A STUDY OF TYPE I HYPERSENSITIVITY (ANAPHYLAXIS)

Dana S. Anstine
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Professor Emmons
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ABSTRACT

Type I hypersensitivity reactions were studied in vitro using the Schulz-Dale Assay. This assay involved the suspension of pieces of sensitized mouse ileum (smooth muscle of the gut) in an organ bath to allow exposure to different treatments. Each piece of ileum represented an atopic animal. Specifically, the effects of histamine were investigated. Ovalbumin, the sensitizing antigen, induced anaphylactic reaction in the pieces of ileum, and antihistamines were added to determine their effectiveness in counteracting the anaphylactic response. Tripelennamine (an ethylenediamine) and chlorpheniramine (an alkylamine) were used as antagonists. Both counteracted the anaphylactic reactions produced by histamine and seemed to be acting as histamine H1-receptor blocking drugs. The assay shows promise for further investigation of the mechanisms and treatments of type I hypersensitivity.

INTRODUCTION

Inflammation, which has an important role in immune response, is one of the responses of tissues to injury manifested in five basic signs (swelling, heat, redness, pain, and loss of function) that result from local changes in vascular permeability. Electrolytes, macromolecules and cells escape into the extracellular fluid as a result of this change in permeability. Inflammation provides a protective mechanism in that it permits protective factors, in the form of antibodies, complement, and phagocytic cells, that are usually present in the circulatory system, to penetrate tissues and enter sites of foreign invasion. Immunologically mediated inflammation is called hypersensitivity (Roitt et al. 1985).

This study involves only those inflammatory reactions occurring within a few minutes after exposure to the antigen (Volk et al. 1982) that are known as anaphylaxis or type I

hypersensitivity. Specifically, type I responses are mediated by an antigen reacting with a target cell (mast cells and/or basophils) sensitized by non-precipitating, heat-labile, reaginic (IgE) antibody (Roy and Karim 1983). These reactions result from an initial exposure to an antigen, that is considered the sensitizing exposure, followed by an immunologic waiting period necessary for IgE immunoglobulin synthesis. Later exposure to the antigen produces anaphylaxis (Barrett 1983).

Mast cells, large round lymphocytes (15 to 20 micrometers in diameter), play an important role in allergic response. Distributed throughout the body in the connective tissues, they are characterized by a cytoplasm that is packed with large granules that store histamine in a complex with heparin. These lymphocytes carry glycoprotein receptors on their cell surface which are specific for the Fc region of IgE, an immunoglobulin produced by B cells. This IgE production involves "antigen presentation via antigen presenting cells", help from T cells, and stimulation of B cells to manufacture IgE (Tizard 1985). IgE is a gamma glycoprotein with light and heavy chains (molecular weight 190,000), found in normal human serum in concentrations of 0.1 to 0.4 micrograms per milliliter. Atopic individuals contain elevated levels of IgE (Roitt et al. 1985).

First, the local mast cells become sensitized by the IgE produced, then unbound IgE enters the circulation and binds to both basophils and tissue-bound mast cells (Roitt et al. 1985). When an antigen cross-links adjacent cell bound IgE molecules,

the sensitized mast cell responds either by expelling the granules from the cell interior to the extracellular environment or by opening channels in the cell cytoplasm permitting the extracellular fluid to penetrate to the granules (Figure 1). This mast cell response occurs rapidly, within a few seconds after the immune complex forms on the surface of the sensitized cells (Tizard 1985).

Many anaphylactic chemical mediators are released as a result of mast cell degranulation, such as the preformed mediators of anaphylaxis. The newly formed mediators of anaphylaxis are then produced from arachidonic acid. The direct effects of these mediators upon tissues produce the pharmacological response of anaphylaxis (Roy and Karim 1983). The physiological effects of these mediators divide them into three main categories, chemotactic agents, inflammatory activators, and spasmogens. Histamine is categorized both as an inflammatory activator, as it can cause vasodilation, edema, and platelet-activating factor (PAF) related damage, and as a spasmogen, because it can directly affect smooth muscle and mucus secretion (Roitt et al. 1985).

I have investigated the effects of histamine, (Figure 2) an anaphylactic mediator. In the mast cell, histamine is held in a complex with heparin. Degranulation causes disassociation of the complex and releases histamine and heparin. As a result, the anticoagulant actions of heparin and the vasodilating and smooth muscle contracting properties of histamine can be observed in an

anaphylactic reaction. The smooth muscle contractions resulting from histamine release cause respiratory distress from the contraction of smooth muscles in the bronchii; the contraction of the smooth muscle in the venules causes a drop in blood pressure, causing compensatory capillary expansion and forcing fluids into the tissue bed (edema). The accompanying capillary engorgement is observed as erythema (Barrett 1983).

As smooth muscle contracts in the presence of histamine, the ileum, an organ of the gut composed of smooth muscle, may be used to investigate the type I hypersensitivity response (Figure 3). Utilization of the ileum is a simpler assay as its response to histamine is less complex than that of other organs, such as the bronchii. Adjacent cross sectional ileal pieces can be used from a single atopic animal. In vitro assays that utilize tissues have an advantage because tissues have the ability to remain in a normal metabolic state for substantial periods of time in a simple physiological medium. Tissue cells can all be accessible to large molecules present in the organ bath; for example, the sensitized mast cells in the experiments are exposed to the antigen. Also, tissues are capable of producing measurable change that is quantitatively related to the severity of the antigen-antibody reaction and can be obtained in usable amounts from one animal (Weir 1978). This assay is also more humane than in vivo assays.

