

Demonstration of human reagin in monkey tissues

III. In vitro passive sensitization of monkey ileum with sera of atopic patients. Physiologic and enhancing experiments

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Comparisons of the response of monkey and guinea pig ileum and human appendix to histamine, bradykinin, serotonin, and SRS-A (slow-reacting substance) are reported. The monkey and guinea pig ileum reacted equally to both histamine and bradykinin. The guinea pig ileum showed a greater response to serotonin than did the other tissues. On the other hand, the monkey ileum reacted more strongly to SRS-A substance than did the guinea pig ileum. The human appendix responded well to both histamine and bradykinin but gave only weak contractions with serotonin. The monkey ileum and human appendix were able to be passively sensitized in vitro by much less serum of allergic patients than was required to sensitize the guinea pig ileum. This sensitization of the guinea pig ileum was not destroyed when the atopic serum was heated at 56° C. for 4 hours. However, this same treatment of serum prevented sensitization of the monkey ileum and human appendix. In vitro sensitization was enhanced 2 to 10 times when serum was diluted in isotonic buffered glucose rather than Tyrode's solution, and also if succinic or maleic acids were added with the antigen for challenge.

It has been previously reported from this laboratory¹ that the monkey ileum can be passively sensitized in vitro by the sera of allergic patients, as demonstrated by the Schultz-Dale method. This technique was from 50 to 500 times more sensitive than the usual passive transfer test to human skin.

Although considerable data concerning the mechanisms of in vitro passive sensitization of guinea pig tissues has been published,²⁻¹⁰ there have been no

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reports of such studies with use of the monkey ileum. In this report the effects of various chemical mediators, as histamine, serotonin, bradykinin, and SRS-A substance on the monkey ileum were studied and were compared with those obtained employing the guinea pig ileum and the human appendix. The ability of these various tissues to fix reaginic antibodies was investigated, as were various methods of enhancing the sensitization (in vitro) of these tissues.

MATERIALS AND METHODS

Antisera

Sera were collected from patients sensitive to ragweed or penicillin and were kept frozen at -20° C. until ready for study. Samples of sera from nonallergic individuals served as controls.

Antigens

Ragweed extract. Undefatted short ragweed pollen, 15 Gm., was extracted in 100 ml. of Coca's solution without the addition of phenol. The clear supernatant was dialyzed for 5 days in the cold room against frequent changes of buffered saline. The nondialyzable solution was then passed through Seitz filters, bottled sterilely in small aliquots, and kept frozen until used.

Benzyl penicillin G. The powder was dissolved in a modified Tyrode's solution* just prior to being used.

Tissues

Strips of ileum. The strips of ileum from the monkey and guinea pig were obtained by the methods previously described.¹

Normal human appendices. Human appendices, obtained as an incidental procedure during hysterectomy, were immediately placed in Tyrode's solution and kept at 4° C. until used. Rings of the human appendix, 4 to 5 mm. thick, were obtained by slicing the tissue in cross sections. Four to five rings were looped together with a silk thread, forming a chain.

Chemical mediators

The isometric contractions of the unsensitized monkey and guinea pig ileum and the human appendix were determined following the addition of various concentrations of the following chemical mediators: (a) histamine (histamine phosphate, U.S.P.) expressed as histamine base†; (b) serotonin (5 hydroxytryptamine creatinine sulphate)‡; (c) bradykinin§; and (d) slow-reacting substance (SRS-A).|| These chemicals were dissolved in Tyrode's solution just prior to each experiment.

*The modified Tyrode's solution used throughout these studies was made up as follows: NaCl, 8.9 Gm.; KCl, 0.2 Gm.; $MgCl_2 \cdot 6H_2O$, 0.1 Gm.; $C_6H_{12}O_6 \cdot H_2O$, 1.1 Gm.; $CaCl_2 \cdot 2H_2O$, 0.066 Gm.; $NaH_2PO_4 \cdot H_2O$, 0.057 Gm.; and $NaHCO_3$, 1.0 Gm. in 1,000 ml. of distilled water.

†Eli Lilly and Company, Indianapolis, Ind.

‡Nutritional Biochemical Corporation, Cleveland, Ohio.

§Sandoz Pharmaceuticals, Division of Sandoz Chemical Works, Inc., Hanover, N. J.

||Supplied by Dr. K. Frank Austen, The Massachusetts General Hospital, Boston, Mass.

Recording equipment

Isometric contractions of the tissues were recorded by means of a transducer attached to an ink-writing polygraph.*

This isometric smooth muscle assay, as adapted for *in vitro* anaphylaxis studies, was thoroughly described in a series of articles by Randall and co-workers¹¹ and by Neu and colleagues.¹² The upper end of the muscle was attached by a silk thread to a force-displacement transducer. The lower end was fixed to a wire hook attached to the tube which introduced air to the muscle chamber. The muscle contractions without shortening represent a force which is recorded from the transducer through the preamplifier and amplifier systems to the ink-writing polygraph. This response may be expressed simply in grams tension for a particular strip of tissue or as an equivalent histamine response.

Calibration of apparatus

As will be noted in Fig. 1, weights ranging from 0.1 to 2.0 Gm. were suspended from the transducer. The different straight-line slopes indicating height of pen displacement are the result of different voltages of the amplifying system used. It was found that, for most experiments, the tension load ranged from 1 to 2 Gm. for the monkey ileum and 0.5 to 1.0 Gm. for the guinea pig ileum and human appendix.

In all these experiments, an initial period of equilibration of the tissue in the muscle bath was necessary in order to obtain optimal responsiveness. This varied from tissue to tissue, but usually equilibration developed in 10 to 15 minutes. When tissue had been stored for 24 hours in the cold room prior to testing, longer periods of time were occasionally required for equilibration. Tests on a particular strip were usually completed within an hour after equilibration.

*Grass Instrument Company, Quincy, Mass. Polygraph, Model 5D, and transducer, Model PT. 03C.

