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Some Pharmacological Properties of Trimethoxy Analogs of Pheniramine, Tripelennamine, and Diphenhydramine

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SOME PHARMACOLOGICAL PROPERTIES OF TRIMETHOXY ANALOGS OF PHENIRAMINE, TRIPELENNAMINE, AND DIPHENHYDRAMINE

BY

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ABSTRACT

Trimethoxy analogs of tripelennamine, diphenhydramine, and pheniramine were studied to determine the influences of the trimethoxy group on the pharmacological activity of known antihistamines. In this investigation, the ability of the parent compounds and their analogs to antagonize the histamine-induced contractions of guinea pig ilia were studied as well as their effects on the systolic blood pressure of male albino rats and on the central nervous system of mice as measured by the actophotometer.

The trimethoxy analogs were comparatively weak competitive antagonists of histamine with the exception of N,N,-Diethyl-N'-(2-pyridyl)-N'-(3,4,5-trimethoxybenzyl) ethylenediamine Dicyclamate (De-TMPBZ) and N,N,-Diethyl-N'-(2-pyridyl)-N'-(3,4,5-trimethoxybenzyl) ethylenediamine Disuccinate (TMPBZ) which were weak noncompetitive histamine antagonists. The parent compounds and their analogs did not significantly alter the systolic blood pressure of rats, but they did cause some minor fluctuations which were of interest.

Intraperitoneal injections of pheniramine in mice caused a significant increase ($P \leq 0.05$) in locomotor activity, but Dma-TMPP, a trimethoxy analog of pheniramine, did not significantly alter locomotor activity. Injections of tripelennamine produced no significant changes in locomotor activity; however, Mor-TMPBZ, a trimethoxy analog of tripelennamine caused a significant decrease in activity.

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MASTER OF SCIENCE THESIS

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I. INTRODUCTION

The 3,4,5-trimethoxyphenyl group frequently occurs in drugs which have a pronounced effect upon the central nervous system. For example, two chemically different compounds, reserpine and mescaline, both contain a 3,4,5-trimethoxyphenyl group, and both affect the central nervous system. It is therefore suspected, that the trimethoxyphenyl group may play an important part in the activity of some centrally active drugs.

DiFazio (1963) synthesized a series of 3,4,5-trimethoxyphenyl analogs of known antihistamines, diphenhydramine (Benadryl ®), tripelennamine (Pyribenzamine ®), and pheniramine (prophenpyridamine, Trimeton ®). These antihistamines represent three different chemical groups which, in addition to their ability to antagonize histamine, have some effect upon the central nervous system. It was hoped that the introduction of the trimethoxy group would result in a new psychotropic drug.

The purpose of this investigation was to elucidate some of the pharmacological properties of the parent compounds and their analogs. This was accomplished by determining their histamine antagonizing abilities, their effects on normal systolic blood pressure, and their effects on the central nervous system, as evidenced by changes in locomotor activity.

II. LITERATURE SURVEY

A search for drugs with the ability to antagonize the physiological effects of histamine has been going on for many years. The first compound shown to have antihistaminic effects was synthesized by Fourneau in 1910 and identified as 929 F (thymoxyethyl diethylamine); it was one of a series of amines with a phenolic ether function. Its antihistaminic effects were described by Bovet and Staub (1937), who showed that 929 F effectively protected guinea pigs against several lethal doses of histamine, antagonized histamine-induced spasms of various smooth muscles and lessened the severity of the symptoms of anaphylactic shock. Two years later, Staub (1939) described the antihistaminic properties of N',N'-diethyl-N^2-phenyl-N^2-ethylethylenediamine (1571 F), which proved to be more active than any previously reported histamine antagonist. However, 929 F and 1571 F were of little clinical value because of their high toxicity. This early work of Staub and Bovet stimulated a great amount of research in the United States with the subsequent discovery of highly effective histamine antagonists, such as diphenhydramine (Benadryl®) by Loew et al. (1945), tripelennamine (Pyribenzamine®) by Mayer et al. (1945), and phenirамine (prophenpyridamine, Trimeton®) by LaBelle and Tislow (1948).

According to Loew (1947), antihistamines are capable of diminishing the following effects of histamine: (1) spasm of smooth muscle located in bronchioles, small intestine and uterus; (2) relaxation of arteriolar smooth muscle as demonstrated by a depressor response to histamine or increased vascularity of a region; (3) increased capillary permeability as indicated by wheal formation and (4) dilatation of capillaries and other vessels as demonstrated by localized flare

reactions. It is generally recognized, however, that antihistamine compounds show little or no effect against histamine-induced gastric secretion (Loew 1947). The diverse actions of histamine, which are antagonized by various antihistamine compounds, indicate a high degree of specificity and serve as a basis for an explanation of antihistamine activity.

A. ANTAGONISM OF HISTAMINE-INDUCED SMOOTH MUSCLE SPASM

(1) Bronchiospasm Usually, antihistamine drugs are selected and partially evaluated by determining their effectiveness in alleviating severe symptoms of, or preventing death as a result of, bronchiocon-striction. This condition is easily induced in guinea pigs with hist-amine, either administered or liberated during anaphylaxis. Friedlaender et al. (1946) expressed antihistamine activity by the number of doses of histamine (2.0 mg./Kg.) necessary to cause death in a guinea pig previously protected by a subcutaneous injection of 3 mg./Kg. of the test drug. They reported values of five doses for diphenhydramine and thirty-seven for tripelennamine. Using a slightly different technique, Winter (1947) calculated the dose of antihistamine necessary to permit survival of 50 percent of the guinea pigs receiving intravenous injec-tions of histamine dihydrochloride, 0.5 mg./Kg. He found that tri-pelennamine was about twelve times more effective than diphenhydramine. In an extensive discussion of the pharmacological properties of tri-pelennamine, diphenhydramine, and pheniramine, LaBelle and Tislow (1955) calculated the therapeutic index of each drug. (The therapeutic index is found by dividing the lethal dose of a drug in 50 percent of the animals (LD_{50}) by the effective dose of a drug in 50 percent of the animals (ED_{50}). In both the ED_{50} and LD_{50} studies in question, the

antihistamines were administered orally to guinea pigs, and in the ED_{50} study, the drug was given one hour prior to an intravenous injection of histamine dihydrochloride (1.1 mg./Kg.). These investigations showed that pheniramine had the highest therapeutic index, a value of 170 (LD_{50} 277 mg./Kg.; ED_{50} 1.63 mg./Kg.); diphenhydramine was next with a therapeutic index of 70 (LD_{50} 284 mg./Kg.; ED_{50} 4 mg./Kg.), followed by tripelennamine with a therapeutic index of 60 (LD_{50} 155 mg./Kg.; ED_{50} 2.59 mg./Kg.).