My primary objective was to observe the counteraction of the agonist, histamine, and its effect on smooth muscle contraction

using antagonists, antihistamines, that are believed to bind to histamine receptors. Histamine binds to histamine receptors on the cell surface of mammalian cells; two types of histamine receptors exist, histamine receptor 1 (H1) and histamine receptor 2 (H2) (Goodman and Gilman 1985). The H1 receptor has more numerous immunological roles than the H2 receptor. It causes smooth muscle contraction, venule dilation and pruritis. H2 induces gastric and mucous secretion and, perhaps, activates suppressor T cells. I studied the effects of two antihistamines, tripelennamine hydrochloride and chlorpheniramine maleate salt (Figure 4). Specifically, my study was designed to determine: 1) Is this an effective assay for studying anaphylactic reactions and their mediation? 2) Do antihistamines counteract anaphylactic contraction of the ileum? 3) If so, which of the two antihistamines is more effective in counteracting the effects of the anaphylactic contractions? 4) How do antihistamines counteract the anaphylactic contractions at the cellular level?

METHODS AND MATERIALS

OUTLINE OF MY INVESTIGATIONAL STRATEGY

1. The mice were sensitized by an intraperitoneal injection of ovalbumin (sensitizing antigen) in phosphate buffered saline (PBS) (see **sensitization** under METHODS).
2. The mice were boosted after 2 weeks and then at 3 week intervals to maintain atopism.
3. The Enzyme-linked Immunosorbent Assay (ELISA) was run approximately 14 days after injection to demonstrate the atopic condition of the animals (see **ELISA** under METHODS).
4. EXPERIMENTAL PROCEDURE

Experimental mice and control mice were sacrificed by cervical dislocation. The ileum of each mouse was removed and sliced into 3 cross sectional pieces for the control and experimental procedures. The sections were then placed in Hank's Basic Salt Solution (BSS) at 37^{o} C. Each piece of ileum was then mounted in the organ bath (37^{o} C.) by clamping one end to a fixed pole and clamping the other end to a moveable pole so that muscle contraction could be recorded by a polygraph (Figure 3). The pieces of ileal tissue were allowed to relax for 10-15 minutes. The sensitizing antigen (ovalbumin) was added to induce anaphylaxis and observed the contraction of the muscles.

Antagonists (antihistamines: either tripelennamine [subjects 1-16] or chlorpheniramine [subjects 17-30]) were added five minutes after the ovalbumin was added to determine which was more effective at counteracting anaphylaxis (See **Schulz-Dale Assay** under METHODS). The results were analyzed using UnkelScope and Psystat computer programs. (See **UnkelScope and Psystat** under METHODS).

METHODS

SENSITIZATION PROTOCOL:

The female Balb C mice were sensitized using injections of 0.5 ml of ovalbumin in PBS (200μg/ml) and a 26 gauge needle. This was the most effective amount for generating atopism. The mice were injected again two weeks after the first injection and then every three weeks following to maintain their atopic states.

ELISA PROTOCOL: (Also see **ELISA Reagents** under Materials)

An ELISA was run on each group of mice that received injections to confirm that the mice were atopic to ovalbumin. The antigen (ovalbumin: 10 μg/ml) was added to the wells, 100 μl/well. The plate was incubated for 1-2 hours at room temperature, then washed with PBS/Tween. Next, 100 μl/well of block (BSA 1mg/ml in PBS) was added. The plate was incubated overnight (>12 hrs) at a temperature of 4° C. The plates were washed again with PBS/Tween, followed by addition of the primary antibody (100 μl/well, mouse serum from the atopic mice collected the previous day) diluted to 1/40 concentration with diluent and incubated at room temperature for 1 hr. After another washing with PBS/Tween, peroxidase labelled goat anti-mouse IgE was added in concentration of 1/2000 in diluent (100 μl/well) and incubated at room temperature for 1hr in darkness. The plates were then washed, and 3,3',5,5' -tetramethylbenzidine (TMB) substrate was added (100μl/well). The plate was allowed to remain in darkness

for 1hr at room temperature. Following which, 25 μl of 2M H_2SO_4 were added to each well to stop the reaction; the plates were read using a plate reader (Dynatech Laboratories Inc. Microelisa Reader).

UNKELSCOPE PROTOCOL:

The UnkelScope program was used to analyze the changes in contractile strength in conjunction with the Schulz-Dale Assay. The program converts data from an analog voltage (from the polygraph) into a digital format, then stores the data for analysis. The parameters were set up to record 50 second intervals of contractions (Figures 5 and 6).

SCHULZ-DALE ASSAY:

The equipment used in this assay consisted of two vessels, one surrounding the other (Figure 3). The outer vessel holds approximately 500-750 ml of liquid while the inner vessel holds 50-60 ml. Water in the outer vessel was changed to maintain a temperature of 37^{o} C. The temperature of the inner vessel was maintained at 37^{o} C by the insulation of the outer vessel. The ileal sections were suspended within the inner chamber in different experimental solutions. Three ileal sections were taken from each mouse at distances 9-10mm, 10-11mm, and 11-12mm from the appendix. Each section represented an individual experiment. In each run, one section of a control (topic) ileum and a matching section of an experimental (atopic) ileum were suspended in the inner vessel. After the ileal sections were allowed to relax for 10-15 minutes, ovalbumin was added (25 mg/ml

in BSS, mixed in proper concentration one day prior to experiment). The ileal sections were allowed to respond to the ovalbumin for 5 minutes, then the antihistamine was added (tripelennamine: 5×10^{-2} μg/ml BSS, chlorpheniramine: 1×10^{-3} μg/ml BSS) (Barnes and Eltherington 1965).

