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# Demonstration of human reagin using monkey tissues

## VI. Pharmacologic studies of in vitro passive sensitization of monkey ileum with sera from atopic patients

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***The response of isolated monkey ileum to various chemical substances which have been described as mediators in the anaphylactic antigen-antibody reaction were studied previously.[2] In this report, several materials that supposedly inhibit these actions in this system are presented. The effect of these antagonists on the prevention of a contraction of a sensitized strip of monkey ileum following the addition of the specific antigen was likewise determined. In this manner, and with all these data available, the probable mediators of the antigen-antibody reaction in the Schultz-Dale-type monkey ileum system could be assessed. It would appear that histamine was the most important mediator of the antigen-antibody reaction in this system. This conclusion is based on the observations listed below. (1) Chlorpheniramine maleate and BP400 were able to suppress the contractions of the monkey ileum produced by histamine and anaphylactic challenge in relatively small dosages (0.05 to 1 μg), respectively. (2) There was no close correlation between the inhibition of the anaphylactic contractions and those produced by serotonin or bradykinin. (3) Phenol, phenylbutazone, salicylic acid, and hydrocortisone each inhibited the contractions produced by each of the chemical mediators (histamine, serotonin, and bradykinin) and the anaphylactic challenge to the same degree. There was no specific action of any of these inhibitory agents, and relatively huge dosages were necessary to produce these effects. Hence, it is assumed that they have a direct action on the ability of the muscle to contract, rather than a specific antagonistic action on any of the mediators. (4) KB-95 demonstrated much more potent anti-bradykinin activity than any of the other drugs tested.***

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Previous reports from this laboratory[1, 2] have shown that the sera of atopic individuals can passively sensitize the monkey ileum in vitro. The addition of the specific antigen to this sensitized strip of tissue in a muscle bath produces a contraction. The exact mechanism of this reaction of the monkey ileum has not as yet been fully elucidated.

It is theorized[3, 4] that the antigen-antibody complex triggers an enzymatic mechanism which releases certain chemical mediators, which produce a muscle contraction. Several of these substances, such as histamine, SRS-A, serotonin, bradykinin, and perhaps acetylcholine, have been implicated in this reaction.

Many studies[5-11] employing the Schultz-Dale technique with guinea pig ileum have shown that histamine, SRS-A, and possibly serotonin are the primary chemical mediators which produce the muscle contraction. Inasmuch as the chief mediators involved in anaphylaxis vary with the species of animal used, it was deemed advisable to study this problem in the isolated monkey ileum.

In a recent report,[2] it was shown that the nonsensitized monkey and guinea pig ileum respond similarly to histamine, SRS-A, serotonin, and bradykinin. In order to further determine the mediators released in the monkey ileum antigen-antibody system, certain substances which inhibit the reactions of these mediators were studied.

## MATERIALS AND METHODS

The following materials were investigated for their ability to prevent contractions elicited by the various chemical mediators: chlorpheniramine maleate,* 9-(N-methyl-piperidylidine-4′)-thioxanthene maleate,† lysergic acid diethylamide,‡ atropine sulfate,§ hydrocortisone sodium succinate,‖ 1-(N-methyl-piperidyl-4′)-3-phenyl-4-benzyl-pyrazolone-5,¶ phenylbutazone,# phenol,** and salicylic acid.††

These antagonists were diluted in Tyrode's solution and the pH adjusted to 7.2 by the addition of NaOH. There was no demonstrable effect on the muscle strips when these substances were added, in the dilutions studied, to the muscle chamber. Eight to 10 minutes after the addition of the antagonist, the muscle was challenged by the addition of the various mediators†† or the specific antigen. The concentration of each of the mediators which gave a maximal response (as measured by this technique‡‡) was kept constant. The minimal amount of each antagonist which competely suppressed muscle contraction in response to each stimulus under investigation was determined.

*Chlor-trimeton, Schering Corporation, Union, N. J.
†BP400, Sandoz Pharmaceuticals, Hanover, N. J.
‡LSD25, Sandoz Pharmaceuticals, Hanover, N. J.
§Burroughs Wellcome and Company, Tuckahoe, N. J.
‖Solu-cortef, The Upjohn Company, Kalamazoo, Mich.
¶KB95, Sandoz Pharmaceuticals, Hanover, N. J.
#Geigy Pharmaceuticals, Yonkers, N. Y.
**Fisher Scientific Company, Pittsburgh, Pa.
††There was not sufficient SRS-A material for study.
‡‡The highest excursion of the pen that could be recorded on the ink-writing Polygraph when a constant tension was applied to the transducer and a constant amplification maintained throughout the experiments.