A more specific index of bronchial spasm consists basically of determining the minimum effective dose (M.E.D.) of a drug which significantly reduces the mortality rate of guinea pigs exposed to a lethal dose of an atomized histamine solution. Using this method, Sherrod et al. (1947) reported the minimum effective dose of diphenhydramine and tripelennamine given intraperitoneally in doses of 1.5 mg./Kg. and 0.3 mg./Kg. respectively. Kaiser and Ichniowski (1956), reporting the ED_{50} of several antihistamines, also found tripelennamine to be significantly more active than diphenhydramine against histamine administered as an aerosol.

The minimum dose of histamine antagonist which will protect an animal against anaphylactic death has been used as a measure of antihistaminic activity. The anaphylactic test, in general, consists of sensitizing the animal with a foreign protein, administering varying doses of the test drug, and injecting the challenging dose of foreign protein. Friedlaender et al. (1946) and Sherrod et al. (1947) using diphenhydramine, and Arbesman et al. (1946) and Mayer (1946) working with tripelennamine, demonstrated antagonism of the anaphylactic reaction in guinea pigs. The prominent feature of anaphylaxis in

guinea pigs is bronchioconstriction. It was found that a dose of 11 mg./Kg. of pheniramine or tripelennamine was sufficient protection against anaphylactic death in guinea pigs, while the same dose of diphenhydramine was not (LaBelle and Tislow, 1955). When the concentration of antihistamine was reduced to 5.1 mg./Kg., no deaths were found with tripelennamine, but several deaths resulted with pheniramine or diphenhydramine.

It is of interest to note that comparatively larger amounts of antihistamines are needed to counteract anaphylaxis than are required to counteract histamine shock. Loew (1947) attempted to explain this phenomenon by assuming that histamine released during anaphylactic shock is in more intimate contact with the effector portion of the cell and is therefore more effective than administered histamine which must be carried by the circulation to the histamine-sensitive cells.

The most prominent feature of anaphylaxis in dogs is hypotension resulting from vasodilation and increased capillary permeability. Other symptoms are respiration irregularities, reaction of smooth muscle, and nervous disorders. Yonkman et al. (1945) reported that tripelennamine diminished hypotension and the other symptoms of anaphylaxis in dogs. An absence of mortality when horse serum was injected into twenty-two sensitized dogs pretreated with diphenhydramine was observed by Wells et al. (1947), who also noted that nine animals or 35 percent of twenty-six animals died.

(2) Intestinal and Uterine Spasm Most compounds prepared as potential histamine antagonists have been evaluated by determining the minimum amount which will relax a histamine-induced spasm of an isolated strip of guinea pig ileum suspended in Tyrode's solution. Loew et al.

(1946) studied histamine antagonism on intestinal muscle and found that a spasm induced with histamine diphosphate, 8×10^{-8} gm./ml., was reduced 75 to 100 percent by diphenhydramine, 2×10^{-8} gm./ml., whereas atropine was effective only in a higher concentration, about 1×10^{-6} gm./ml. In 1946, Mayer showed that a dilution of tripelennamine, 2×10^{-8} gm./ml., was sufficient to prevent the spasmogenic action of histamine, 1×10^{-7} gm./ml., and occasionally, 1×10^{-6} gm./ml. Tripelennamine was also shown to be only weakly antagonistic to the spasmogenic action of acetylcholine. LaBelle and Tislow (1955) were also interested in the anticholinergic activity of pheniramine and diphenhydramine. Their results showed that both drugs were very weak antagonists of acetylcholine, an indication that the antihistamines are significantly active only in the presence of histamine.

Schild presented a paper in 1947 which suggested the use of pA_x as a measure of drug antagonism in isolated tissue. He defined pA_x as the negative logarithm to the base ten of the molar concentration of an antagonistic drug which will reduce the effect of a multiple dose (x) of an active drug to that of a single dose. Thus, if diphenhydramine at a concentration of 10^{-7} molar in the bath fluid reduces the effect of two micrograms of histamine to that produced by one microgram of histamine, the pA_2 of diphenhydramine antagonism of histamine is 7.00. An advantage of using pA_x is that some indication of the nature of the antagonism can be seen by calculating the difference between pA_2 and pA_{10}. Marshall (1955) used Schild's method to determine the pA_2 and pA_{10} of diphenhydramine, tripelennamine, and pheniramine antagonism of histamine. His pA_2 values were: (1) diphenhydramine 8.14; (2) tripelennamine 9.00 and (3) pheniramine 7.82. He concluded from his data

that diphenhydramine and tripelennamine probably antagonize histamine by competitive inhibition, while pheniramine is definitely a competitive inhibitor. Later work by Van Rossum (1963), using a modification of Schild's technique, showed diphenhydramine to be a competitive histamine inhibitor with a pA_2 value of 7.70.

Sherrod et al. (1947), using anesthetized dogs, showed that a histamine-induced intestinal spasm was blocked by tripelennamine and diphenhydramine. The ability of these antihistamines to reduce smooth muscle tonus, or spontaneous motility, in intact dogs was also noted. They found that tripelennamine, when injected intravenously, stimulated intestinal and uterine activity, whereas diphenhydramine partially decreased tonus and motility of the intestine.

The demonstration of antihistamine antagonism of the oxytocic action of histamine on the uterus, has been very difficult, because many of the common antihistamines exhibit a spasmogenic action. According to Mayer (1946), tripelennamine administered alone has the ability to effect contraction of an isolated guinea pig uterus; however, tripelennamine will modify the contractions brought about by histamine to such an extent that antihistamine activity must be concluded. Sherrod et al. (1947) showed that tripelennamine, injected intravenously into anesthetized dogs, was capable of effecting contractions of the uterus, while the same dose of diphenhydramine failed to do so. Both drugs had the ability to antagonize the spasmotic effect of injected histamine.

From the experimental data concerning antihistamine action on smooth muscle, it may be concluded that antihistaminic drugs do not prevent spasms nor exert a prominent spasmolytic effect, except under

conditions in which histamine has produced increased tonus, hypermotility, or spasm. This type of evidence is often used as proof that antihistamines are specific antagonists of histamine and not general spasmolytics.