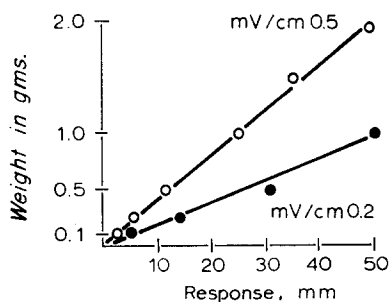


Fig. 1

The weight in grams applied to the transducer is plotted against the pen displacement, with the use of varying degrees of amplification (MV/CM) as indicated by the different slopes.

In vitro sensitization

Strips of ileum 2 to 2.5 cm. long or ring chains of human appendix were placed in test tubes containing approximately 5 ml. of human serum diluted with either Tyrode's or other solutions (described below). These tissues were placed in a 37° C. water bath for different periods of time (in vitro sensitization). The sensitized strip of ileum was then suspended in a muscle chamber and washed several times with Tyrode's solution. The highest dilution of anti-serum that was still able to sensitize the different tissues, as evidenced by a muscle contraction after the addition of the specific antigen, was determined.

It was found that high concentrations of some antigens, such as ragweed pollen extract, caused nonspecific contractions of unsensitized tissues. Therefore, each antigen was titrated prior to use and the appropriate concentration which did not produce this type of reaction was utilized to challenge the sensitized strip. (Usually 0.3 to 0.5 ml. of the optimal dilution of antigen was added to the 10 ml. capacity muscle bath.)

Determination of histamine equivalent

Prior to challenge of a sensitized strip with the specific antigen, the response to various concentrations of histamine was obtained. Following desensitization (the subsequent additions of the same antigen, until there were no further specific contractions), the histamine response was again determined. With these

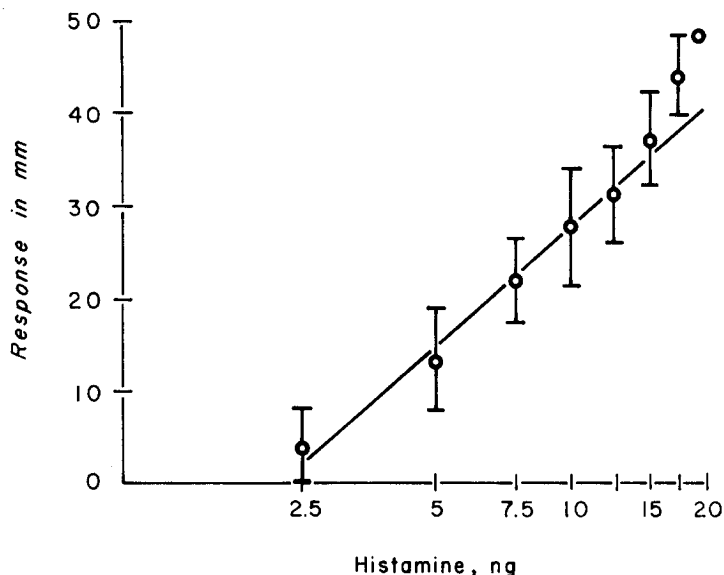
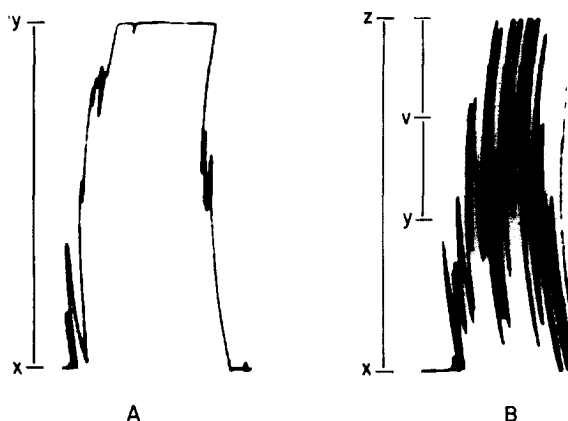


Fig. 2

A reference curve was constructed by means of adding increased concentrations of *histamine* plotted against the displacement of the pen, with a constant tension and amplification. This figure represents the average value as obtained from 15 different strips of monkey ileum, each originating from a different animal. For each point the standard deviation was calculated, demonstrating the physiologic variation from animal to animal in response to exogenous histamine.

**Fig. 3**

Evaluation of histamine equivalent.

A, demonstrates the sustained contraction obtained after the addition of antigen to the muscle bath containing the sensitized tissue. The pen displacement from the base line *x* to the apex of contraction *y* is measured and the histamine equivalent determined from the dose-response curve (Fig. 2).

B, illustrates the pattern of sustained contractions and alternate isometric contractions and relaxation of the tissue. The amount of response was empirically determined as midway between the sustained contraction *xy* and the maximal contraction relaxation pattern *xz*. This mean distance of *xv* was the amount considered, and the histamine equivalent was determined from this measurement.

data and the standard-dose-response curve previously determined (Fig. 2), the histamine equivalent of the antigen stimulation could be calculated by merely measuring the height of pen displacement.

In some experiments, instead of the usual sustained contraction (Fig. 3, *A*), the ileum developed alternate contractions and relaxation (Fig. 3, *B*). The determination of the histamine equivalent in this latter type of response is described in the legend for Fig. 3.

In the figures and tables throughout this paper, the concentrations of reagents are expressed in amounts added to the muscle bath.

Methods of enhancement

*Isotonic buffered glucose (IBG).** Sera from allergic patients were diluted with varying mixtures of Tyrode's solution and isotonic buffered glucose solution (IBG) for sensitization of monkey and guinea pig ileum and human appendices. After determining the optimal time of sensitization, the minimal concentration of antiserum that was able to sensitize each tissue was explored.

Succinic and maleic acid. These chemicals (in concentrations of 0.05 mM.) were added to the tissue either during sensitization with the antiserum, just prior to the challenging antigen, or mixed with the antigen at time of challenge. The muscle response with and without these chemicals was compared for the

*Isotonic buffered glucose, henceforth referred to as IBG in this presentation, was made up as follows: $C_6H_{12}O_6 \cdot H_2O$, 55.1 Gm.; $NaHCO_3$, 43.0 mg.; distilled water, 1,000 ml.