The monkey ileum was passively sensitized in vitro by the technique previously described.[1] The concentration of the same serum (1 to 500 dilution) for sensitization and antigen (0.5 ml. of 1 to 1,000 dilution of 5 per cent ragweed extract) for challenge was kept constant throughout all these experiments. The various antagonists were added to the musele bath which contained the passively sensitized strip of monkey ileum prior to the addition of the challenging antigen. This type of reaction will be referred to throughout the paper and tables as the anaphylactic response (or anaphylaxis). At least 6 strips of tissue were used for each determination. The results reported are the average of many experiments with each antagonist and each mediator or allergen and were quite reproducible. The variability of inhibition of response was ± 20 per cent.

In the figures and tables presented, the concentrations of the agents are expressed as amounts added to the 10 ml. tissue bath.

## RESULTS

### 1. The effects of an antihistaminic

Chlorpheniramine maleate was used as a classical histamine antagonist. As shown in Table I, 0.05 μg of this substance produced complete inhibition of the histamine response. Three micrograms, sixty times the amount necessary to inhibit the histamine response, was needed to inhibit completely the serotonin response. One hundred micrograms of chlorpheniramine maleate were required to block the bradykinin response.

The antianaphylactic action of this drug on sensitized monkey ileum was

**Table I.** *Effect of chlorpheniramine maleate on the response of mediators and anaphylaxis of the monkey ileum*

| *Inhibiting substance: Chlorpheniramine maleate (μg)* | *% Inhibition of challenging agents** | | | |
|---|---|---|---|---|
| | *Histamine (20 ng.)†* | *Serotonin (0.1 μg)* | *Bradykinin (20 μg)* | *Anaphylaxis‡ (10 ng. histamine equivalent)* |
| 100 | | | 100 | |
| 50 | | | 70 | |
| 10 | | | 50 | |
| 3 | | 100 | N.D. | |
| 2 | | 75 | N.D. | |
| 1 | | 40 | 0 | 100 |
| 0.5 | | 40 | | 80 |
| 0.1 | | 40 | | 40 |
| 0.05 | 100 | N.D. | | 20 |
| 0.02 | 45 | N.D. | | N.D. |
| 0.01 | 40 | N.D. | | 0 |
| 0.005 | 25 | N.D. | | |
| 0.0025 | 0 | N.D. | | |

N.D. = not done.

*$\frac{Eo - Ei}{Eo} \times 100$: Eo = response without inhibitor; Ei = response with inhibitor.

†Nanogram (0.001 microgram).

‡Responses to ragweed extract of strips sensitized with the serum (1 to 500) of a ragweed-sensitive patient.

very pronounced, but approximately 20 times more material was necessary to inhibit this reaction than that required to prevent the histamine response.

### 2. The effect of antiserotonin agents

*A. 9-(N-Methyl-piperidylidine-4')-thioxanthene maleate (BP400).* This compound has been described as having potent antiserotonin activity in other species. Its chemical structure is closely related to the phenothiazines.[12] The effects of this drug on the monkey ileum are shown in Table II. Although 2.5 μg of BP400 inhibited the muscle response produced by serotonin, only 0.1 μg was necessary to prevent the response induced by histamine. Hence its ability to inhibit histamine was almost equivalent to that of chlopheniramine. The effect of BP400 on the bradykinin response was very weak and 50 μg were required to abolish this response.

The antianaphylactic activity of this drug was high and only 1 μg of BP400 was necessary to prevent the contraction induced by the specific antigen. This was less than was necessary to inhibit the serotinin reaction but 10 times more than was required to abolish the histamine contraction.

*B. Atropine and LSD25.* It was assumed that the monkey ileum had two serotonin receptors.[13, 14] Hence both atropine and LSD25 were studied for their effects individually and in combinations against serotinin and the various other stimulators. The effect of LSD25 alone against the serotonin response of the monkey ileum was very weak (ED50: 10 μg against 0.1 μg of serotonin); even 100 μg of LSD25 did not completely inhibit the serotonin response.