PSYSTAT:

The program was developed by Professor Joseph B. Thompson of the psychology department of Washington and Lee University to compute an analysis of variance. The data were evaluated from the 16 experiments using tripelennamine and the 14 experiments using chlorpheniramine. The program determined the significance of the changes in contractions of the ileum, concluding whether or not they were a result of the additions of ovalbumin and antihistamines, or merely a result of individual differences within each set of mice (set 1 exposed to tripelennamine, set 2 exposed to chlorpheniramine). The results of the two sets were compared to one another using subject by treatment anova (S(A)xB Anova), which can determine not only the significance of changes within a group but also the significance of the changes comparatively between two groups.

Materials:

antihistamines

Tripelennamine hydrochloride (Sigma # T6137)

Chlorpheniramine maleate salt (Sigma # C3025)

Bovine Serum Albumin (BSA) (Sigma #1520)

Peroxidase labelled goat anti-mouse IgE (Nordic Immunological Laboratories)

Female Balb C mice

UnkelScope (c) MIT 1984

PBS

Sodium chloride 8.00 g L^{-1}

Potassium chloride 0.70 g L^{-1}

Disodium hydrogen phosphate (Na_2HPO_4) 0.20 g L^{-1}

Potassium di-hydrogen phosphate 0.20 g L^{-1}

BSS

Sodium chloride 8.00 g L^{-1}

Calcium chloride 0.20 g L^{-1}

Magnesium sulfate 0.20 g L^{-1}

Potassium chloride 0.40 g L^{-1}

Potassium di-hydrogen phosphate (KH_2PO_4) 0.10 g L^{-1}

Sodium bicarbonate 1.27 g L^{-1}

Glucose 2.00 g L^{-1}

ELISA REAGENTS

Coating Buffer: (Carbonate Buffer, pH 9.6)

1.59 g Na_2CO_3

2.93 g $NaHCO_3$

Dissolve in 1000 ml distilled water.

Diluent:

Add 50 µl Goat Serum to 50 ml PBS

PBS-Tween:

.5 ml Tween-20/1 L

Ovalbumin:

10 µg/ml in Coating Buffer

NGS-PBS: (normal goat serum) (1%)

1 ml NGS/100 ml PBS

BSA-PBS: (bovine serum albumin) (0.1%)

.1 g BSA/100 ml PBS

TMB Substrate:

10 mg 3,3',5,5'-tetramethylbenzidine (TMB) (Miles code #:98-050)

.1 M acetate/citric acid buffer (pH 6.0)

2M Sulfuric Acid

1 ml DMSO (Dimethylsulfoxide)

4 µl H_2O_2 (Hydrogen Peroxide 30%)

A. Mix 10 mg TMB with 1 ml DMSO

Take 0.25 ml of A, mix dropwise into 25 ml citric acetate buffer

Just before use add 4 µl H_2O_2

RESULTS

I investigated anaphylactic reactions at the tissue level. At this level, I attempted to mediate the type I inflammatory response by counteracting the effects of the chemical mediator, histamine, after it had been released as a result of mast cell degranulation. The Schulz-Dale assay was used; the assay has demonstrated that in vitro anaphylaxis is dependent upon cell bound antibody and has helped to establish the antigen specificity of anaphylaxis, specific immunologic sensitization, and the efficacy of drugs in mimicking or preventing anaphylaxis. This involved the use of antagonists tripelennamine hydrochloride (an ethylenediamine) and chlorpheniramine maleate salt (an alkylamine). I compared the effects of these different antihistamines to determine which more effectively counteracted the anaphylactic contractions of the smooth muscle. The significance of the response was evaluated using the "Psystat" computer program.

The ELISA showed that the serum of all animals subjected to the sensitization procedure had IgE specific for ovalbumin; consequently, the animals were atopic. All ELISA experiments were run in triplicate with suitable controls.

Samples of contractions were recorded while the ilea were in BSS, after addition of ovalbumin in BSS, and after addition of antihistamine in BSS. These were analyzed using the UnkelScope Utilities Mode to Edit Data. Analyses of data were made using

measurements of ileal contractile strength taken from each of the five 10 second intervals for both control and experimental ileal contractions for each sample (Figure 6). The ilea from atopic animals responded to the antigen by increased (twofold) magnitude of contraction (Figure 7, Table 1). Generally, in subjects 1-30 in Table 2, the contractile level after the addition of antigen was greater than that of the basal level. This response was shown to result from the addition of the ovalbumin and its interaction with sensitized mast cells as ilea from topic animals did not respond to the antigen, and when BSS was added in place of ovalbumin contractile strength did not increase.

After addition of the antigen had elicited increased contractile strength, the ilea of subjects 1-30 (Table 2) generally responded to the additions of both tripelennamine and chlorpheniramine by returning to basal level contractions within the five minute time period. It was determined statistically that the changes in the ileal contractile strength were strong, significant, and a result of the additions of the antigen and the antagonists. Such results could have only occurred by chance less than once in 100,000 times ($P = <.00001$). The basal levels of contraction (.667, .581) and the contractile levels after the addition of either antihistamine (.619, .669) did not differ significantly from one another (Figure 7, Table 1). Therefore, the antihistamines had similar effects. Topic pieces did not respond to the antigen or the antagonists, thus the contractile changes must have resulted from mediation of the anaphylactic

reaction. This mediation of the anaphylactic response was shown to be a result of the additions of antagonists by the following controls. Atopic pieces were exposed to different sequences of additions to the organ bath. The first was immersed in BSS, then the bath was filled with BSS two more times. It did not show significant variation of contractile strength. The next piece was immersed in BSS, then immersed in BSS with antihistamine, and again immersed in BSS. No variance in contractile strength occurred. Thus, the variance in contraction showed during the course of the experiments in which antihistamine in BSS was added after ovalbumin in BSS did not result from simply being added later in the sequence of immersions or from the tiring of the ileum over time. This was also evidenced by another control, where an atopic ileal piece was immersed in BSS, immersed in BSS with ovalbumin, then immersed in BSS again. This piece showed increased contraction after the addition of ovalbumin, but did not return to basal level after the second immersion in BSS.