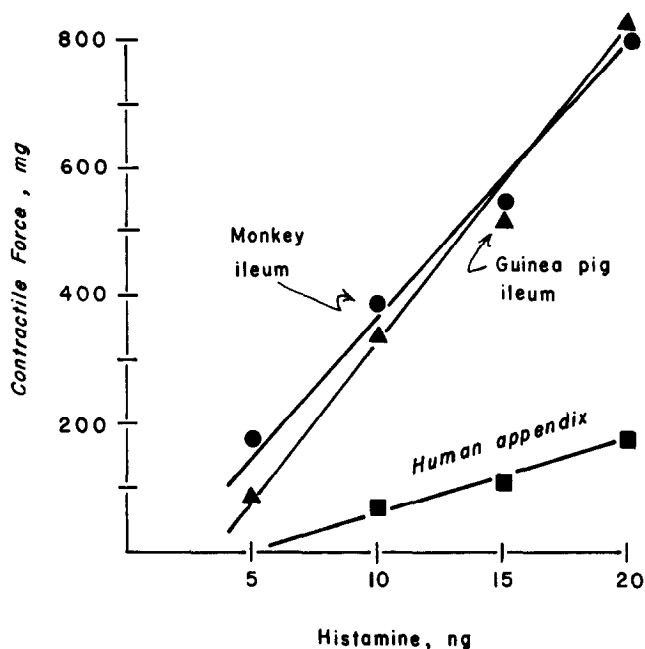


Fig. 4

The response of *monkey ileum*, *guinea pig ileum*, and *human appendix* to various concentrations of *histamine*. The abscissa indicates the amount of *histamine* in nanograms added to the bath. The ordinate is the mean *contractile force* expressed in milligrams. These data represent the mean results obtained from 10 strips of each tissue.

three tissues studied, as was the minimal amount of antiserum that could still passively sensitize the tissue *in vitro*.

Heparin. Sera from atopic patients were serially diluted with IBG to which 1.4 U. per milliliter of heparin was added. These mixtures were then used to sensitize the various tissues. Similar dilutions of the same serum were made up with IBG alone. The highest dilution of serum (in various diluents with or without heparin) which still was able to passively sensitize the tissues was determined.

EDTA.* Similar experiments as described with heparin were performed by adding EDTA (4 mM.) to the IBG as a diluent of the sensitizing serum.

RESULTS

Effect of chemical mediators on monkey and guinea pig ileum and human appendix

The reactivity of the monkey and guinea pig ileum and the human appendix to histamine, serotonin, bradykinin, and SRS-A substance was determined by testing several strips of each tissue with each chemical in varying concentrations in order to obtain the dose-response curves.

Histamine. The monkey ileum responded regularly to dosages of 10 to 20 ng.

*EDTA, ethylenediaminetetraacetic acid disodium salt. Eastman Organic Chemicals, Distillation Products Industries, Rochester, N. Y.

of histamine base added to the 10 ml. muscle chamber. Certain strips would contract with as little as 2 to 5 ng. The guinea pig ileum was as reactive as the monkey ileum to histamine. The human appendix was less sensitive to histamine than either the monkey or the guinea pig ileum. At least 20 ng. of histamine was necessary to produce a significant response. These differences in response are demonstrated in Fig. 4, where the response to histamine is measured as the contractile force. The response in millimeters can be calibrated from the slopes as indicated in Fig. 1.

Serotonin. More than 100 ng. of serotonin was needed to cause a regular reproducible contraction of the monkey ileum. The guinea pig ileum reacted slightly more readily to serotonin than did the monkey ileum. However, the human appendix was considerably less responsive to serotonin. Five hundred to 1,000 ng. was necessary to produce meager contractions of the human appendix. The differences of response to serotonin are shown in Fig. 5.

Bradykinin. The monkey ileum reacted to bradykinin; 10 to 20 ng. usually produced constant contractions. Twenty to 50 ng. was needed to produce an equivalent response in the guinea pig ileum. The human appendix responded much less to bradykinin. The wide variation of responses in these three tissues is illustrated in Fig. 6.

SRS-A. An antihistamine (chlorpheniramine maleate, 0.5 mg.) capable of totally inhibiting a histamine contraction was placed in the muscle bath 15 to 30 minutes prior to the addition of the SRS-A substance.

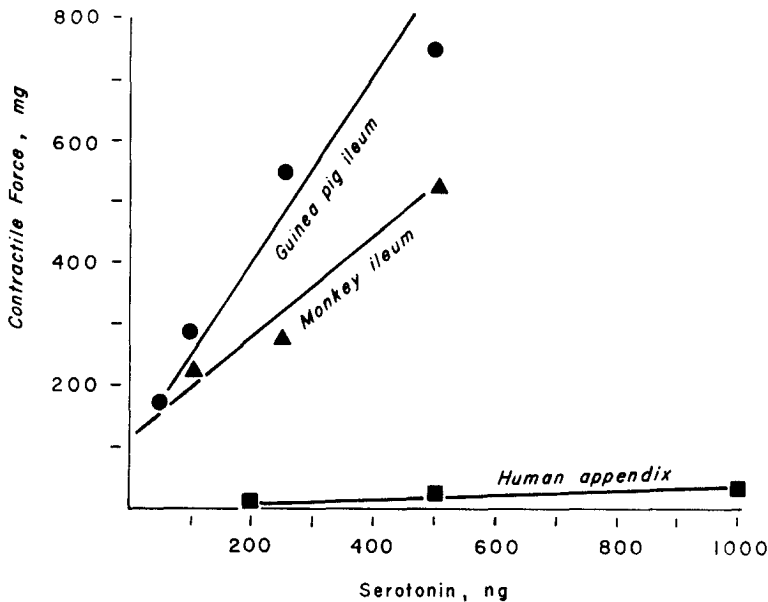


Fig. 5

The response of *monkey ileum*, *guinea pig ileum*, and *human appendix* to various concentrations of *serotonin*. The abscissa indicates the amount of *serotonin* in nanograms added to the bath. The ordinate is the mean *contractile force* expressed in milligrams. These data represent the mean results obtained from 10 strips of each tissue.

