^{-1a}	Comparison at 1/40 dilution of serum			Wheal Size	Percentage Inhibition	p[c]
		Wheal Size (mm)[2]	Percentage Inhibition	p[c]			
Saline Control...	160	111±10	—		152±31	—	—
undiluted.....	80	42±21	62	0.1	51±16	67	0.01
1/5.....	40	64±34	43	NS	64±5	58	0.01
1/40.....	80	62±33	44	NS	121±9	21	NS

[a]Maximal dilution of serum which gave a positive passive cutaneous anaphylaxis reaction (wheal size >5 mm x 5 mm).
[b]5 x 10^6 cells injected per site.
[c]n=4.

An aliquot of the extract was dialyzed against a large volume of saline solution (200 times volume/change, 3 changes over 2 days). After dialysis, the volume of the extract usually increased ≈50%. It was concentrated back to the original

volume by evaporation under N_2 gas at 60° and then diluted. As can be seen in table 3, an analysis of the sera demonstrated that the dialyzed extract was still active in inhibiting IgE formation at concentrations between 1:5 and 1:160.

TABLE 3. The effect of intraperitoneal administration of a dialyzed extract of *Angelica polymorpha* on the production of reaginic antibody formation in the mouse.

Dilution of Extract	HPCA Reaction			
	Titer^{-1a}	Comparison at 1/20 dilution of serum		
		Wheal Size (mm)[2]	Percentage Inhibition	P[b]
Saline Control	40	70±38	—	—
1/5	<20	0	100	—
1/40	<20	7±8	90	0.01
1/160	20	33±17	53	0.1
1/320	80	98±30	(40)	NS

[a]Maximal dilution of serum which gave a positive cutaneous anaphylaxis reaction (wheal size >5 mm x 5 mm)).
[b]n=4.

The potent immunosuppressive activity of the *Angelica* extract against the primary response encouraged the testing of the extract against a secondary immunological response. In these studies the mice were injected intraperitoneally with 1 μg of DNP_3–OA in the presence of 0.05 ml alhydrogel in a 0.5 ml saline on Day 0 and again on Day 36. Groups of mice were killed on designated days, and the pooled sera from the animals were assayed for their content of reaginic antibodies by passive cutaneous anaphylaxis. Figure 2 shows that the antigenic peak response occurred between Days 41 and 42, and thereafter the titers fell. Little or no reaginic antibodies were detected on Days 37 through 39. Dosing with the extract of *Angelica* was started on Day 32 and was continued daily up to but not including, the day of sacrifice. The results summarized in table 4 show that the extract effectively blocked the reaginic antibody response elicited by the booster injection. Four dilutions of the extract ranging from ⅓ to 1/100 were tested, and in most cases at least 50% inhibition was observed.

The sera collected from the mice were also assayed for their total content of IgG by ELISA, and the values for the extract-treated animals were not significantly different from the values of the control animals (±10%). Additionally, there was no significant effect on the production of IgM as measured by the Jerne Plaque assay (±10%) (15).

Attempts were made to evaluate the effect of *Angelica* on antigen specific IgG utilizing a direct and indirect plaque-forming cell assay by challenging with DNP–SRBC or DNP–OA–SRBC (SRBC=sheep red blood cell) with or without concomitant or subsequent challenge of goat anti-mouse IgG (Fc fragment, γ-chain specific, Cappel Labs). DNP–SRBC was prepared according to Eisen et al (16). DNP–OA–SRBC was prepared by coating SRBC with DNP_3–OA. Freshly washed SRBC adjusted to a 2.5% cell suspension were incubated for 15 minutes in tannic acid (0.05 mg/ml) at room temperature. After several washings in PBS the cells were incubated for 15 minutes in DNP_3–OA, 1 mg/ml. The coated SRBC were washed three times and adjusted to a 10% cell concentration for plaquing. However, following the same immunization schedule and dosing regime as described in Methods, we were unable to detect any plaque formation for DNP– or DNP–OA specific IgG, thereby preventing such an analysis.