An ileum from a topic mouse was also run in BSS for over two hours with no significant change in the contractile strength after the ileum had become acclimated (10 minutes). The remaining portion of the ileum also continued contractions from within the petri dish for more than two hours. This demonstrated that the changes in the strength of contraction were not likely to have resulted from only the length of time of the experiment, as the individual experiments were completed in less than 20-25 minutes, and all pieces from an individual animal were used

within approximately 75 minutes from the time of sacrifice.

DISCUSSION OF RESULTS AND CONCLUSION

In type I hypersensitivity (anaphylaxis), smooth muscle contraction results from activation of the H1 receptors, although responses vary widely among species and individuals (Goodman and Gilman 1985). These receptors, components of the cell surface, have not yet been isolated or identified, nor is the nature of their interaction with histamine that elicits the cellular response fully understood. The antihistamines used in this experiment acted as histamine antagonists by blocking H1-receptors. These experiments illustrated the competitive nature of the blockers and the importance of histamine in eliciting the contraction of the smooth muscle of the gut, as the antihistamines counteracted the contractions completely, returning the ilea to basal levels of contraction.

This model demonstrates that the mechanisms of anaphylaxis are similar in man and mouse. As in man, sensitized mast cells must be present in the vascular and connective tissue connected to the ileum and in its intracellular spaces. Degranulation must release histamine, which binds to H1 receptors present on the ileal cell surfaces. The antagonists were able to bind to these receptors, counteracting the anaphylactic reaction. Both antagonists had equivalent effects in blocking the activation of the H1 receptors. As they produced comparable effects, similarities between the shapes and chemical composition of the two may suggest something about the nature of the H1 receptor.

However, although the anaphylactic contractions were counteracted, the antagonists merely suppressed in varying degrees the symptoms ascribed to the pharmacological activity of the interaction of histamine and the H1 receptor. Only a small phase of the immediate hypersensitivity reaction has been examined. Other mediators were released whose effects have not been considered here. The histamine antagonists do not lessen the intensity of the antigen-antibody reaction, which is the root cause of type I hypersensitivity.

This assay could be adapted for the use of different drugs or tissues to explore the mechanisms and treatments of type I hypersensitivity. Each ileal section represents the anaphylactic response of an atopic animal when challenged with an antigen. An investigation of the causes of type I hypersensitivity is necessary to discover how to prevent the reaction rather than addressing the symptoms once the anaphylactic condition has already occurred. T cell deficiency or defect may result in allergic responses; there is substantial evidence that specifically implicates suppressor T cells in the IgE response (Roitt et al. 1985). Atopic individuals often possess a reduced number of suppressor T cells. IgE production by B cells seems to be regulated by T cells that consist of two types cells, suppressor T cells ($CD8^+$) and helper T cells ($CD4^+$) (Ishizaka 1984). One approach to mediate the immune anaphylactic response is to induce the production of suppressor T cells by attaching a

non-immunogenic polymer to an antigen, allowing these T cells to regulate the immune response. Dissociation between the IgE response and the IgG response in rodents infested with nematodes suggests that IgE antibody response is mediated not only by antigen specific helper and suppressor T cells but also by some mechanism selective for IgE. Two T cell factors, the glycosylation enhancing factor (GEF) and the glycosylation inhibiting factor (GIF), have an affinity for IgE and selectively regulate the IgE response (Ishizaka 1984). By switching the nature of the IgE binding factors, the IgE response can be regulated. Thus, GEF enhances the reaction while GIF acts as a suppressor. The factors are produced by T cells that are exposed to culture supernatants of macrophages and monocytes from animals treated with adjuvants such as Bordella pertussis or aluminum hydroxide gel, as well as other experimental conditions (Ishizaka et al. 1985). If the responsiveness of IgE binding factors is determined by the balance between GIF and GEF in vivo, administration of GIF might be an effective suppressor for the IgE response (Ishizaka et al. 1987). This suggests that other cell mediators could exist that may have an effect on the type I inflammatory response at the cellular level.

Much of the recent knowledge concerning the induction of the allergic inflammatory response that has been gained from animal studies has clinical relevance. Atopic diseases seem difficult to approach with animal models in that most animals are not atopic; however, the basic mechanisms of IgE regulation in

anaphylactic reactions are similar in man and mouse. Clinical evidence also indicates that new knowledge on proliferation of mast cells, basophils, and mucous cells derived from animal studies is relevant to the allergic inflammatory response in humans (Bjorkstein and Ahlstedt 1983). Another approach to mediation of the inflammatory response could be the prevention of mast cell degranulation. If cross-linkage of the Fc receptors on the mast cells could be prevented, degranulation would not result, and the inflammatory response could not occur. The Schulz-Dale assay demonstrated the necessity of cell bound (cytotropic) antibody, and has proved to be very useful in establishing the antigen specificity of anaphylaxis, specific immunologic desensitization, and determining drug efficacy in mimicking or preventing anaphylaxis (Barrett 1983). This evidence suggests further that the experiments that I performed using a mouse model have particular clinical relevance for the study of Type I inflammatory response. The results clearly demonstrate that the atopic mice were produced, that an anaphylactic response was elicited <u>in vitro</u>, and that the antihistamines had similar effects in counteracting the effects of histamine by returning the ileal contractions to basal levels.