B. ANTAGONISM OF THE VASCULAR EFFECTS OF HISTAMINE

In most carnivores, the intravenous injection of small amounts of histamine precipitates a rapid, transient hypotension due primarily to arteriolar dilatation and some capillary dilatation. Sherrod et al. (1947), using anesthetized dogs, found that intravenous doses of 30 mg./Kg. of tripelennamine and diphenhydramine diminished the depressor action of histamine to the same degree (i.e., 55 percent). LaBelle and Tislow (1955) also used dogs and showed a depression of the effects of histamine in animals pretreated with 8 mg./Kg. of either diphenhydramine, tripelennamine or pheniramine. From the data, it would appear that these drugs possessed equal potency in regard to antagonizing the depressor effect of histamine in dogs even though their ability to antagonize the bronchioconstrictive action of histamine in guinea pigs varied significantly. The work of Loew et al. (1946) and Wells et al. (1946), however, showed that the percent inhibition of the depressor action of histamine varied only slightly when the dose of diphenhydramine was significantly varied.

This antagonism of the hypotensive effect of histamine was not attributable to an atropine-like action, since Sherrod et al. (1947) reported that tripelennamine was unable to diminish the depressor effects of acetylcholine. Loew et al. (1946) demonstrated that intravenous injections of diphenhydramine decreased the depressor effect of histamine and were also effective in reducing but not abolishing

the depressor effect of acetylcholine. The anticholinergic action of diphenhydramine was shown to be relatively weak, since it required only 1/100 as much atropine to duplicate the effect.

When the antihistamine drugs are injected intravenously, they cause only slight effects in normal blood pressure. Cats which had been injected intravenously with tripelennamine in doses of 10 to 200 micrograms showed only minor fluctuations in blood pressure after the smaller doses, and a slight sustained rise after the larger doses (Yonkman et al. (1946). Loew et al. (1946) reported a 6 to 10 percent drop in blood pressure for about one minute, followed by an 8 to 10 percent increase in blood pressure lasting three to eight minutes in dogs injected with low doses of diphenhydramine. When the dose of diphenhydramine was increased to 3 mg./Kg., the diphasic alterations in blood pressure were similar but of greater magnitude. According to LaBelle and Tislow (1955), pheniramine administered at a rate faster than 6 mg./minute produced a transient fall in blood pressure, followed by a rise in blood pressure in dogs. Neither the pressor nor the depressor responses of the antihistamine drugs were of sufficient degree or duration to suggest a relationship to their antagonism of histamine, which lasted for one to two hours.

C. ANTAGONISM OF LOCALIZED CUTANEOUS RESPONSES TO HISTAMINE

The experimental studies concerning antihistamine antagonism as related to capillary dilatation and increased capillary permeability are almost wholly concerned with cutaneous responses to histamine. An intradermal injection of histamine causes a localized edema or wheal formation, which is mainly the result of increased capillary permeability independent of nervous factors. The accompanying flare,

however, is mainly dependent on vasodilation produced through reflex action. Loew (1950) felt that any analysis of the wheal and flare responses, as influenced by antihistamine drugs, must include consideration of influences on increased capillary permeability, vasoconstriction, vasodilation and the nervous factors related to the flare.

In the preceding section, it was observed that tripelennamine, diphenhydramine, and pheniramine did not produce a significant vasodilator or vasoconstrictor effect in intact animals. It therefore seems unlikely that changes in vascularity induced by antihistamines would account for a decrease of the wheal and flare response to histamine. However, Haley and Harris (1949) found some interesting results in their experiments on the capillary effects of antihistamines. They studied the effects of antihistamines topically applied to rat mesoappendix, and their results showed that tripelennamine at the concentration of 0.01 moles, pheniramine at 0.01 moles, and diphenhydramine at 0.001 moles all produced some vasoconstriction. They felt this vasoconstrictor response might in part account for antihistamine antagonism of histamine-induced capillary dilatation.

Diphenhydramine (Leavitt and Code, 1947), tripelennamine (Mayer, 1946), and pheniramine (LaBelle and Tislow, 1955) have been shown to possess some local anesthetic capabilities. The local anesthetic properties of all three antihistamines were studied by LaBelle and Tislow (1955) by means of the blink reflex in guinea pigs. They found diphenhydramine to be the best local anesthetic of the three. Tripelennamine and pheniramine exhibited some local anesthetic properties, but to a lesser degree than diphenhydramine. In a clinical study involving over 2000

patients, Fitzpatrick and Orr (1955) showed that a 2 percent solution of tripelennamine was a safe and potent urethral anesthetic. Very few failures of anesthetic activity were recorded. Therefore, it would seem that injection in localized areas or topical application of suitable quantities of these antihistamines could affect cutaneous reactions by means of a local anesthetic action.

Experiments by Leavitt and Code (1947) demonstrated that both diphenhydramine and procaine given intradermally in man definitely diminished cutaneous responses to histamine. However, after the local anesthetic effect of both drugs had subsided, some other action of diphenhydramine persisted and diminished the cutaneous reaction to histamine. It therefore seemed likely that the local anesthetic activity of diphenhydramine only partially accounted for its inhibitory influence on cutaneous reaction to histamine. Aaron and Abramson (1947) introduced tripelennamine and procaine into human skin by iontophoresis one and one-half hours before introducing histamine. Tripelennamine inhibited the histamine wheal and flare reaction, but procaine did not. This indicated that the action of tripelennamine was a result of antagonism of histamine and not a local anesthetic action.

The oral administration of diphenhydramine by Friedlaender and Feinberg (1946) and of tripelennamine by Arbesman et al. (1946) to humans frequently diminished the effects of intracutaneous injections of histamine. The highest incidence and degree of supression of cutaneous responses to histamine occurred when threshold doses of histamine were injected in subjects treated with antihistamine drugs.

D. CENTRAL NERVOUS SYSTEM EFFECTS

The antihistaminic drugs have been used clinically to relieve the

discomforts of seasonal hay fever, perennial vasomotor rhinitis, asthma, urticarial dermatoses, allergic dermatitis, and serum reactions. Feinberg (1950), in a review of the clinical uses of antihistamines, pointed out that these drugs were capable of symptomatic relief only and did nothing to relieve the cause of the abnormality. The clinical usefulness of these compounds varied with patients, depending on their susceptibility to the drug's side effects. One of the most important side effects of antihistamine drugs is their action on the central nervous system. They can cause stimulation resulting in restlessness, nervousness, or insomnia, but most frequently they cause depression resulting in sedation. Tripelennamine, pheniramine, and especially diphenhydramine are capable of producing somnolence in some persons. This condition seems to be the result of some type of depression of the central nervous system, independent of their histamine antagonism.