It was noted that 1 to 1.3 units of SRS-A* substance was necessary to produce a contraction of the monkey ileum. This contraction began about 30 to 40 seconds after the material was placed in the bath and reached a maximum in 3 to 4 minutes (Fig. 7). However, it required 9 to 10 units of SRS-A to elicit the typical response of the guinea pig ileum. The contractions started about 1.5 minutes after challenge and reached the peak at 6 to 7 minutes. Due to lack of material, the effect of SRS-A on the human appendix was not studied.

Antigen-antibody ratio

Varying doses of antigen. Several monkey ileum strips were sensitized with a 1:50 dilution of serum from a ragweed-sensitive patient. They were challenged with varying concentrations of ragweed extract. As is shown in Fig. 8, a 1:500 dilution of antigen produced less response than did concentrations between 1:1,000 and 1:4,000. Amounts of ragweed extract in dilutions greater than 1:10,000 failed to produce a contraction.

Varying concentrations of serum. Strips sensitized with varying concentrations of one serum were each challenged with the same dose of antigen.† When serum concentrations were too high, the response was markedly diminished (Table I). When the serum concentration was markedly diminished, it could no

*SRS-A unit as defined by Brocklehurst and Austen is the amount necessary to produce a characteristic contraction of the guinea pig ileum equal in amplitude to a response produced by 5 mg. of histamine base.

†0.4 ml. of 1: 2,000 dilution of a 15 per cent extract of short ragweed pollen equivalent to 16 PNU.

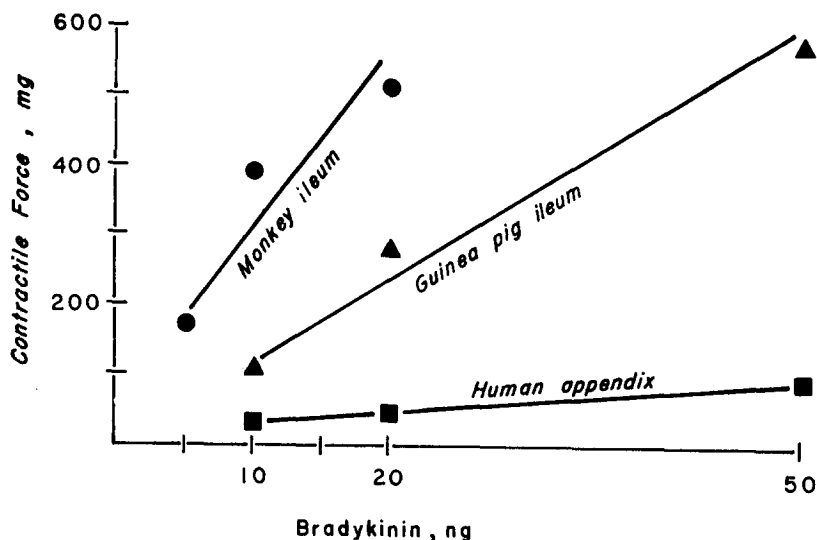
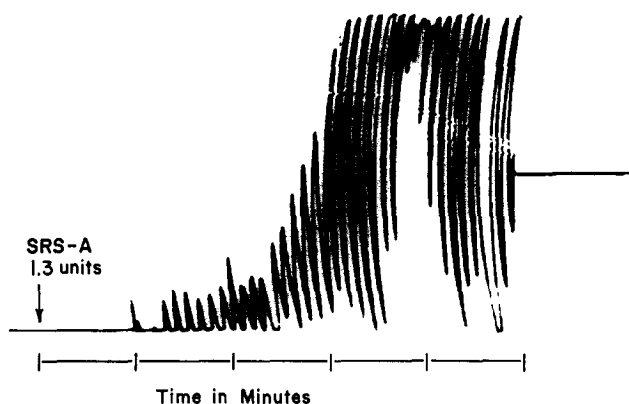


Fig. 6

The response of *monkey ileum*, *guinea pig ileum*, and *human appendix* to various concentrations of *bradykinin*. The abscissa indicates the amount of *bradykinin* in nanograms added to the bath. The ordinate is the mean *contractile force* expressed in milligrams. These data represent the mean results obtained from 10 strips of each tissue.

**Fig. 7**

The response of the monkey ileum to 1.3 units of SRS-A substance after the previous addition of 0.5 mg. of chlorpeniramine maleate.

Table I. Comparative responses of *in vitro* sensitization of monkey ileum with serum (from allergic patient) in various diluents

Dilution of serum	Serum diluent expressed in histamine equivalents, nanograms*	
	Isotonic buffer glucose†	Tyrode‡
1:20	3.5	7
1:100	10	14
1:500	11	13
1:1000	18	13
1:5000	12	No response
1:10,000	No response	No response

*Nanogram = 0.001 microgram.

†Tissue incubated at 37° C. for 12 minutes.

‡Tissue incubated at 37° C. for 60 minutes.

longer sensitize the tissue. This variation of response was observed in both monkey and guinea pig ileum, and also when the serum was diluted either in Tyrode's or IBG. The monkey ileum, when sensitized with serum diluted in IBG, demonstrated a bell-shaped curve (Fig. 9).

Enhancement of sensitization

Various diluents. The monkey ileum was sensitized with the same concentration of human atopic serum diluted with varying mixtures of Tyrode's and isotonic glucose solutions. As shown in Table II, the sensitizing activity gradually increased (as evidenced by muscle response) with increasing amounts of the isotonic glucose in the mixture. However, when the 100 per cent IBG was used as the diluent of serum, and the monkey ileum strip was sensitized for one hour, it did not respond either to histamine or antigen challenge.