The effect of atropine alone on serotonin response in monkey ileum was also extremely weak; as much as 2 mg. in the bath did not completely inhibit the response induced by 0.1 μg of serotonin. However, when both drugs were tested simultaneously, they induced potent antiserotonin activity in the monkey ileum

**Table II.** *Effect of BP400 on the responses to mediators and anaphylaxis of the monkey ileum*

| *Inhibiting substance BP400 (μg)* | *% Inhibition of challenging agents** | | | |
|---|---|---|---|---|
| | *Histamine (20 ng.)* | *Serotonin (0.1 μg)* | *Bradykinin (20 ng.)* | *Anaphylaxis† (10 ng. histamine equivalent)* |
| 50 | | | 100 | |
| 10 | | | 50 | |
| 2.5 | | 100 | N.D. | |
| 1 | | 90 | 0 | 100 |
| 0.2 | | 70 | | 57 |
| 0.1 | 100 | 70 | | 50 |
| 0.05 | 75 | 50 | | 14 |
| 0.01 | 65 | 20 | | N.D. |
| 0.0025 | 40 | N.D. | | N.D. |

N.D. = not done.

*$\frac{Eo - Ei}{Eo} \times 100$: Eo = response without inhibitor; Ei = response with inhibitor.

†Responses by ragweed extract of strips sensitized with serum (1 to 500) of a ragweed-sensitive patient.

(Table III). By contrast, huge doses of the combination of these substances were needed to inhibit the histamine, the bradykinin, and the anaphylactic responses.

### 3. The effect of antibradykinin agents

Although there is no known drug with highly specific antibradykinin activity, the following chemicals were tested for this property:

*A. Phenylbutazone.* Phenylbutazone (Table IV) showed the strongest effect on a bradykinin response. It had slightly less inhibitory activity on the reaction induced by histamine and produced the weakest protection against a serotonin

**Table III.** *Effect of combination of LSD25 and atropine on the responses to mediators and anaphylaxis of the monkey ileum*

| *Inhibiting substances* | | *% Inhibition of challenging agent** | | | |
|---|---|---|---|---|---|
| *Atropine (μg)* | *LSD25 (μg)* | *Histamine (20 ng.)* | *Serotonin (0.1 μg)* | *Bradykinin (20 ng.)* | *Anaphylaxis† (10 ng. histamine equivalent)* |
| 100 | 50 | 100 | | 100 | |
| 50 | 25 | 75 | | 90 | 100 |
| 30 | 15 | 50 | | N.D. | N.D. |
| 20 | 10 | 25 | | 50 | 40 |
| 10 | 5 | N.D. | | N.D. | 20 |
| 2 | 1 | N.D. | 100 | N.D. | 0 |
| 1 | 0.5 | | 75 | | |
| 0.5 | 0.25 | | 25 | | |
| 0.2 | 0.1 | | 0 | | |

N.D. = not done.

*$\frac{Eo - Ei}{Eo} \times 100$: Eo = response without inhibitor; Ei = response with inhibitor.

†Responses by ragweed extract of strips sensitized with serum (1 to 500) of a ragweed-sensitive patient.

**Table IV.** *Effect of phenylbutazone on the responses to mediators and anaphylaxis of the monkey ileum*

| *Inhibiting substance phenylbutazone (mg.)* | *% Inhibition of challenging agent** | | | |
|---|---|---|---|---|
| | *Histamine (20 ng.)* | *Serotonin (0.1 μg)* | *Bradykinin (20 ng.)* | *Anaphylaxis† (10 ng. histamine equivalent)* |
| 2.4 | | 85 | | |
| 1.8 | | 60 | | |
| 1.2 | 100 | 50 | 100 | |
| 0.9 | 50 | N.D. | 95 | 100 |
| 0.6 | 0 | 37 | 75 | 42 |
| 0.3 | | N.D. | 0 | 0 |
| 0.06 | | 0 | | |

N.D. = not done.

*$\frac{Eo - Ei}{Eo} \times 100$: Eo = response without inhibitor; Ei = response with inhibitor.

†Responses by ragweed extract of strips sensitized with serum (1 to 500) of ragweed-sensitive patient.