Loew et al. (1946), in his original study of the pharmacological properties of diphenhydramine, observed no drowsiness in dogs which had received 10 mg./Kg. subcutaneously. Winter et al. (1948) obtained similar results in mice, rats, dogs, guinea pigs and monkeys. When diphenhydramine was given to mice in sublethal amounts, Graham (1947) observed powerful central excitement and incoordination. LaBelle and Tislow (1955), studying the effects of pheniramine, found that oral doses of about 12 mg./Kg. produced slight drowsiness in dogs. When the dose was increased, they noted anorexia, decreased and slower responses, tremors of the head, apprehension, hypersensitivity and jumpiness. The dogs often lost their ability to stand and frequently had mild convulsions. The symptoms leading to their deaths were alike. The animals suddenly went into tonic-clonic convulsions with opistho-

tonus, while their bodies were in tremor. LaBelle and Tislow noted that the main symptoms of death pointed to involvement of the central nervous system. Mayer et al. (1946) observed no drowsiness as a result of low doses of tripelennamine, but when given in toxic amounts, it caused the animals to become excited and convulsive.

The measurement of drowsiness in experimental animals is very difficult, if not impossible, to quantify. Such is not the case with humans, because they have the advantage of being able to explain their condition to the investigator. Beck (1947) made note of the common side effects in patients receiving diphenhydramine or tripelennamine. The most common side effects were depression of the C.N.S. resulting in drowsiness, lassitude and inability to concentrate. Pearson (1957) made a study of the psychomotor effect of diphenhydramine on air force trainees. He found that subjects who received 50 mg. of the drug were unable to perform a practiced test as well as did placebo control subjects. Pearson concluded that the effects of diphenhydramine were such that they should not be used in circumstances where perceptual motor skills are required.

Numerous workers have tried to study the C.N.S. actions of the antihistamines with varying degress of success. Winter (1948) studied the effects of diphenhydramine and tripelennamine on the sedative action of hexobarbital in mice. He found that the mean waking time was prolonged about 10 percent by tripelennamine and about 40 percent by diphenhydramine. He concluded that whatever potentially sedative effect antihistamines might have in animals was masked by a coexisting excitatory effect, and that, in low doses, the two effects might cancel each other. In 1952, Way and Herbert reported that sodium pentobarbital

increased the intraperitoneal LD_{50} of tripelennamine and diphenhydramine in rats. It did not affect the death rate significantly in animals overdosed orally with diphenhydramine and tripelennamine, although convulsions were less severe when preceded by the barbiturate. They also reported that when the animals were injected with a low dose of sodium pentobarbital (30 mg./Kg.) as an antidote, they died from convulsions, whereas a higher dose (60 mg./Kg.) caused death from respiratory depression. To explain the difference in causes of death, Way and Herbert suggested that the degradation products of the antihistamines, which are present in greater amounts after oral administration, may enhance the actions of pentobarbital and contribute to its overall toxicity.

Lightstone and Nelson (1954) showed that diphenhydramine and tripelennamine significantly prolonged pentobarbital sleeping time in rats. In an attempt to explain this effect, they suggested that the drugs did not seem to interfere with liver detoxification of pentobarbital. When there was a high enough plasma concentration of the barbiturate, it appeared that diphenhydramine caused an increase in the rate of entrance of pentobarbital into the brain. They concluded that the mechanism by which diphenhydramine and tripelennamine prolonged pentobarbital sleeping time was independent of their common side effects.

Another method used to determine the C.N.S. effects of the antihistamines is the use of trained animals. Winter and Flataker (1951) trained rats to climb a vertical rope. The climbing time was noted, as well as the behavior of the animals. In general, diphenhydramine and tripelennamine gave evidence of cerebral depression and muscular incoordination resulting in failure or prolongation in the climbing

time of the trained rats. Tripelennamine seemed to have a more pronounced effect on the behavior of the trained rats than that of diphenhydramine. The depressant effects of diphenhydramine and tripelennamine were counteracted by the administration of amphetamine or caffeine, which suggested to the investigators that the site of action was probably the cerebral cortex.

In order to gain more information about the effects of antihistamines in the C.N.S., the locomotor activity of rats injected with tripelennamine and diphenhydramine has been studied. Heinrich (1951) found that administration of diphenhydramine, 22.8 mg./Kg./day, or tripelennamine, 21.6 mg./Kg./day, in the diet had no effect on the spontaneous running of adult rats in activity cages. A more complete study was made by Baird and Boyd (1954) who placed drug-treated rats in Wahman vertically-revolving drums and recorded locomotor activity. They administered tripelennamine orally and subcutaneously in amounts from 1 mg./Kg. to lethal doses and found that tripelennamine had a statistically insignificant effect on locomotor activity. They also found that subcutaneous injections of diphenhydramine in doses up to 25 mg./Kg. had no effect on locomotor activity. When they gave diphenhydramine orally in doses of 100 and 200 mg./Kg., significant increases in activity were noted. Doses of 200 mg./Kg. caused convulsions and death in 80 percent of the animals. Therefore, Baird and Boyd concluded that diphenhydramine in toxic doses stimulated the C.N.S. and caused an increase in locomotor activity.

It can be said that all antihistamines possess, in varying degrees, the pharmacological properties discussed in the preceding pages. They all have the ability to antagonize most of the pharmacological actions

of histamine. Antihistamines also reduce the intensity of allergic and anaphylactic reactions, and it is this property that serves as the basis for their major therapeutic applications. The C.N.S. effects of antihistamines have proven to be interesting and somewhat baffling to many scientists. It was primarily the ability of antihistamines to affect the C.N.S. that led to the synthesis of the trimethoxy analogs of tripelennamine, diphenhydramine, and pheniramine, which are discussed in the following pages.

III. INVESTIGATION

A. OBJECTIVES

This study was concerned with the determination of some of the pharmacological properties of trimethoxyphenyl analogs of tripelennamine, diphenhydramine, and pheniramine. The experimental compounds were studied by observing their effects on the following parameters: (1) antagonism of histamine-induced contractions of isolated guinea pig ileum; (2) systolic blood pressure of male albino rats: and (3) exploratory activity of male albino mice.

The ultimate goal of this investigation was to provide information concerning the activity and the mechanisms of action of the parent compounds and their analogs, thus enabling the pharmaceutical chemist to more accurately predict the effects of trimethoxyphenyl substitutions on known compounds.