Hence, various periods of time for sensitization of the monkey and guinea pig ileum with the use of isotonic buffered glucose (IBG) as the diluent were

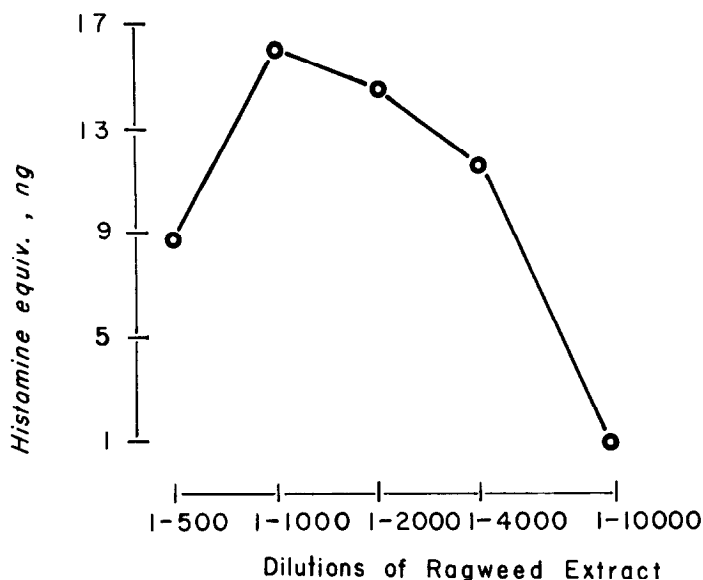


Fig. 8

Various strips of monkey ileum were passively sensitized in vitro with a constant amount of serum from a ragweed-sensitive patient. The strips were each challenged with serial dilutions of a 15 per cent extract of short ragweed pollen. The response to the additions of these antigens is expressed in histamine equivalents (nanograms).

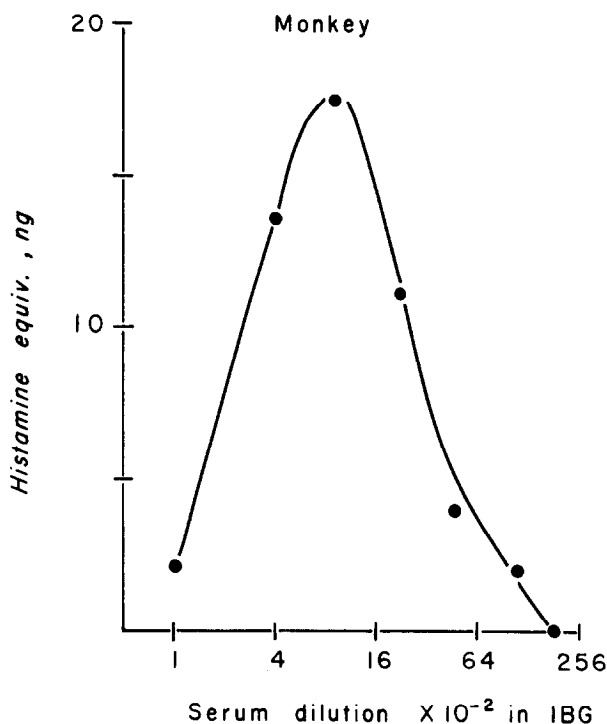


Fig. 9

Strips of *monkey* ileum were passively sensitized in vitro with varying concentrations of *serum* from a ragweed-sensitive patient. Each strip was challenged with the same concentration of ragweed pollen extract. The response to this challenge is expressed as histamine equivalents (nanograms).

Table II. *Comparison of responses of in vitro sensitization of monkey ileum* with atopic serum diluted in various proportions of IBG and Tyrode's solutions*

Ratio of diluents		Response expressed in histamine equivalents (nanograms)
Tyrode's	IBG	
100	0	5
90	10	20
50	50	20
10	90	22
0	100	No response

*Sensitized for one hour.

Table III. *Effect of varying incubation period in isotonic buffered glucose for sensitization*

Incubation period (minutes)	Response to antigens (histamine equivalent)	
	Guinea pig ileum (ng.)	Monkey ileum (ng.)
5	0	4
10	12	13
15	9	9
20	8	8
30	8	4
60	6	0

studied. These results (Table III) suggest that the optimum sensitization period with IBG is between 10 and 15 minutes. The time of sensitization was essentially the same even when various concentrations of antisera were used in testing either the monkey or guinea pig ileum.

As will be noted in Fig. 10 and Table I, when the sensitizing serum was diluted in IBG,* the response to antigen challenge increased and the amount of serum still capable of sensitizing the tissue was much less.

The results of sensitization of both the monkey and guinea pig ileum with the sera of 11 patients (sensitive either to ragweed or penicillin) when diluted in Tyrode's solution or IBG are shown in Table IV. When the sera were diluted in IBG, the titer for sensitization was from 2 to 10 times greater than when Tyrode's was used in the monkey ileum system. There was also some enhancement of sensitization of the guinea pig ileum. Considerably higher serum dilutions could passively sensitize the monkey ileum than they could the guinea pig ileum. In other experiments, it was found that the human appendix can be passively sensitized in vitro essentially to the same degree as the monkey tissue.

Heparin. There was no enhancement of sensitization when 1.4 U. per milliliter of heparin was added to IBG as the diluent of serum for sensitization. It appeared that the heparin seemed to decrease the sensitization in the monkey ileum system (Table V). For example, the titer of one serum diluted in IBG without heparin was 10,000, whereas with heparin added it was 5,000.

*Tissue sensitized for 10 to 15 minutes.

Table IV. Comparison of *in vitro* sensitization of monkey and guinea pig ileum by sera from allergic patients diluted in IBG and Tyrode's solutions

Serum	Monkey ileum serum diluent		Guinea pig ileum serum diluent	
	IBG* titer†	Tyrode‡ titer	IBG titer	Tyrode titer
KO	15,000	5,000	200	50
LV	10,000	2,000	200	50
IL	10,000	1,000	200	200
LA	7,000	3,000	<10	N.D.§
FA	15,000	5,000	50	50
DO	20,000	3,000	<10	<10
CH	10,000	5,000	N.D.	N.D.
SO	10,000	3,000	50	100
SH	7,000	500	<10	<10
RI	20,000	1,000	100	<10
WA	20,000	10,000	10	<10

*Tissue incubated at 37° C. for 12 minutes.

†Titer = the reciprocal of the highest dilution of serum, still capable of passively sensitizing the tissue, as evidenced by a muscle contraction following challenge with the specific antigen (ragweed or penicillin G).

‡Tissue incubated at 37° C. for 60 minutes.

§N.D., not done.

Table V. Effect of heparin on the *in vitro* sensitization of monkey ileum

Dilutions of serum	Serum diluted in IBG (response expressed in histamine equivalents, nanograms)	
	Heparin (1.4 U./ml.) added	Without heparin
1:10	0	N.D.*
1:100	7	N.D.
1:500	N.D.	12
1:1,000	15	20
1:5,000	7	10
1:10,000	0	5
1:15,000	0	0
1:20,000	0	0

*N.D., not done.