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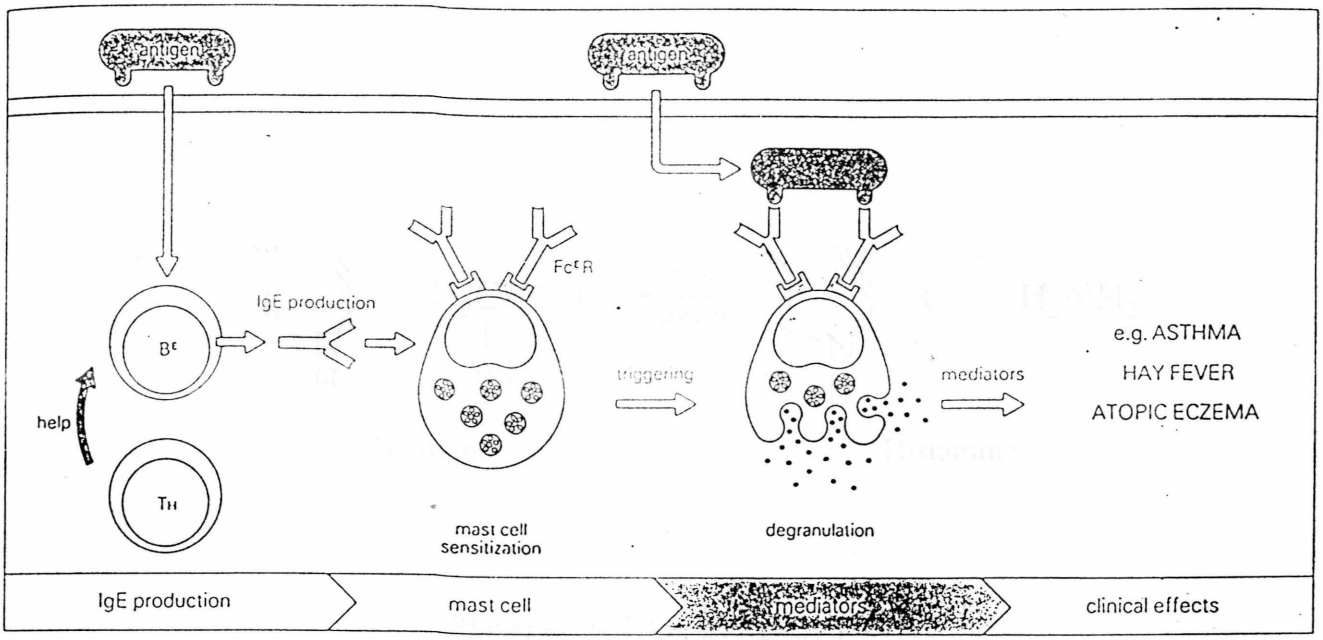
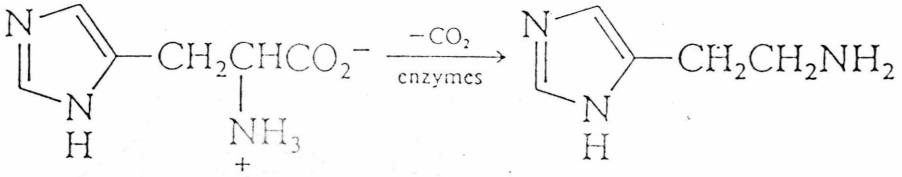


Fig.1 Overall scheme for Type I hypersensitivity. Antigen stimulates B^{ε} cells to produce specific IgE with T cell help. This antigen-specific IgE binds to mast cells via Fc^{ε} receptors ($Fc^{\varepsilon}R$) thus sensitizing them. When antigen subsequently reaches the sensitized mast cell, it crosslinks surface bound IgE and the cell degranulates, releasing mediators which cause the symptoms associated with Type I hypersensitivity.

Figure 1 (Roitt et al, 1985)



Histidine Histamine

Figure 2 (Carey 1987)

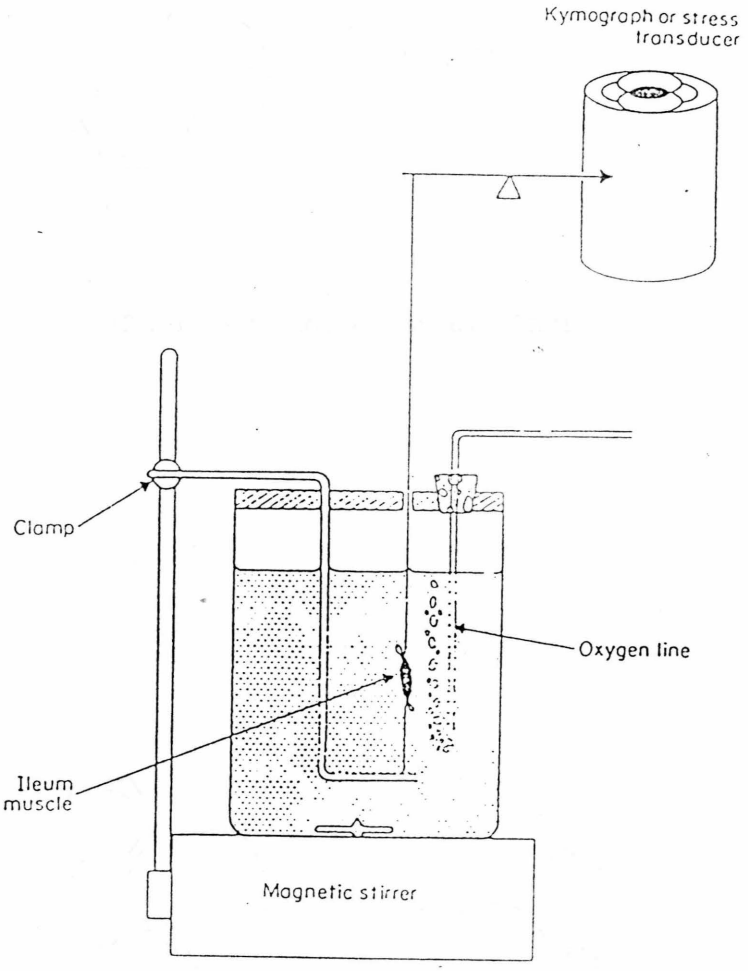
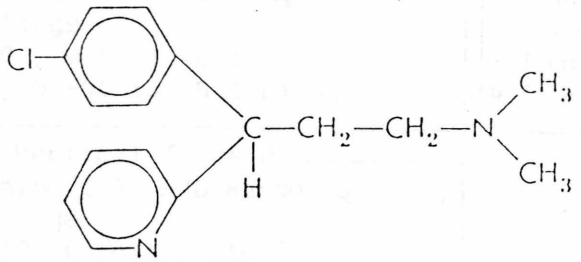
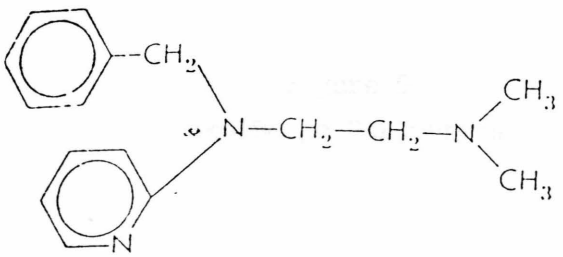