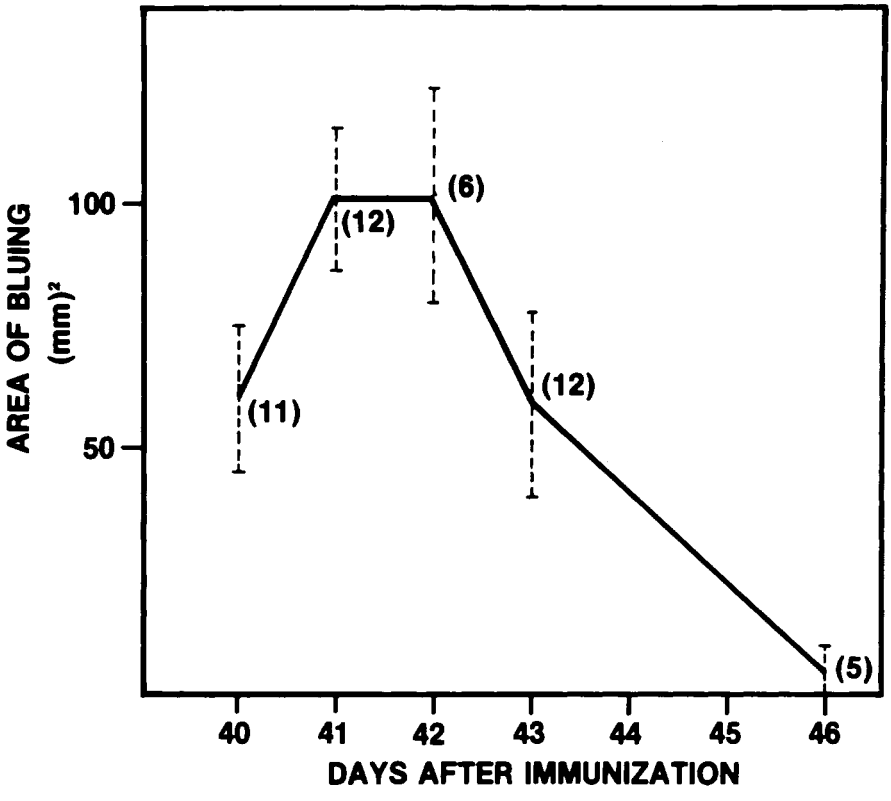

FIG. 2. The induction of reaginic antibodies in mice by a second intraperitoneal injection of 1 μg DNP_s-OA which was given 36 days after the initial injection of 1 μg DNP_s-OA. Serum reaginic antibodies were determined by heterologous passive cutaneous anaphylaxis. The sera were diluted 1/40. In each experiment there were fifteen mice, and the numbers in the parentheses represent the number of experiments.

TABLE 4. The effect of oral administration of an extract of *Angelica polymorpha*[a] on production of reaginic antibody formation in the mouse elicited by a booster injection of the antigen (secondary response).

Dilution of Extract	Percentage inhibition as measured by passive cutaneous anaphylaxis reaction		
	Day		
	40	41	43
1/3	90 (0.01)[b]	36 (NS)	55 (0.05)
1/10	62 (0.05)	49 (0.05)	53 (0.05)
1/50	33 (NS)	51 (0.05)	66 (0.05)
1/100	76 (0.05)	32 (NS)	100 —

[a]The amount of soluble material in the undiluted supernatant in this experiment was 0.1 gm/ml compared to 0.21 gm/ml used in the other experiments.
[b]Numbers in parentheses are the levels of significance.

Extracts of *Angelica* were also tested for their ability to interfere with other steps of the allergic response. Its effect on the release of mediators from the mast

TABLE 5. The effect of an extract of *Angelica polymorpha* on heterologous and homologous passive cutaneous anaphylaxis and the release of histamine from passively sensitized rat lung fragments.