EDTA. The sensitizing activity of IBG alone and with the addition of 4 mM. of EDTA as diluent of the atopic sera were compared. A typical experiment of one serum is illustrated in Table VI. The titer without EDTA added to the diluent was 10,000, but with EDTA it was only 1,000.

Maleic and succinic acids. In preliminary experiments it was found that when 0.05 mM. of either succinic or maleic acid (other concentrations of these substances were not as effective) was added to the antigen for challenge of the sensitized tissue, enhancement of the reaction occurred. These experiments were performed with IBG as the diluent. The enhancing activity was observed in both monkey (Table VII) and guinea pig ileum (Table VIII), when sensitized with either sera from ragweed- or penicillin-sensitive patients.

Succinic acid produced slightly greater enhancement than did maleic acid, but the end point was about 2 to 5 fold higher when either acid was used

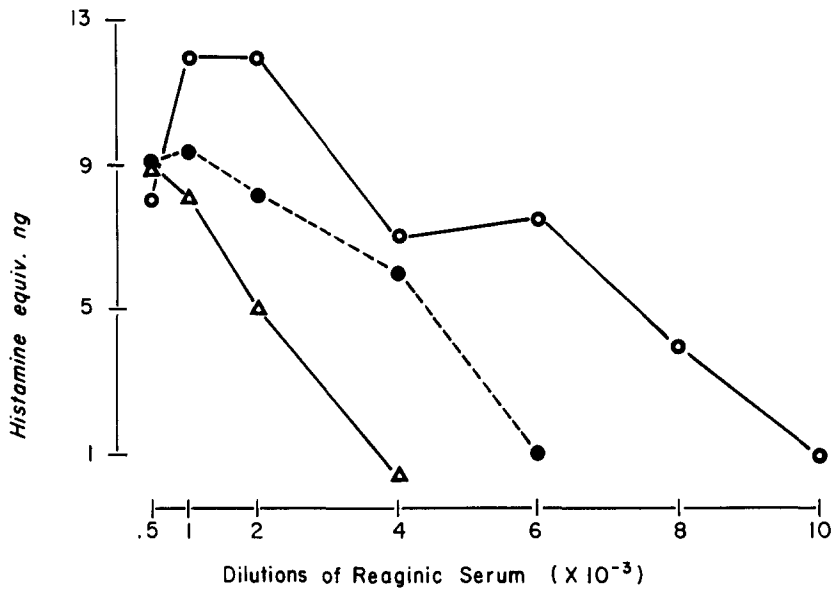


Fig. 10

○—○ serum diluted with IBG, sensitized for 12 minutes.
△—△ serum diluted with Tyrode's solution, sensitized for 60 minutes.
●—● serum diluted with equal parts of IBG and Tyrode's solution, sensitized for 60 minutes.
The strips were challenged with a constant amount of antigen. The response is recorded in histamine equivalents.

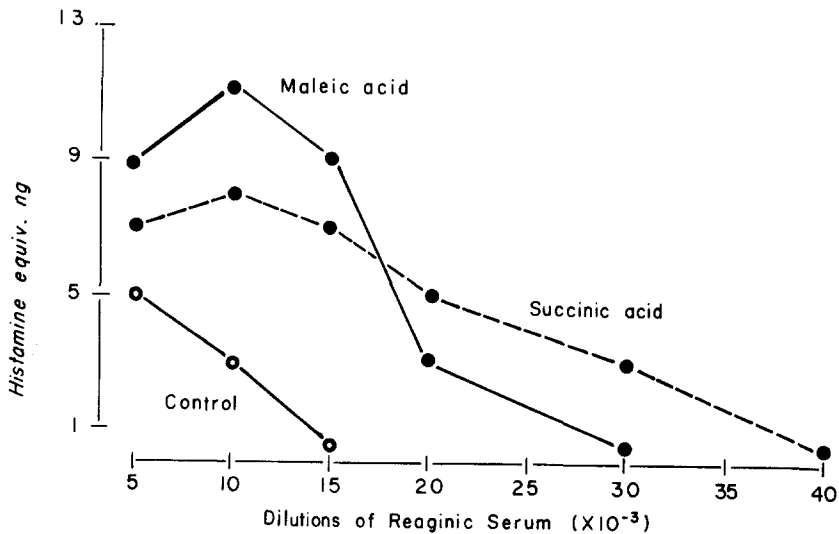


Fig. 11

Comparison of muscle contractions of monkey ileum passively sensitized in vitro, when challenged with antigen alone and with the addition of 0.05 mM. of succinic or maleic acid.

Table VI. *Effect of EDTA on the in vitro sensitization of monkey ileum*

Dilutions of serum	Serum diluted in IBG (response expressed in histamine equivalents, nanograms)	
	With EDTA (4 mM.)	Without EDTA
1:10	15	5
1:100	29	20
1:1000	5	20
1:5000	0	10
1:10,000	0	7
1:15,000	0	0

Table VII. *Effect of succinic and maleic acids on the in vitro sensitization of monkey ileum*

Dilutions of serum	Antigen challenge (response expressed in histamine equivalents, nanograms)		
	With maleic acid*	With succinic acid*	Without acids
1:10,000	13	10	2
1:15,000	10	16	0
1:20,000	5	9	0
1:30,000	9	9	0
1:50,000	0	6	0
1:70,000	0	0	0

*0.05 mM. of either acid was added with the antigen at time of challenge.

Table VIII. *Effect of succinic and maleic acids on the in vitro sensitization of guinea pig ileum*

Dilutions of serum	Antigen challenge (response expressed in histamine equivalents, nanograms)		
	With maleic acid*	With succinic acid*	Without acids
1:200	4	N.D.†	2
1:500	13	7	0
1:700	2	7	0
1:1,000	0	4	0
1:1,500	0	0	0

*0.05 mM. of either acid was added with the antigen at time of challenge.

†N.D., not done.