B. MATERIALS AND METHODS

(1) Compounds The experimental compounds were synthesized by Dr. L.D. DiFazio (1963), Department of Pharmaceutical Chemistry, University of Rhode Island. Glass distilled water was used as the solvent throughout the study since the synthesized compounds, as well as the parent compounds, diphenhydramine hydrochloride (Benadryl ®)[1], pheniramine maleate (prophenpyridamine, Trimeton ®)[2], and tripelen-

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[1] Kindly supplied by Dr. A.C. Bratton, Parke-Davis Laboratories, Ann Arbor, Michigan

[2] Kindly supplied by Dr. R.E. Thompson, Schering Laboratories, Bloomfield, New Jersey

namine hydrochloride (Pyribenzamine®)[1], were all available in the form of water soluble salts.

The trimethoxyphenyl analogs of tripelennamine are listed below.

a. N,N-Dimethyl-N'-(2-pyridyl)-N'-(3,4,5-trimethoxybenzyl) ethylenediamine Disuccinate (TMPBZ),

b. β-(4-Morpholino)-N-(2-pyridyl)-N-(3,4,5-trimethoxybenzyl) ethylamine Dicyclamate (Mor-TMPBZ),

c. N,N,-Diethyl-N'-(2-pyridyl)-N'-(3,4,5-trimethoxybenzyl) ethylenediamine Dicyclamate (DE-TMPBZ),

d. β-(1-Piperidino)-N-(2-pyridyl)-N-(3,4,5-trimethoxybenzyl) ethylamine Dicyclamate (Pip-TMPBZ),

e. β-(1-Pyrrolidino)-N-(2-pyridyl)-N-(3,4,5-trimethoxybenzyl) ethylamine Dicyclamate (Pyl-TMPBZ).

The trimethoxyphenyl analog of diphenhydramine is 2-(3,4,5-trimethoxybenzhydryloxy)-N,N-dimethylethylamine Succinate (TMBD). The trimethoxyphenyl analogs of pheniramine are:

a. 1-(2-pyridyl)-1-(3,4,5-trimethoxyphenyl)-3-dimethylamino-propane Cyclamate (TMT),

b. ω-Dimethylamino-3,4,5-trimethoxypropiophenone Hydrochloride (Dma-TMPP).

The structural formulas of all the above compounds are shown in Figures I-III.

(2) Isolated Tissue Study The ability of the parent compounds and their analogs to antagonize histamine-induced contractions of intestinal smooth muscle was studied on isolated guinea pig ileum. Each guinea pig[2] was deprived of food twenty-four hours before being sacrificed. The ileum was removed and a section 1.5-2.0 centimeters

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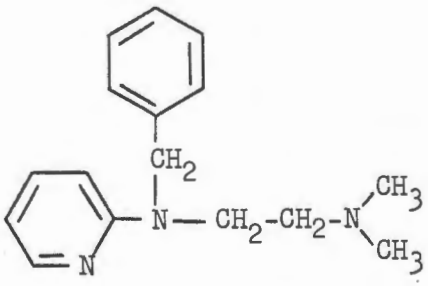
[1] Kindly supplied by Dr. J. Cooper, CIBA Laboratories, Summit, New Jersey

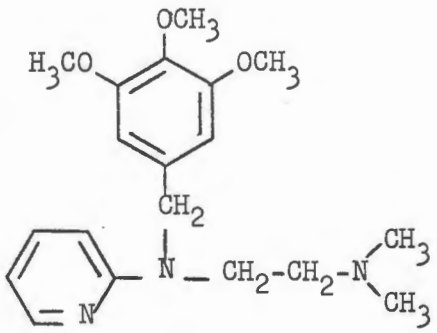
[2] Purchased from Albino Farms, Red Bank, New Jersey

FIGURE 1

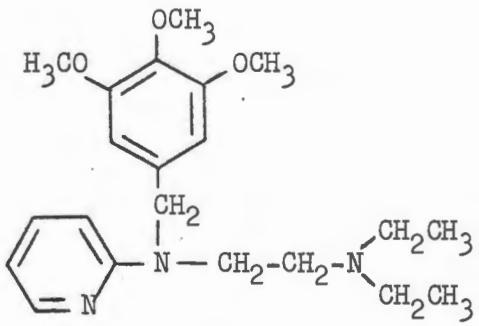
TRIPELENNAMINE AND ITS TRIMETHOXY ANALOGS