Procedure	Protocol	Dilution of Extract	Control Wheal Size (mm)	Percentage Change	n	P
I. Heterologous passive cutaneous anaphylaxis...	Mouse anti-DNP-OA serum ± *Angelica* extract ↓ 15 min @ 37° Inject mixture into dorsal skin of rat ↓ 3 hrs Rats challenged (i.v.) with DNP_{20}-BGG + Evans blue dye ↓ 30 min Size of wheal measured	1/3	113±25	− 5	3	NS
II. Homologous passive cutaneous anaphylaxis...	A. Rat anti-OA serum ± *Angelica* extract ↓ 15 nin @ 37° Injected mixture into dorsal skin of rat ↓ 48 hrs Rats challenged (i.v.) with ovalbumin + Evans blue dye ↓ 30 min Size of wheal measured	undiluted 1/5 1/10 1/20	147±20	− 1 +18 −11 +29	4 4 4 4	NS NS NS NS
	B. Rat anti-OA serum injected into dorsal skin of rat ↓ 48 hrs 0.5 of *Angelica* extract given intravenously ↓ 0.5 min Rats challenged (i.v.) with ovalbumin + Evans blue dye ↓ 30 min Size of wheal measured	undiluted	166±33	− 5	6	NS
III. Release of histamine from passively sensitized rat lung fragments.	A. Rat lung fragments incubated with rat anti-OA serum ↓ 90 min @ 37° Lung fragments washed ↓ Lung fragments challenged with ovalbumin ± *Angelica* extract ↓ 30 min @ 37° Amount of histamine released measured	1/10 1/20	N.A. N.A.	−12 + 9	3 3	NS NS
	B. Rat lung fragments incubated with rat anti-OA serum ± *Angelica* extract ↓ 90 min @ 37° Lung fragments washed ↓ Lung fragments challenged with ovalbumin ↓ 30 min @ 37° Amount of histamine released measured	1/5 1/20 1/40	N.A. N.A. N.A.	−11 − 9 0	2 2 2	

cell was studied in both heterologous and homologous passive cutaneous anaphylaxis tests. The former consisted of bleeding mice eleven days after they had been immunized with DNP_3–OA and then mixing dilutions of the serum with *Angelica* extract. After incubation the mixtures for 15 minutes at 37°, 0.1 ml of the mixture was injected into each of 4 sites on the dorsal skin of 6 rats. Two to four hours later the recipient rats were challenged intravenously with DNP_{20}–BGG antigen and Evans blue dye. Thirty to 45 minutes later the rats were killed by cervical dislocation, and the dorsal skin was reflected to reveal lesion areas. The results in table 5 (Procedure I) show that the incubation of *Angelica* extract with mouse serum had no significant effect on the response.

The effect of extracts of *Angelica* on homologous passive cutaneous anaphylaxis response was done in rats. The animals were injected intradermally on their shaved backs with rat anti-ovalbumin serum and then challenged with ovalbumin. In one experiment the extract of *Angelica* was mixed with the antiserum, and in another the extract was given (iv) shortly before the rats were challenged with ovalbumin. The results of these experiments are summarized in table 5 (Procedure II); it was found that the extract of *Angelica* did not significantly affect the size of the wheals.

The extract was also examined for its effect on the release of histamine from passively sensitized rat lung fragments. The extract was tested for its effect on the sensitization phase and the releasing phase. The results summarized in table 5 (Procedure III) demonstrate that the extract of *Angelica* did not significantly interfere with these processes.

It may be concluded from these studies that there is some validity to the reported use of "Tang-Kuei" for the prevention and relief of allergic symptoms. Extracts of the root were examined in various experimental tests designed to measure certain aspects of the allergic response. While the extract did not seem to affect the sensitization or release of mediators from the mast cell, it presumably inhibited the production of IgE antibodies in a selective manner (i.e., no significant effect on the production of IgG antibodies). A strong suppression of both primary and secondary reaginic responses was observed. To elucidate the mechanism of this suppression seems worthy of further investigation since these results may ultimately lead to a novel approach for treating atopic diseases.

MATERIALS AND METHODS

PLANT MATERIAL.—The plant material was purchased from a local Chinese market or from Meer Co., New York, N. Y.