Table IX. *Comparison of in vitro sensitization of monkey ileum and human appendix by serum of allergic patients*

Serum of patient	Patient sensitivity	Monkey ileum titer	Human appendix ring titer
KO	Ragweed	15,000	10,000
LV	Ragweed	10,000	10,000
FE	Penicillin	5,000	2,000
WA	Penicillin	7,000	5,000
VO	Penicillin	2,000	5,000
DA	Penicillin	7,000	7,000
JA	Penicillin	2,000	2,000

(Fig. 11). The same effect was observed when these acids were added to the tissue bath 10 minutes prior to challenge with antigen, but the effects were slightly less than when antigen and acid were added simultaneously. However, when the same concentrations of these acids were added to the sera diluted in IBG at the time of sensitization of the tissues, no enhancing effect was noted.

Comparison of in vitro sensitization of monkey ileum and human appendix

The minimal amounts of serum from 2 ragweed and 5 penicillin-sensitive patients who were able to passively sensitize the monkey ileum and the human appendix are illustrated in Table IX. The titers obtained with the monkey ileum and human appendix were essentially the same. However, these titers were 10 to 100 times higher than when the guinea pig ileum was used. (Table IV).

When each of these sera was heated at 56° C. for 4 hours, it lost its ability to sensitize the monkey ileum and human appendix, but the end point and degree of sensitization of the guinea pig ileum was not affected by such treatment.

DISCUSSION

Recently, a report from this laboratory¹ has shown that the monkey ileum could be passively sensitized in vitro by sera of allergic patients. Heating the sera at 56° C. for 4 hours, or treatment with 2-mercaptoethanol (2ME) prevented sensitization of the tissues. There was some correlation between the titer obtained by this method and the usual P-K test, but the monkey ileum technique was 50 to 500 times more sensitive. Tollackson and Frick¹⁵ presented data showing that the human uterus and ileum could also be sensitized in vitro by atopic sera. However, the tissue responses and end points determined were much less than the results obtained in our laboratories. Chopra and co-workers¹⁶ and Goodfriend, Kovacs, and Rose¹⁷ reported that the human appendix and monkey lung could also be sensitized in vitro by reagin, but their quantitative tests indicate that the degree of sensitization is not as great as when the monkey ileum is sensitized and then challenged as described above. In this presentation, we found that the passive sensitization of the monkey ileum is a highly sensitive and reliable method of measuring antibodies in the serum of atopic patients. The human appendix, when cut into a series of 4 to 5 rings, could act as an adequate substitute for the monkey ileum. Both of these tissues were capable of being sensitized with a much smaller amount of atopic sera than that necessary to sensitize the ileum of the guinea pig. Heating the sensitizing sera at 56° C. for 4 hours did not affect the ability to sensitize the guinea pig ileum, but this treatment prevented (almost completely) sensitization of both the monkey ileum or human appendix. This is strong circumstantial evidence that these latter two tissues fix reagin, whereas probably only IgG antibodies fix to guinea pig tissues.

However, as in many other biologic tests, there were certain difficulties encountered with this method. There frequently were individual variations of response with tissues from different animals of the same species, as well as

with various strips of tissue from the same animal. At times, certain strips of monkey ileum had spontaneous contractions that were too great to permit their use immediately. Under such circumstances, the tissue was either kept in the cold room (4° C.) overnight or cooled at -1° to -2° C. for 10 to 30 minutes prior to testing, and the ileum became more stable and could be used. The reliability and reproducibility of this technique was from 70 to 80 per cent when ilei of various monkeys or guinea pigs were tested. It should be emphasized that each test was repeated several times with various concentrations of the sensitizing sera.

In the data presented, it was observed that either an excess of antigen for challenge or an excess of serum for sensitization depressed the response of the monkey ileum. Liacopoulos, Halpern, and Frick¹⁸ also found that an excess of antigen for challenge diminished the response of the Schultz-Dale reaction with the use of the guinea pig system. Austen and colleagues³² have also reported that an excess of antigen inhibits the release of histamine from sensitized rat mast cells. More recently Mongar⁵ and Mongar and Winne⁶ described a bell-shaped curve with an *in vitro* sensitization of guinea pig ileum indicating a decrease in sensitization with excess antibody. The bell-shaped curve that was described in the present communication (Fig. 9) is similar to that observed by Mongar.

Mongar and Winne⁶ proposed that this bell-shaped curve could be explained on the basis that "single attachments of antibody to the tissue are ineffective and no sensitization can occur until two or three attachments are achieved. In high concentrations, the antibody cannot combine readily with two receptors because most of them are already occupied by singly attached molecules." They assumed that the effect, histamine release, is proportional to the fraction of the receptors occupied by attached antibody molecules. However, it is difficult to explain from this hypothesis why excess of antigen also inhibits the histamine release.

As many investigators¹⁹ have already confirmed, antibody must be capable of fixing to the tissue in anaphylaxis. A complex of two antigen molecules and one antibody molecule do not produce skin reactivity, but the complexes containing two or more antibody molecules do produce this reaction.²⁰ Furthermore, Ishizaka, Ishizaka, and Campbell²¹ have shown that the most active soluble antigen-antibody complex is Ag_3Ab_2 or greater. It thus would seem that the antigen-antibody ratio probably plays some role in this bell-shaped dose-response curve. These findings are similar to a prozone effect seen in other serologic reactions.

Although there have been many reports on the reactivity of the guinea pig ileum to various mediators,^{4, 11, 13, 22-24} none compared these responses under the same experimental conditions. In the studies presented, it was found that the guinea pig response to histamine was slightly greater than that described by Binaghi and Osler.¹³ It is recognized that these responses will vary with differences in initial tension, portion of ileum used, method of handling the tissue, and ionic environment, as well as variations in the amplifying system. The response of the guinea pig tissue to bradykinin was of the same order of magnitude as that reported by Khairallah and Page.²³ The reactivity of SRS-A substance

in the guinea pig was approximately the same as that reported by Chakravarty.²⁴ Employing the technique described, it was noted that the guinea pig ileum responded best to histamine, then to bradykinin, and least to serotonin, when comparing these materials on a weight basis.