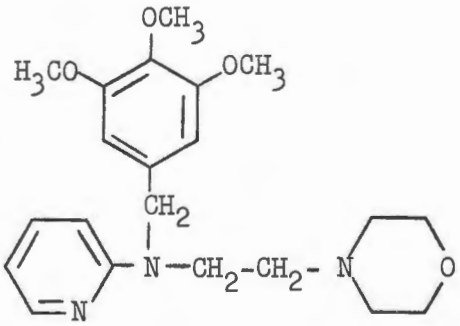
TRIPELENNAMINE



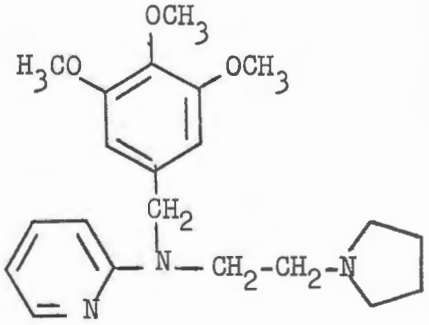
TMPBZ



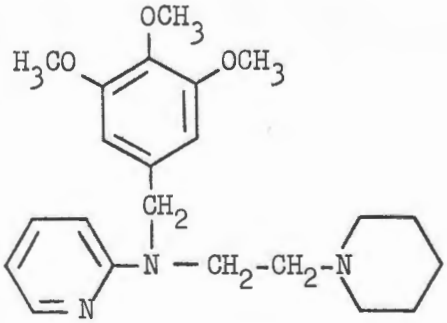
DE-TMPBZ



MOR-TMPBZ



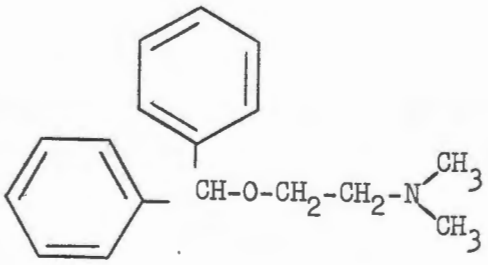
PYL-TMPBZ



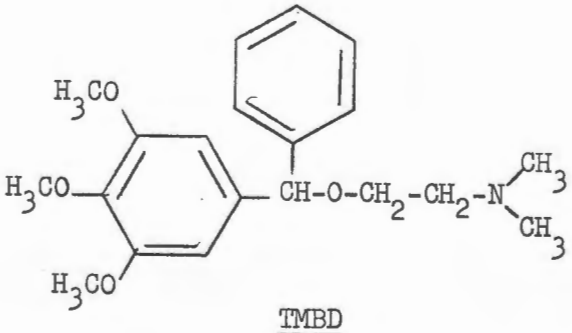
PIP-TMPBZ

FIGURE 2

DIPHENHYDRAMINE AND ITS TRIMETHOXY ANALOG



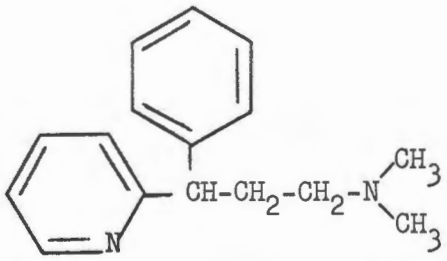
DIPHENHYDRAMINE



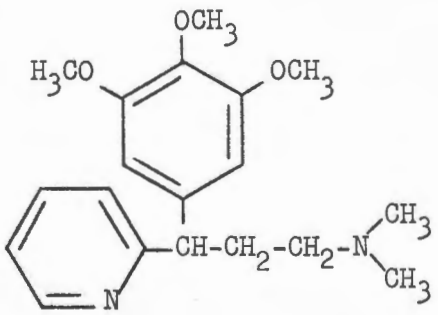
TMBD

FIGURE 3

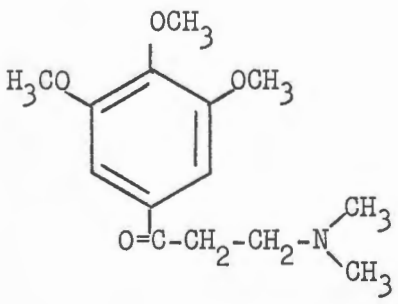
PHENIRAMINE AND ITS TRIMETHOXY ANALOGS



PHENIRAMINE



TMT



DMA-TMPP

was suspended in a tissue bath containing thirty milliliters of aerated Tyrode's solution. The Tyrode's solution was maintained at 37° centigrade by suspending the entire tissue bath in a thermostatically-regulated water bath[1]. The contractions of the tissue were recorded on a slowly-moving sooted kymograph by means of an isotonic writing lever.

TABLE 1

SEQUENCE OF HISTAMINE DOSES ADDED FOR CUMULATIVE DOSE-RESPONSE CURVES

The amount to be added to the tissue bath		Total molar concentration in the tissue bath
Ml.	Molar Concentration	X 10^{-8}
0.03	10^{-5}	1
0.06	10^{-5}	3
0.21	10^{-5}	10
0.06	10^{-4}	30
0.21	10^{-4}	100
0.06	10^{-3}	300
0.21	10^{-3}	1000
etc.		etc.

Cumulative dose-response curves, as described by Van Rossum and Van Den Brink (1963), were made with each experimental compound. To make these curves, increasing amounts of histamine diphosphate were added to the tissue bath until a maximum contraction was effected. A contraction was considered maximum when subsequent doses of histamine

[1] Precision Scientific Company, Chicago, Illinois

resulted in no further contraction of the ileum (Figure 4). The histamine was added to the bath in a stepwise sequence of one-half $\log_{10}$ intervals (Table 1). Whenever possible, the total amount of added solution was kept below 1.5 milliliters (i.e., 5 percent of the tissue bath volume).

In order to observe the antagonistic ability of the antihistamines and their analogs, it was necessary to make dose-response curves of histamine alone, as well as dose-response curves of histamine in the presence of the antagonists (Figures 4 and 6). The experimental drugs were allowed to equilibrate for two minutes in the tissue bath before histamine was added. Each drug was tested at three dose levels on three different guinea pig ilia.

The dose-response curves were evaluated by measuring the contractions in millimeters produced by each cumulative dose of histamine and expressing it as a percent of the maximum height of the completed curve. The percentages were plotted on a linear scale as millimeters on the ordinate (100 percent equal 100 millimeters) and the logarithms of the doses were plotted on the abcissa, using thirty millimeters for one $\log_{10}$ interval (Figures 5 and 7). The theory and method used to evaluate the dose-response curves is described by Van Rossum (1963) and is briefly discussed below.

a. Evaluation of Histamine Cumulative Dose-Response Curves

Histamine is a stimulant drug and is therefore referred to as an agonist. It is generally believed that agonists produce a stimulus as a result of receptor occupation and in this way produce an effect. Stimulant drugs have both an affinity for the receptor site and an intrinsic activity, and are characterized mainly by these properties.

The affinity of the agonist for the receptor site is often used as an estimate of drug potency and is expressed as a pD_2 value. pD_2 is the negative logarithm of the molar dose necessary to produce a response that is 50 percent of the maximum effect. In order to find the pD_2 values of histamine, each dose-response curve was plotted according to the previously described procedure. The logarithm of the molar dose of histamine required to induce a 50 percent response was then read from the graph and converted to its negative logarithm.

b. Evaluation of Competitive Antagonists Competitive antagonists are inactive as such. They have an affinity for a specific receptor site but have no intrinisic activity; therefore, they do not have the ability to generate a stimulus. In order to study the effects of a competitive antagonist, it must be combined with a stimulant drug (i.e., an agonist). A dose-response curve of the agonist in the presence of a given concentration of the competitive drug has a shape essentially identical with the curve when the antagonist is absent. When the competitive drug is present, a 50 percent response can be reached only with a higher concentration of the agonist; therefore, the curves are shifted to the right on the log dose-axis (Figures 5 and 7). This shift (x), is equal to the concentration of the agonist in the presence of the antagonist (A_B) divided by the concentration of the agonist in the absence of the antagonist, (A_o).

$$x = A_B/A_o$$

The value of x can be used as an estimate of drug potency, which, in turn, is related to the affinity of the antagonist for the receptor site. The affinity of the antagonist is expressed as the negative logarithm of the molar concentration that caused the shift x, and is

designated pA_x. The pA_x value is then converted to a pA_2 value, which is the negative logarithm of the molar concentration of the antagonist that will cause a shift of a factor 2. The relationship between pA_x and pA_2 is:

$$pA_2 = pA_x + \log(x-1)$$

The experimental procedure for competitive antagonists consisted of alternating histamine dose-response curves with dose-response curves of histamine in the presence of the experimental drug (Figures 4 and 6). The logarithms of the molar concentrations of histamine used to complete each dose-response curve were then plotted according to the previously described procedure. A_o and A_B were read from these graphs and x and pA_x were calculated. Once x and pA_x were known, pA_2 was easily determined.

c. Evaluation of Non-Competitive Antagonists Non-competitive antagonists, like competitive antagonists, are inactive as such. These drugs do not have an affinity for the specific receptors of the agonist but interact with different receptors and, thereby, influence stimulus formation or stimulus effectuation. It can be seen from Figures 8 and 10, that the main action of the non-competitive antagonist is a depression of the dose-response curve of the agonist. This depression can be used to estimate the affinity of the antagonist for its receptor site, and is also a measure of the potency of the drug. The negative logarithm of the molar concentration that causes a depression (x) of the dose-response curve is called pD'_x. pD'_x is always converted to pD'_2, which is the negative logarithm of the molar concentration of the non-competitive antagonist that causes a depression of 50 percent.