Fig. 3 Organ bath and recording apparatus for the demonstration of immediate hypersensitivity *in vitro*

Figure 3 (Hudson and Hay 1980)



Chlorpheniramine (an alkylamine)



Tripelennamine (an ethylenediamine)

Figure 4
(Goodman and Gilman 1985)

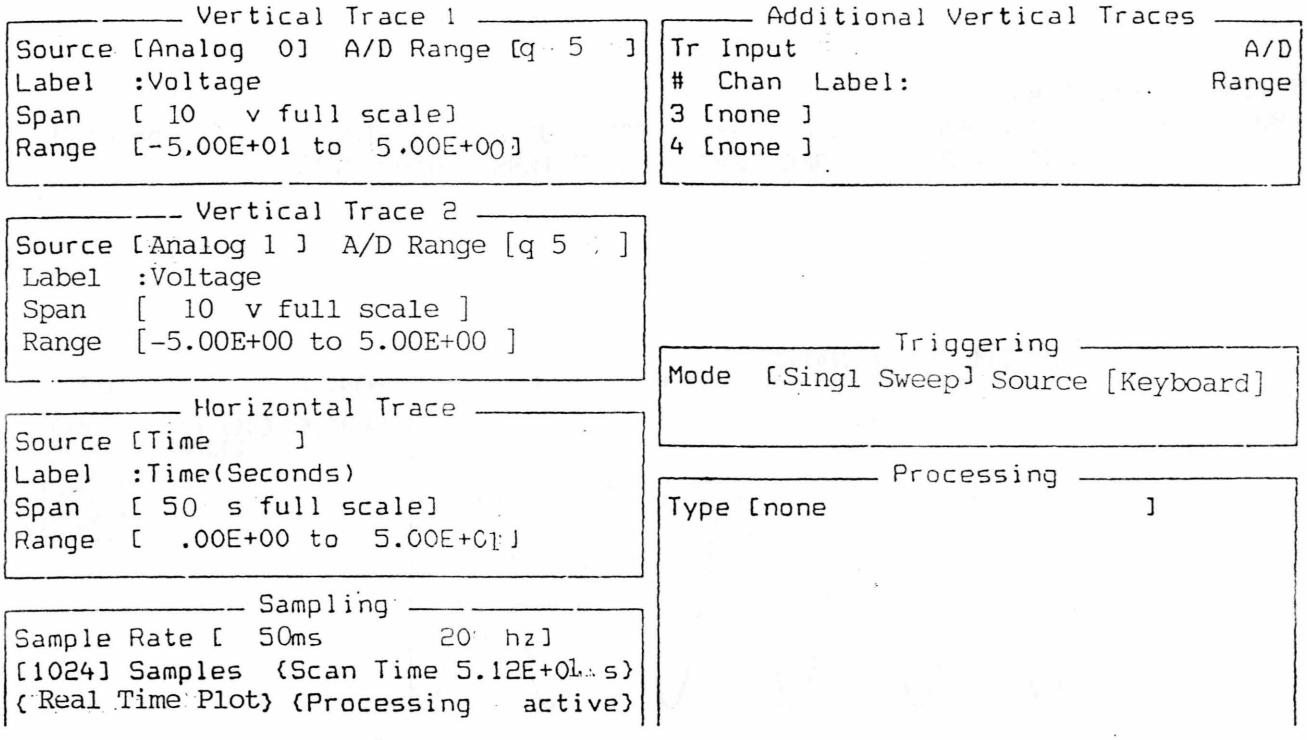


Figure 5

UnkelScope Parameters

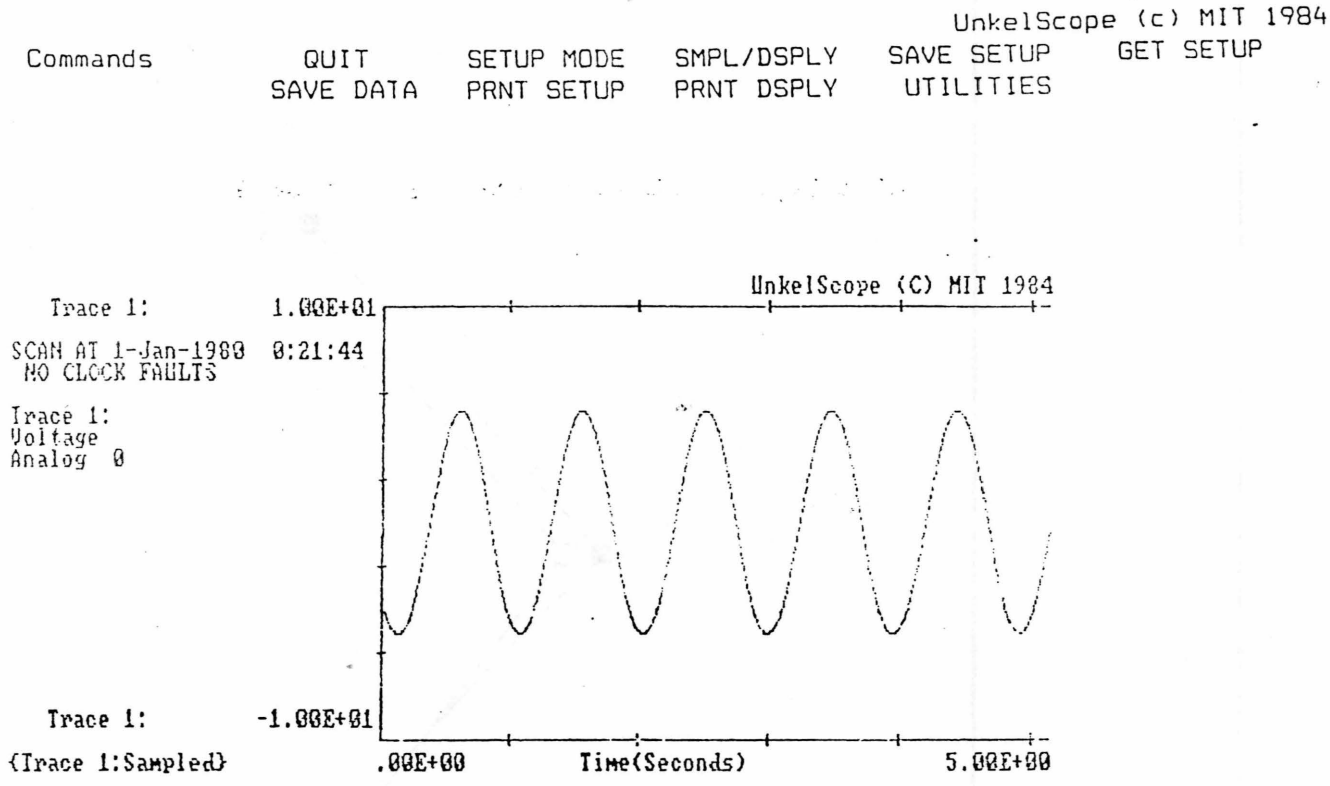


Figure 6

Example of UnkelScope Sample/Display Mode
displaying five 10 second intervals

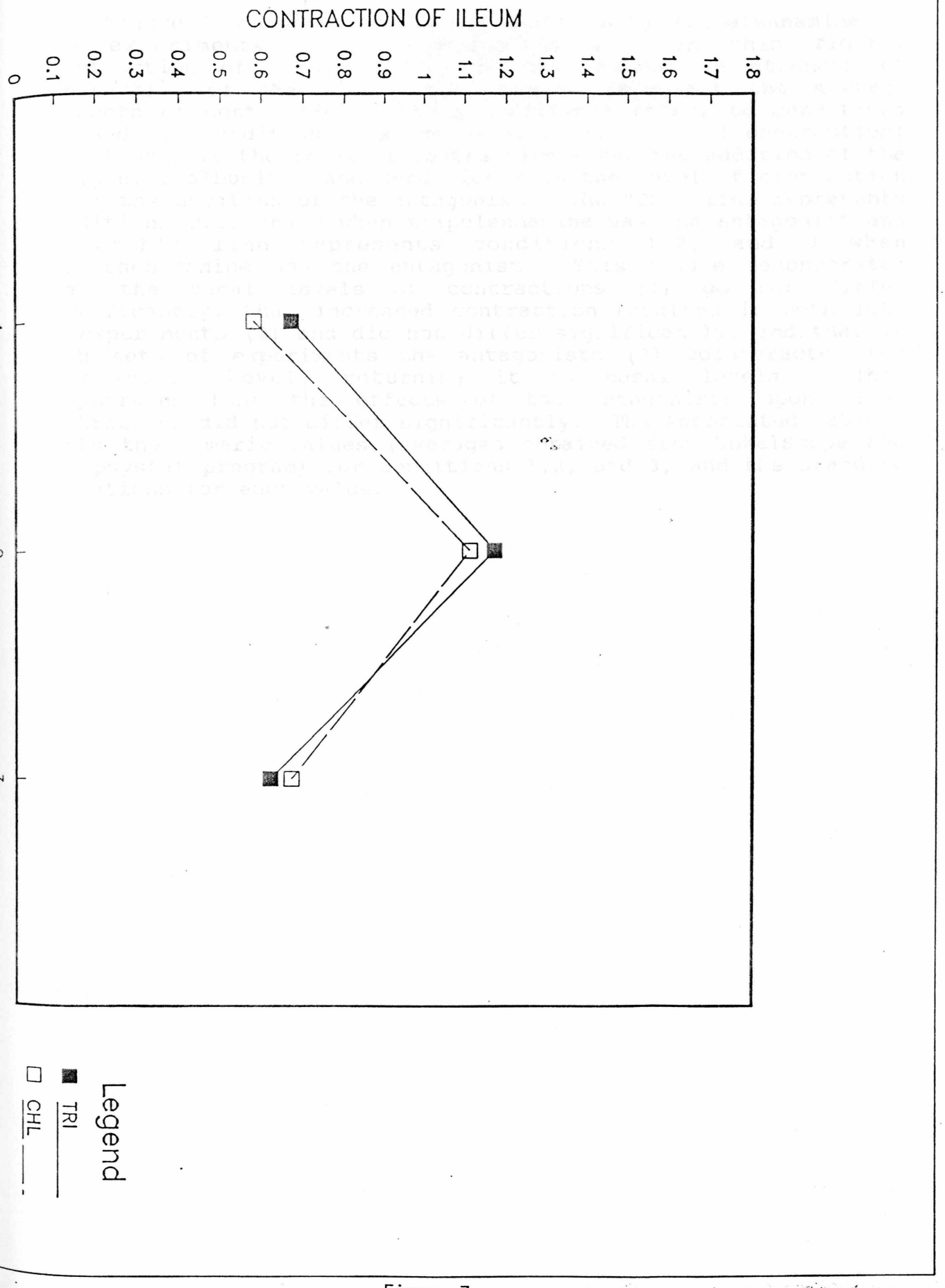

CONTRACTION OF ILEUM
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
1.1
1.2
1.3
1.4
1.5
1.6
1.7
1.8
Legend
TRI
CHL

Figure 7

Legend for Figure 7 cont.

Figure 7 compares the experiments using tripelennamine to the experiments using chlorpheniramine. In this figure, "contraction of ileum" refers to the changes in strength of contraction of the ileum, the numbers represent the average strength of contraction; "drug condition" refers to conditions 1,2,and 3. Condition 1 is the basal level of ileal contraction; condition 2 is the level of contraction after the addition of the antigen, ovalbumin; and condition 3 is the level of contraction after the addition of the antagonist. The "Tri" line represents conditions 1,2, and 3 when tripelennamine was the antagonist and the "Chl" line represents conditions 1,2, and 3 when chlorpheniramine was the antagonist. This figure demonstrates that the basal levels of contractions (1) do not differ significantly, that increased contraction resulted in both sets of experiments (2) and did not differ significantly, and that in both sets of experiments the antagonists (3) counteracted the contractile level, returning it to basal levels. This illustrates that the effects of the antagonists upon ileal contraction did not differ significantly. The Associated Table 2 lists the numeric values (averages obtained from UnkelScope and the psystat program) for conditions 1,2, and 3, and the standard deviations for each value.