There are only a few publications on the effect of these mediators on human tissue. Chopra and colleagues¹⁶ reported the histamine sensitivity of the human appendix ranged from 0.1 to 1.0 μ g. In the present studies, it was found that when the human appendix was made into a chain of rings, the variability of the histamine response was less and a constantly higher degree of sensitivity was observed. Bennett²⁵ stated that the human ileum had a much greater response to serotonin than to histamine. Fishlock²⁶ also reported that the human ileum contracted well to serotonin, but the human colon relaxed, rather than contracted, when this material was added. In our experiments, the human appendix was found to have only a meager response to serotonin but reacted more readily to histamine and bradykinin. Apparently this tissue responds to serotonin somewhere between the reaction of the human ileum and colon. Brocklehurst³³ showed that isolated human bronchioles reacted to SRS-A.

The only report on the effect of these mediators on the monkey tissue was that of Brocklehurst²² who described the effects on the bronchioles of monkeys. In our studies, it was noted that monkey ileum and guinea pig ileum responded essentially the same to histamine, but the monkey ileum did not respond quite as well as the guinea pig to stimulation by serotonin. On the other hand, when SRS-A substance or bradykinin was added to the monkey ileum, it was more reactive than was the guinea pig ileum.

The end point for *in vitro* sensitization of the monkey ileum was 2 to 10 times higher when IBG was used as the diluent of the serum rather than when Tyrode's solution was employed. Binaghi and co-workers²⁷ reported similar enhancement of sensitization in the guinea pig ileum. However, in our hands, the degree of enhancement with the use of the guinea pig ileum was not as great as with the monkey ileum. Perhaps the discrepancy in our results could be due to the fact that we used atopic serum for sensitization, whereas Binaghi used a rabbit antiserum. In addition, we did not find any stronger sensitization beyond 15 minutes of incubation in IBG. According to Binaghi's report,²⁷ the period of sensitization in IBG was 50 to 100 times faster than that in Tyrode's. This could be interpreted that 15 minutes of incubation time in IBG corresponded to approximately 10 hours of incubation in Tyrode. Halpern and colleagues² reported the strongest sensitization in Tyrode's solution occurred after 8 hours of incubation, but Mongar and Schild³ stated that the optimal time for sensitization in Tyrode's solution was 2 hours. When the monkey ileum was sensitized with sera diluted in IBG longer than 20 to 30 minutes, the responses to both histamine and antigen challenge were diminished.

Neither the mechanism of *in vitro* sensitization nor enhancement in IBG is entirely explained. Passive sensitization can be considered to be a fixation of preformed antibody to tissue or cells containing histamine.⁵ Nielsen, Terres, and Feigen⁷ reported a close correlation between adsorption and sensitization, as both depended on concentration. However, Brocklehurst and co-workers^{9, 10} and

Feigen, Nielsen, and Terres⁸ have shown that sensitization is not simple adsorption. Antibody which does not sensitize is adsorbed as readily as that which does. Others observed that both γ_1 and γ_2 -globulins of the guinea pig could be adsorbed to the chopped guinea pig lung, but sensitization was more readily produced by γ_1 -globulin than by γ_2 -globulin.²⁹ Feigen, Nielsen, and Terres⁸ inferred that the sensitization could be due to a reaction involving a change in covalent binding. Mongar⁵ and Mongar and Winne⁶ also mentioned the multiattachment theory of passive sensitization.

Binaghi²⁷ reported that the enhancing sensitization of reduced ionic strength medium was not due to the chemical nature of each electrolyte, but was rather dependent upon the ionic strength. The enhancing mechanism of IBG could, therefore, be assumed to be due to less electrolyte in medium producing greater attachment of antibody to the tissue,¹⁰ presumably due to physiochemical change of the medium or antibody combining site.

The potentiating action of maleic and succinic acids on the release of mediators by antigen-antibody reaction has been observed by Austen and Brocklehurst,²⁸ Baker, Bloch, and Austen,²⁹ and Noah³⁰ in the chopped guinea pig lung system. Similar action of these chemicals in the monkey and guinea pig Schultz-Dale experiments presented here was found. There was no enhancing activity when these substances were added to the serum at the time of sensitization, but only when they were added just prior to or simultaneously with the challenging antigen. This is in agreement with the observations of other investigators.²⁸⁻³⁰ Austen²⁸ has postulated that the enhancement of the muscle response seems to be due to potentiation of some step activated by the antigen-antibody interaction and common to both the release of histamine and slow-reacting substance.

Levy and Osler³¹ reported on the enhancing activity of heparin in in vitro passive sensitization of human leukocytes, but did not explain the mechanism involved. When heparin was added to the serum diluted in IBG for in vitro sensitization of either guinea pig or monkey ileum, no such enhancement was noted in our experiments.

Osler, Lichtenstein, and Levy¹⁴ reported that the passive sensitization of human leukocytes by reaginic sera could be enhanced by the addition of EDTA. In the studies presented, we did not observe this enhancement effect.

SUMMARY AND CONCLUSIONS

1. The monkey and guinea pig ileum reacted equally to both histamine and bradykinin. The guinea pig ileum showed a greater response to serotonin than did the other tissues. On the other hand, the monkey ileum reacted more strongly to SRS-A substance than did the guinea pig ileum. The human appendix responded well to both histamine and bradykinin but gave only weak contractions with serotonin.

2. The monkey ileum and human appendix were able to be passively sensitized in vitro by much less serum of allergic patients than was required to sensitize the guinea pig ileum. This sensitization of the guinea pig ileum was not destroyed when the atopic serum was heated at 56° C. for 4 hours. However, this

same treatment of serum prevented sensitization of the monkey ileum and human appendix.

3. A bell-shaped dose-response curve was observed with the use of different concentrations of serum from atopic patients. Excess antigen for challenge also reduced the muscle response.

4. In vitro sensitization was enhanced 2 to 10 times when the serum was diluted in IBG rather than Tyrode's solution. The optimum time period for sensitization with serum diluted in IBG was from 10 to 15 minutes.

5. Maleic and succinic acids added with the antigen for challenge potentiated the response approximately threefold.

6. The addition of heparin and/or EDTA did not increase the sensitization over IBG as diluent alone.

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