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| | Anaphylaxis† (10 ng. histamine equivalent) |
|---|---|
| | 100 |
| | N.D. |
| | 40 |
| | 20 |
| | 0 |

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| Anaphylaxis† (10 ng. histamine equivalent) |
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response. Phenylbutazone's antianaphylactic activity was slightly greater than its antihistaminic and antibradykinin properties.

*B. 1-(N-Methyl-pipendyl-4')-3-phenyl-4-benzyl-pyrazolone-5 (KB95).* KB95 is a modified pyrazol derivative[15] but is not similar to other known pyrazol compounds; it represents, together with piperylon, a prototype of a new class of piperidinopyrazol compounds. The results (Table V) indicated that KB95 has a relatively potent antibradykinin action as compared with other substances studied. Only 15 μg were necessary to prevent the bradykinin response, whereas 100 μg of chlorpheniramine, 50 μg of BP400, and 1,200 μg of phenylbutazone were needed to produce the same pharmacologic effect. KB95 was more effective against serotonin than was phenylbutazone.

**Table V.** *Effect of KB95 on the responses to mediators and anaphylaxis of the monkey ileum*

| Inhibiting substance KB95 (μg) | % Inhibition of challenging agent* | | | |
|---|---|---|---|---|
| | Histamine (20 ng.) | Serotonin (0.1 μg) | Bradykinin (20 ng.) | Anaphylaxis† (10 ng. histamine equivalent) |
| 15 | | | 100 | |
| 10 | 100 | 100 | 80 | 100 |
| 6 | 100 | 90 | 80 | 83 |
| 3 | 90 | 70 | 75 | 66 |
| 2 | 70 | N.D. | N.D. | N.D. |
| 1 | N.D. | 30 | N.D. | 46 |
| 0.6 | 20 | 20 | 50 | N.D. |
| 0.3 | N.D. | 10 | 20 | 0 |

N.D. = not done.

*$\frac{Eo - Ei}{Eo} \times 100$: Eo = responses without inhibitor; Ei = responses with inhibitor.

†Responses by ragweed extract of strips sensitized with the serum (1 to 500) of a ragweed-sensitive patient.

**Table VI.** *Effect of salicylic acid on the responses to mediators and to anaphylaxis of the monkey ileum*

| Inhibiting substance salicylic acid (mg.) | % Inhibition of challenging agent* | | | |
|---|---|---|---|---|
| | Histamine (20 ng.) | Serotonin (0.1 μg) | Bradykinin (20 ng.) | Anaphylaxis† (10 ng. histamine equivalent) |
| 14 | 100 | 100 | 100 | 100 |
| 10 | 70 | N.D. | N.D. | 75 |
| 7 | N.D. | 95 | 50 | N.D. |
| 5 | 60 | N.D. | N.D. | N.D. |
| 3.5 | N.D. | 75 | 0 | N.D. |
| 0.7 | N.D. | 35 | | N.D. |

N.D. = not done.

*$\frac{Eo - Ei}{Eo} \times 100$: Eo = responses without inhibitor; Ei = responses with inhibitor.

†Responses by ragweed extract of strips sensitized with the serum (1 to 500) of a ragweed-sensitive patient.

**Table VII.** *Effect of hydrocortisone on the responses to mediators and to anaphylaxis of the monkey ileum*

| *Inhibiting substance hydrocortisone (mg.)* | *% Inhibition of challenging agent** | | | |
|---|---|---|---|---|
| | *Histamine (20 ng.)* | *Serotonin (0.1 μg)* | *Bradykinin (20 ng.)* | *Anaphylaxis† (10 ng. histamine equivalent)* |
| 10 | 100 | 100 | 100 | 100 |
| 5 | 75 | 80 | 75 | -25 |
| 1 | 0 | 0 | 0 | 0 |
| 0.5 | 0 | 0 | 0 | 0 |
| 0.1 | 0 | 0 | 0 | 0 |

*$\frac{Eo - Ei}{Eo} \times 100$: Eo = responses without inhibitor; Ei = responses with inhibitor.

†Responses by ragweed extract of strips sensitized with the serum (1 to 500) of a ragweed-sensitive patient.