TABLE 1

Table of the means (in volts) of ileal contractions under the conditions of basal level, ovalbumin, and antagonists (antihistamines: tripelennamine and chlorpheniramine) from Figure 7 and accompanying standard deviations.

Experimental conditions	Mean	Standard Deviation
Tripelennamine (Tri):		
Basal level	0.667	± 0.326
Ovalbumin	1.173	± 0.470
Tripelennamine	0.619	± 0.309
Chlorpheniramine (Chl):		
Basal level	0.581	± 0.237
Ovalbumin	1.106	± 0.389
Chlorpheniramine	0.669	± 0.268

Legend: Table 1

The basal levels of ileal contraction do not differ significantly from one another; nor do the levels of chlorpheniramine and tripelennamine differ significantly from one another. Ovalbumin produced significantly stronger contractions (twofold) than the levels of basal contraction, and the antihistamines significantly reduced (twofold) the levels of anaphylactic contraction resulting from the addition of ovalbumin. The levels of ileal contraction after the additions of tripelennamine and chlorpheniramine do not differ significantly from the basal levels. Apparently, these antihistamines restored the ilea to basal levels of contraction.

TABLE 2

Average levels of contraction of ileal sections (in volts) according to treatment of each ileum.

Subject	Basal Level	Antigen (OVA)	Antihistamine (Tripelennamine)
1	0.70	1.89	0.69
2	0.37	0.42	0.20
3	0.32	0.58	0.21
4	1.12	1.23	0.85
5	0.58	1.08	0.59
6	0.92	0.64	0.38
7	0.67	1.13	0.51
8	0.34	1.26	0.53
9	0.39	1.76	1.01
10	0.90	1.67	1.34