EXTRACTION OF ANGELICA POLYMORPHA ROOTS.—The roots were sliced with a surgical blade. Seventeen g of slices were boiled over low heat for 3 hrs in 300 ml distilled water in a 600 ml beaker covered with a watch glass (simulation of decoction). After boiling the volume of the mixture was about 150 ml. The decoction was allowed to cool to room temperature, then homogenized for one minute in a Waring blender at full speed. The homogenate was first centrifuged at 30,000 x g for 10 minutes, and the resulting supernatant was spun again at 105,000 x g for one hour. The supernatant was adjusted to a final volume of 2.7 ml per 1 gram of original dry root by evaporation under N_2 gas at 60–80°. The amount of soluble material in the resulting supernatant was determined to be 0.21 gm/ml. Solid NaCl was added to the extract to achieve a final concentration of 0.9%. In the experiments to be described, the extract was further diluted from this stock solution with isotonic saline solution.

DOSING OF MICE.—Once a day the mice received 0.2 ml of either the extract which contained 0.21 gm of soluble material per ml (see above) or 0.2 ml of the indicated dilution of the extract. The route of administration and the timing with respect to immunization are indicated in the text.

PREPARATION OF HAPTEN-PROTEIN CONJUGATES.—The haptenized proteins were prepared similarly to the procedure described by Ishizaka and Okudaira (5). To prepare dinitrophenyl-ovalbumin (DNP_3–OA), fifty mg each of dinitrobenzene sulfonic acid (Eastman) and sodium carbonate were dissolved in 1 ml of distilled water, and the resulting resolution was added to 5 ml of a solution of water containing 100 mg ovalbumin (Calbiochem). The mixture was stirred for 4 hrs at 37° and then dialyzed against water. To prepare dinitrophenylbovine gamma globulin (DNP_{20}–BGG), two gms of bovine gamma globulin (Calbiochem) were dissolved in 80 ml of distilled water containing 1.696 gms of sodium carbonate. After the addition of 300 mg of dinitrobenzene sulfonic acid, the mixture was stirred 18 hrs at room temperature and then dialyzed against water.

IMMUNIZATION OF MICE.—(C57BL/6 x DBA/2)F_1, male, age 8–12 weeks, mice (Jackson Laboratories or Microbiological Associates) were injected intraperitoneally with 1 μg of DNP_3-OA in the presence of 1 mg of alhydrogel (Accurate Chemical and Scientific Corp.) in 0.5 ml saline, as recommended by Levine and Vaz (6), and Lee and Sehon (7).

MEASUREMENT OF SERUM REAGINIC ANTIBODIES [HETEROLOGOUS PASSIVE CUTANEOUS ANAPHYLAXIS (HPCA)].—Fundamentally, the technique of Ovary (8) was utilized as modified by Mota and Wong (9). Volumes of 0.1 ml of diluted serum were injected intradermally into four separate sites on the shaved dorsal skin of male rats (Charles River, 200–250 gm). Two to three hours later the rats were challenged by an intravenous injection of 1 mg of DNP_{20}-BGG in 0.5 ml of saline containing 1% Evans blue dye (Eastman). Thirty to sixty minutes later the rats were killed by cervical dislocation and the dorsal skin was reflected. To measure the extent of cutaneous anaphylaxis (bluing) the product of two perpendicular axes (mm x mm) of each of the four wheals was used. The products were averaged for each aninal. For each sample, a minimum of four rats were used. A dilution of the control serum that gave a wheal size≈100 mm^2 was chosen, and the results were calculated by comparing the average wheal diameter produced by the drug at this dilution with the average diameter of wheals from control animals receiving vehicle alone. Occasionally, to verify the results, the sera were compared by a maximal dilution for giving a positive passive cutaneous anaphylaxis reaction, and in all cases the two methods gave comparable results.