**Table VIII.** *Effect of phenol on the responses to mediators and to anaphylaxis of the monkey ileum*

| *Inhibiting substance phenol (mg.)* | *% Inhibition of challenging agent** | | | |
|---|---|---|---|---|
| | *Histamine (20 ng.)* | *Serotonin (0.1 μg)* | *Bradykinin (20 ng.)* | *Anaphylaxis† (10 ng. histamine equivalent)* |
| 250 | 100 | N.D. | N.D. | N.D. |
| 125 | 62 | 95 | 100 | 85 |
| 90 | N.D. | N.D. | N.D. | 65 |
| 50 | N.D. | 30 | 30 | N.D. |

N.D. = not done.

*$\frac{Eo - Ei}{Eo} \times 100$: Eo = responses without inhibitor; Ei = responses with inhibitor.

†Responses by ragweed extract of strips sensitized with the serum (1 to 500) of a ragweed-sensitive patient.

**Table IX.** *Comparison of ED50 inhibition of the various agents in Schultz-Dale experiments in the monkey ileum*

| *Drugs studied* | *ED50 inhibition to the response of:* | | | |
|---|---|---|---|---|
| | *Histamine (20 ng.)* | *Serotonin (0.1 μg)* | *Bradykinin (20 ng.)* | *Anaphylaxis (10 ng. histamine equivalent)* |
| BP400 | 0.007 | 0.05 | 10 | 0.1 |
| Chlorpheniramine maleate | 0.025 | 1.4 | 10 | 0.2 |
| KB95 | 1.5 | 2 | 0.6 | 1.3 |
| Atropine + LSD | 30 + 15 | 0.7 + 0.35 | 20 + 10 | 26 + 13 |
| Phenol | 90 | 70 | 70 | 60 |
| Phenylbutazone | 900 | 1,200 | 500 | 700 |
| Salicylic acid | 2,500 | 2,500 | 7,000 | 2,500 |
| Hydrocortisone | 4,000 | 3,500 | 4,000 | 8,800 |

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| *igent** | |
|---|---|
| *in* ) | *Anaphylaxis† (10 ng. histamine equivalent)* |
| | 100 |
| | -25 |
| | 0 |
| | 0 |
| | 0 |

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| *ient** | |
|---|---|
| n | *Anaphylaxis† (10 ng. histamine equivalent)* |
| | N.D. |
| | 85 |
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*ts in Schultz-Dale*

| *of:* | |
|---|---|
| | *Anaphylaxis (10 ng. histamine equivalent)* |
| | 0.1 |
| | 0.2 |
| | 1.3 |
| | 26 + 13 |
| | 60 |
| | 700 |
| | 2,500 |
| | 8,800 |



*C. Salicylic acid.* The effects of salicylic acid on the monkey ileum are presented in Table VI. These results indicated that salicylic acid is equally effective in inhibiting the responses to various mediators and the anaphylactic reaction. These findings were similar to those with phenylbutazone, except that inhibitory activity on the bradykinin response was slightly less. As a whole, the dose required for inhibition of each of these mediators was quite large, namely, from 1 to 14 mg. in a 10 ml. bath.

### 4. Miscellaneous inhibitory agents

*A. Hydrocortisone (Table VII).* Huge doses of hydrocortisone (5.0 to 10 mg.) inhibited all responses equally. These results suggested that hydrocortisone had a toxic rather than specific inhibitory activity on these responses. The antianaphylactic activity of hydrocortisone on the monkey ileum was extremely weak, as shown by the fact that even 5 mg. of hydrocortisone did not prevent a contraction of sensitized gut. In a few experiments it appeared that this dose of steroid actually enhanced the anaphylactic response.

*B. Phenol.* Results in Table VIII indicated that approximately 100 to 250 μg significantly prevented the responses by the various mediators as well as by antigen. The inhibitory effect of phenol on all these responses was similar.

### 5. Comparison of ED50 inhibition of used agents

As presented in Table IX, all agents were arranged in order of the potency of antihistaminic action. It is of interest that this order of antihistaminic potency exactly correlates with antianaphylactic potency, whereas antiserotonin or antibradykinin action did not correlate.

If these antagonists were compared on a weight basis, the most potent inhibitory activity on anaphylactic and histamine response was obtained with BP400. The next most effective agent was chlorpheniramine maleate.

Each of the top four agents tested (Table IX) showed some selective antagonistic action. The lower four agents (phenol, phenylbutazone, salicylic acid, and hydrocortisone) did not demonstrate any selective action against a particular mediator, and huge doses were required to produce inhibition.