11	1.25	1.09	0.59
12	0.36	0.55	0.31
13	0.86	1.53	0.79
14	0.34	0.81	0.38
15	0.41	1.72	0.59
16	1.14	1.40	0.93
			(Chlorpheniramine)
17	0.77	1.40	0.65
18	0.58	1.03	0.59
19	0.85	1.00	0.72
20	0.66	1.76	1.01
21	0.14	0.35	0.26
22	0.59	1.13	0.80
23	0.38	0.76	0.54
24	0.97	0.68	0.46
25	0.50	1.48	1.17
26	0.23	1.33	0.41
27	0.76	1.08	0.56
28	0.44	1.40	0.60
29	0.78	0.67	1.11
30	0.49	1.41	0.48

Legend: Table 2

The variation in contraction was observed from the additions of ovalbumin and the antihistamines. Each number was obtained by averaging five samples of contraction measurements from each of five 10 second sections in the UnkelScope Edit Data function (Figure 6). A higher number indicates a stronger contraction.

The first column (Subject) represents each ileal piece. The second column (Basal level) expresses the average basal measure of contraction. The third column (Antigen OVA) expresses the average measure of contraction after the addition of ovalbumin. The last column (Antihistamine) expresses the average measure of contraction after the addition of antihistamine. In the first section the antihistamine is tripelennamine, in the second, chlorpheniramine. Looking at each subject, generally the contractions increased from "Base" to "Antigen" and then decreased from "Antigen" to "Antihistamine". The "Base" levels did not differ significantly from the "Antihistamine" levels and "Antigen" levels were significantly larger than both "Base" and "Antihistamine". Differences across the rows were a result of the additions of ovalbumin and the antihistamines. Differences down the columns may have resulted from different tautness of particular ilea connected to the polygraph.

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