MEASUREMENT OF IGE-PRODUCING CELLS [HETEROLOGOUS ADOPTIVE CUTANEOUS ANAPHYLAXIS (HACA)].—The method of Kind and Macedo-Sobrinho (10) was used to measure the immunocompetence of the cellular suspensions. Splenic cellular suspensions were prepared by squeezing the spleens through a cytosieve. The cells released were collected in antibiotic-free tissue culture medium 199 (Gibco). They were washed three times with tissue culture fluid and, following leukocyte cell count determinations, were further diluted to reflect various concentrations. Volumes of 0.1 ml of the cell suspensions were injected into four discrete areas of the shave dorsal skin of male rats (Charles River, 200–250 gm). Three to four hours after the adoptive transfer of mouse cells, the recipient rats were injected intravenously with 1 mg of DNP_{20}-BGG in 0.5 ml saline containing 1% Evans blue dye. The animals were killed, and the relative number of IgE-producing cells was measured in terms of the diameter of the inflamed (bluing) skin area as described above.

MEASUREMENT OF SERUM IgG ANTIBODIES.—An enzyme-linked immunosorbent assay (ELISA) was used to measure mouse serum IgG. Bascially, the method of Engvall and Perlmann (11), as described by Hoffman (12), was carried out with some modifications. In brief, the alkaline phosphatase (Sigma, St. Louis, MO) was conjugated to purified mouse IgG (Cappel Laboratories, Inc.) in the presence of glutaraldehyde (Sigma) as described by Hoffman (12). After the conjugation, the sample was diluted to 1 ml with Tris-HCl buffer pH 8.0 and applied to a Bio-gel A5M (Bio-Rad, Richmond, Calif.) column (2 x 40 cm). Major protein peak which contained conjugate activity was found in the void volume as determined by blue dextran 2000 (Pharmacia, Uppsala, Sweden). ELISA was performed by incubating overnight at 25°, the enzyme conjugate, standard IgG or unknown serum samples in plastic tubes (Falcon Plastic No. 2038, 10 x 75 mm) coated with 1 μg/ml of goat anti-mouse IgG antibody (Cappel Laboratories, Inc.). The bound enzyme conjugate IgG was assayed by providing the substrate *p*-nitrophenyl phosphate (Sigma) and estimating the product formation of *p*-nitrophenol at 400 nm on a Gilford 240 spectrophotometer.

HOMOLOGOUS PASSIVE CUTANEOUS ANAPHYLAXIS.—The rat anti-ovalbumin serum was prepared in a manner similar to that described by Orr and Blair (13). Adult female Charles River albino rats (≈250 g) were injected with 0.2 ml of egg albumin (1 mg/ml) in saline solution i.m. and 0.5 ml heat killed *Bordatelia pertussis* U.S.P. (≈2 x 10^{10} Organisms, Eli Lilly Co.) i.p. Ten days later these animals were also injected sc with 0.2 ml (=1,500 larvae)*Nippostrongylus Brasiliensis* suspended in 0.156 M saline. Ten to 14 days later the animals were bled, and serum was prepared and titered.

The backs of unanesthetized rats were shaved and 0.1 ml of an appropriately diluted antiserum was injected intradermally at four sites on the back. Forty-eight hours later the rats were injected iv with 5 mg Evans blue and 5 mg egg albumin in saline solution. Thirty minutes later the animals were killed, and the dorsal skin was reflected. The extent of cutaneous anaphylaxis (bluing) was measured as described above.

PASSIVE SENSITIZATION AND IMMUNOLOGICAL CHALLENGE OF RAT LUNG FRAGMENTS.—The preparation of rat lung fragments was similar to that described by Orr and Blair (13).

Rat lung fragments were sensitized with 1:10 diluted high titer rat anti-OA serum (3 ml/gm of tissue) for 90 min at 37° under an atmosphere of 5% CO_2 and 95% O_2. Following sensitization, the fragments were rinsed (3 x) and randomly selected to form individual samples (75 mg/tube), each consisting of approximately 12 to 15 sections. The tissues were then challenged with 25 μg of 5 x crystallized ovalbumin in 1 ml of Tyrode's solution in a test tube for 30 min at 37° under the atmosphere of 5% CO_2 and 95% O_2.

The amount of histamine released into the Tyrode's solution was measured by bioassay utilizing the guinea pig ileum preparation (14). In the calculation, the spontaneous release was substracted from gross release.

STATISTICAL ANALYSIS.—Student's *t* test was used, and the significance level was set at 0.05.

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