## DISCUSSION

Results obtained with chlorpheniramine maleate in the monkey ileum indicated that the agent has potent antihistaminic action and practically no antibradykinin action. The effect of this drug on serotonin response is of interest, since small doses are partially inhibitory, but very large doses are necessary for complete inhibition. These results are similar to those reported by Gaddum,[13, 14] who studied the inhibitory action of antihistaminics to serotonin response in the guinea pig ileum.

The effects of antihistaminics on the Schultz-Dale–type anaphylactic reaction (employing the guinea pig ileum) have been reported by many investigators.[6-8, 17] All these reports have confirmed that antihistaminics themselves do not inhibit the anaphylactic reaction as well as the histamine response. The results presented indicate that approximately 20 times more chlorpheniramine

maleate was necessary to prevent the anaphylactic reaction (in the monkey ileum sensitized by reaginic antibody) than the histamine-induced contraction. It must be pointed out that the anaphylactic response was equivalent to the response of 10$\mu$g of histamine, whereas inhibition studies of 20 $\mu$g of histamine were reported. Hence, in reality the difference between inhibition of anaphylactic response and histamine response is about twice that described. However, the antianaphylactic activity of chlorpheniramine was significantly greater than the inhibitory activity on the responses to bradykinin. These results suggest that when the monkey ileum is sensitized with reaginic antibody, bradykinin probably does not play an important role in the muscular response following the addition of antigen.

Why antihistamine does not abolish the anaphylactic reaction to the same degree as the histamine reaction is still not clear. Two hypotheses are proposed[9, 17]: (1) The difference in concentration of endogenously liberated histamine and exogenously added histamine near the effector cells may be important because mast cells are closely embedded between smooth muscle cells.[16] (2) Other kinds of mediators, which may not be inhibited by an antihistaminic, may be involved.

BP400 showed marked antihistaminic, antianaphylactic, and antiserotonin activity and weak antibradykinin activity. In the guinea pig ileum or rat uterus, BP400 also has pronounced antiacetylcholine effects as well as antiserotonin and antihistaminic activity. Moreover, in higher concentrations, it inhibits bradykinin and oxytocin action.[12] Leme[18] observed a competitive antagonistic effect of thioxanthene (BP400) on bradykinin responses in the guinea pig. These reports are quite similar to our results with monkey ileum.

Fishlock[19] reported that LSD completely blocked the response of serotonin on circular muscle of the human ileum. We observed that neither atropine nor LSD alone completely blocked the effect of serotonin on monkey ileum. However, the combination of atropine and LSD could inhibit the response of the monkey ileum to serotonin. These results appear to agree with the findings of Geiger and colleagues[5] and Gaddum and colleagues[13] that there are two kinds of serotonin receptors in guinea pig ileum. Hence one could postulate that the monkey ileum has similar receptors.

The inhibitory action of this combination on the anaphylactic response was very weak. Even the doses which completely inhibited the serotonin response did not show any inhibitory action on an antigen-induced contraction. These findings suggest that serotonin probably plays little or no role in the anaphylactic reaction of the monkey ileum. However, Geiger[5, 6] reported that in Schultz-Dale–type experiments with the guinea pig ileum, serotonin plays some role in muscular contraction. Fink,[20] by using the same methods as Geiger, reported that a combination of antihistaminic, atropine, and LSD could inhibit all responses to serotonin, histamine, and anaphylaxis.

The effects of the combination of atropine and LSD on the histamine and bradykinin response, as well as on anaphylaxis, could well be nonspecific, since the dose which was needed for inhibition was high. Brocklehurst[8] has mentioned that atropine, $10^{-6}$ Gm. per milliliter, prevented the SRS-A contraction non-

specifically, but Berry[21] observed that neither atropine nor LSD reduced the response to SRS-A.

Collier,[22] Bonta,[23] and Mitchell[24] have reported that phenylbutazone can prevent the bradykinin response of smooth muscle. Our results indicated that phenylbutazone seems to have relatively potent antibradykinin action as compared to its ability to inhibit the other mediators studied. However, it was not clear whether this activity is specific or nonspecific.

These data indicate that phenylbutazone, in relatively high dosages, can inhibit the muscle contraction of the monkey ileum produced by the addition of the various mediators and anaphylaxis. These results agree with the observations of other investigators using the guinea pig ileum.[9, 21] However, others[9, 25, 26] reported that phenylbutazone acts as a nonspecific inhibitor of muscle contraction and generally suppresses the cell function.