The relationship of pD'_x to pD'_2 is:

$$pD'_2 = pD'_x + \log(x-1)$$

In this study, the pD'_2 values were found by dividing the maximum height of the histamine dose-response curve in the presence of the antagonist ($E_{AmB'}$) by the maximum height of the histamine dose-response curve in the absence of the antagonist (E_{Am}). By knowing this ratio, it was possible to calculate x and pD'_x since:

$$\frac{E_{AmB'}}{E_{Am}} = \frac{100}{x}$$

pD'_2 was then calculated by following the proper formula.

(3) Blood Pressure Study The effects of diphenhydramine, tripelennamine, pheniramine, and their trimethoxyphenyl analogs were measured on systolic blood pressure by cannulation of the carotid artery. Male albino rats of the Sprague-Dawley strain[1] weighing 222-293 grams were used. The recording system consisted of a mercury manometer connected to a glass cannula via a fluid bridge of 0.9 percent saline. The blood pressure fluctuations were recorded on a slowly-moving sooted kymograph. All the animals used in this study were deprived of food for twenty-four hours before they were anesthetized with 1.1 gm./Kg. of urethan, injected intraperitoneally. To prevent the blood from clotting in the cannula, 0.1 milliliters of heparin sodium, 1:1000, was put into the tip of the cannula.

All the compounds studied were dissolved in glass distilled water and were injected intravenously via the femoral vein. The doses of the drugs were calculated on a molar basis in order to allow for a more

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[1] Charles River Breeding Farm, North Wilmington, Massachusetts

accurate comparison of the parent compounds to their analogs. Each rat was given only on injection of a drug before it was discarded.

After a compound was injected, the duration and degree of any change in the blood pressure was recorded. All drug-induced increases or decreases in blood pressure were measured as mmHg and were reported as a percent change from the normal blood pressure. The duration of a response was measured in minutes and seconds using a Franz Kymo Timer[1]. The response was considered ended when the blood pressure returned to normal, ± 5 mm Hg.

(4) Activity Study Male albino Charles River[2] mice weighing 18-24 grams were used for the activity study. The animals were housed in a soundproof, windowless room maintained at about 50 percent relative humidity with a constant temperature of 20° ± 1° centigrade. The room was artificially lighted daily from 7:00 a.m. to 6:00 p.m. Each animal was fed four grams of Purina Rat Chow[3] daily at 3:30 p.m. and had unlimited access to water. All the mice were allowed to acclimate to the room and feeding schedule for ten days before the study began.

Four multibeam actophotometers[4], located in the above described room, were employed to determine the effect of tripelennamine, pheniramine, Mor-TMPBZ, and Dma-TMPP on the exploratory activity of mice.

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[1]Franz Manufacturing Company, New Haven, Connecticut

[2]Charles River Breeding Farm, North Wilmington, Massachusetts

[3]Ralston Purina, St. Louis, Missouri

[4]Metro Scientific, Inc., Carle Plase, Long Island City, New York

The actophotometer consists of a circular cage with a grid floor. Twelve equally spaced holes are located about one centimeter from the grid floor around the inside diameter of the cage. From these holes, six light beams radiate through a red filter and across the cage to activate their respective photoelectric cell. When a light beam is broken, it causes a digital counter located in an adjacent room to advance one unit. In this manner, it is possible to obtain an estimate of exploratory activity per unit time.

The light beams and photocells were adjusted for maximum sensitivity by varying the angle of incidence of light to the photocell and by adjusting the voltage to the counter relay and photocell. Mendillo (1965) found that each cage had its own sensitivity characteristics, resulting in varying total counts per unit time from one cage to another. Therefore, the experiment was designed so that data from both vehicle-treated and drug-treated animals were obtained from the same cages.

Table 2 outlines the experimental design used in this study and represents one run. In each run, three groups of mice were used with four mice per cage. As each animal was used only once, a total of forty-eight mice were used in one run. The entire study consisted of six runs, which were conducted on three consecutive days between the hours of 9:00 a.m. and 3:00 p.m. Following injection with a drug or vehicle, ten minutes was allowed for systemic absorption. The animals were then placed in their respective activity cages. Transferring the mice and turning on the counters took approximately thirty seconds. Total counts per cage for thirty minutes as well as incremental counts per ten minutes and fifteen minutes were recorded.

TABLE 2

EXPERIMENTAL DESIGN OF THE ACTIVITY STUDY

CAGE	I	II	III	IV
Group I	Vehicle-Treated	Vehicle-Treated	Vehicle-Treated	Vehicle-Treated
Group II	Vehicle-Treated	Pyribenzamine 7.6×10^{-2}M/Kg.	Mor-TMPBZ 7.6×10^{-2}M/Kg.	Mor-TMPBZ 1.52×10^{-1}M/Kg.
Group III	Vehicle-Treated	Pheniramine 1×10^{-1}M/Kg.	Dma-TMPP 1×10^{-1}M/Kg.	Dma-TMPP 2×10^{-1}M/Kg.

The vehicle-treated animals received an intraperitoneal injection of 0.2 ml. of glass-distilled water.

All drugs were dissolved in glass-disstilled water and were injected intraperitoneally in an approximate volume of 0.2 ml.