KB-95 had more potent inhibitory activity than did phenylbutazone on the responses to the mediators and to anaphylaxis in the monkey ileum. Its antiserotonin activity was much greater than that of phenylbutazone if compared on a weight basis. Only 15 $\mu$g of KB-95 was necessary to inhibit the serotonin stimulus, whereas even 2.4 mg. of phenylbutazone could not cause 100 per cent inhibition. KB-95 had much stronger antibradykinin activity than did any of the other drugs tested. In our experimental results, KB-95 suppressed essentially equally the responses to histamine, serotonin, bradykinin, and anaphylaxis.

Several observers[9, 24, 27, 28] have reported that acetylsalicylic acid (ASA) inhibits the action of histamine, SRS-A, and anaphylaxis in the guinea pig. Mitchell[24] noted an antibradykinin effect of this drug. However, Mongar[9, 26] has reported that salicylates depress the oxygen consumption and contractility of smooth muscle in about the same concentration at which they inhibit histamine release.

In our studies, we found that huge doses (14 mg.) of ASA were necessary to suppress contractions of the monkey ileum when subsequently challenged by each of the mediators or by specific antigen. These findings with the monkey ileum agree with the reports of other investigators listed above. It would appear from the lack of specific inhibition and the large amounts necessary that ASA has a nonspecific effect on the contractility of the smooth muscle itself.[9, 26]

Hydrocortisone had practically no inhibitory activity on the responses of the monkey ileum to any of the mediators. It also lacked antianaphylactic activity. This observation agrees with work reported by many previous investigators[28-32] that the corticosteroids will not prevent anaphylactic shock of the intact animal. On the other hand, Trethewie[29, 30] noted that cortisone acetate depressed the response of the gut to exogenous histamine and SRS-A in the guinea pig. However, other investigators[21, 28] could not confirm these findings.

Phenol, in amounts much less than either phenylbutazone or hydrocortisone, was able to prevent equally the contractions of the monkey ileum produced by each of the mediators tested and the anaphylactic challenge. Several reports[16, 26] have demonstrated that phenol can prevent histamine release and also have a direct suppressive action on the muscle. Hence, in view of these reports and our findings, one could assume that phenol does not have a specific action on any

particular mediators. Nevertheless, from a practical point of view, one must take into account that this substance can suppress the anaphylactic response, and therefore antigen used for challenge of sensitized tissue in experimental systems should be devoid of phenol.

During the course of these investigations, it was necessary to determine whether the specific antagonists fix to the monkey ileum or whether their presence in the muscle bath alone produce their effects. Hence, in comparative experiments, after the particular antagonist was added to the tissue for the prescribed period, the tissue was washed six to eight times prior to the addition of the specific mediator (agonist) or the specific antigen. The response of the tissues was the same whether the ileum was washed or not prior to exposure to the particular stimulant. This would indicate that the antagonists studied remain fixed to the tissue and prevent the contraction of the gut.

The comparison of ED50 inhibition of all the agents studied here indicated that in the monkey ileum system there was good correlation between the inhibitory activity to histamine and anaphylactic responses, but not between the anaphylactic reactions and bradykinin responses. However, there are several reports[7, 33, 35] which suggest that bradykinin has some role in anaphylaxis in certain animal species.

Mitchell[24] mentioned that bradykinin is thought to be a second phase mediator of the inflammatory response. In the first phase there is an increase of capillary permeability resulting from histamine activity. Plasma protein then enters the tissue spaces, and kallikrein, which is produced by damaged cells, releases bradykinin from $\alpha_2$-globulin. These reports can explain the results that bradykinin may not be involved in the Schultz-Dale reaction of monkey tissues as it was performed in vitro under blood-free conditions.

Comparative results of serotonin responses appear to show some supplementary participation in the anaphylactic response. Serotonin has been reported to be an active chemical mediator in the rat uterus[10] and mouse uterus.[20] However, there is no unaminity of opinion on the relationship of serotonin release in the guinea pig to anaphylactic reactions. Some investigators[4] felt that serotonin is liberated, whereas others[7, 10, 20] could not varify these findings. Although there are no previous reports on the role of serotonin in anaphylaxis of the monkey ileum, serotonin probably does not play an important role in this reaction.

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