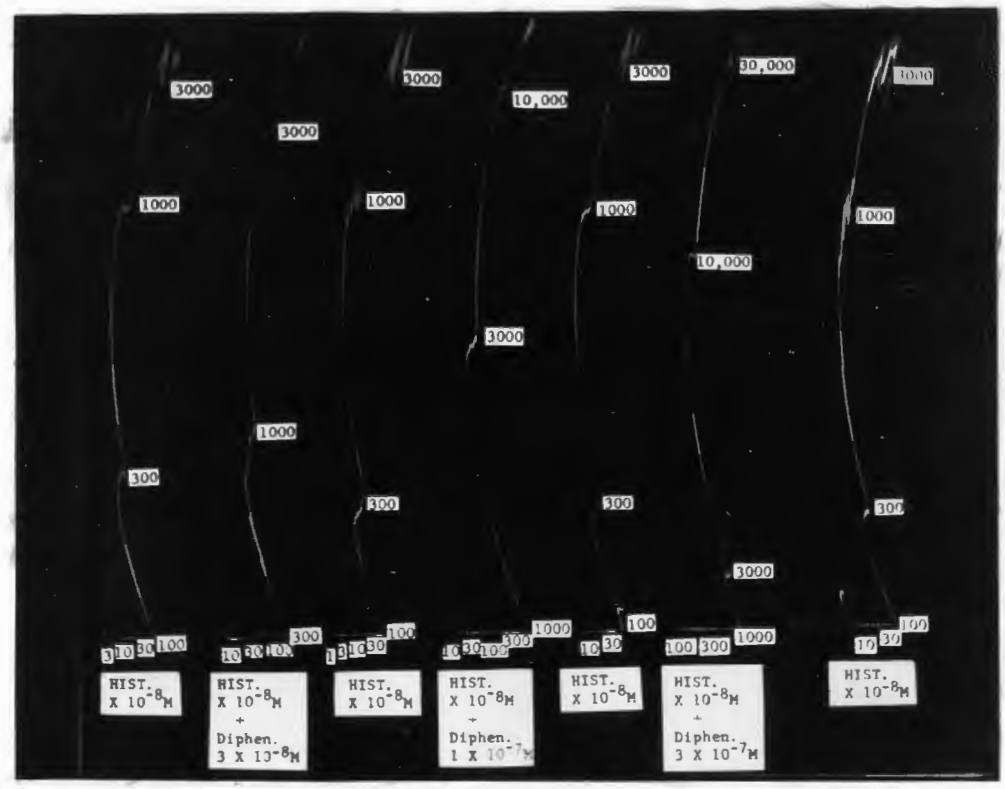
The data were analyzed by comparing the counts per unit time from the drug-treated animals to their respective controls. The comparisons were evaluated by calculating the student's "t" value (Snedecor 1956). The following comparisons per unit time were made: (1) incremental counts per ten minutes; (2) incremental counts per fifteen minutes; and (3) total counts per thirty minutes.

IV. RESULTS

All experimental results are contained in this section.

FIGURE 4

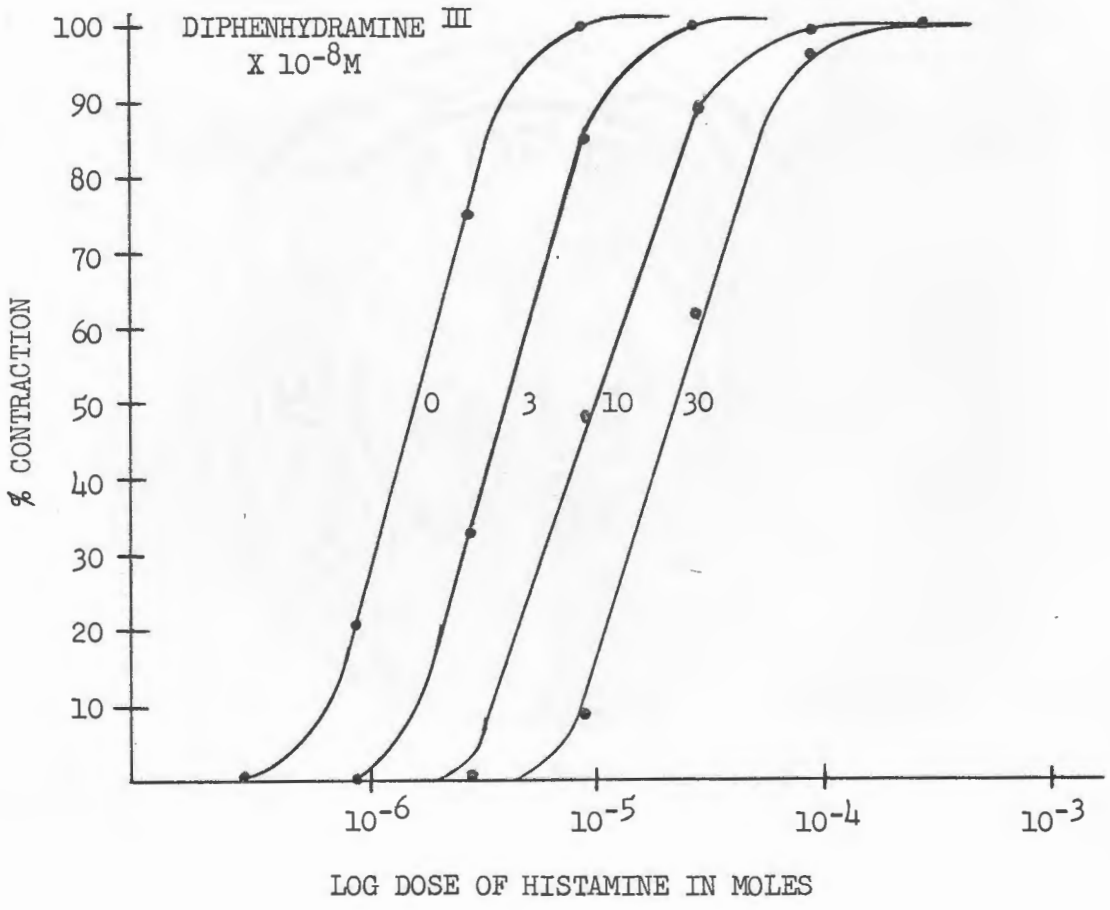
CUMULATIVE DOSE-RESPONSE CURVES OF HISTAMINE ON THE GUINEA PIG ILEUM IN THE PRESENCE AND ABSENCE OF DIPHENHYDRAMINE



Note that as the concentration of diphenhydramine (a known competitive antagonist) is increased, a higher dose of histamine is needed to reach the same level.

FIGURE 5

CUMULATIVE DOSE-RESPONSE CURVES AS CALCULATED FROM FIGURE 4



Note the progressive shift of the dose-response curves as induced by the competitive antagonist. From this shift, the drug's affinity toward the receptor site can be calculated as a pA_2 value.

TABLE 3

DIPHENHYDRAMINE HYDROCHLORIDE ANTAGONISM TO HISTAMINE-INDUCED CONTRACTIONS OF ISOLATED GUINEA PIG ILEUM

EXPERIMENT	RESPONSE TO AGONIST				RESPONSE TO AGONIST PLUS ANTAGONIST					
	NO.	MAX. RESP. (mm)	A_o	pD_2	NO.	B. 10^{-q}	MAX. RESP. (mm)	pA_x	A_B	pA_2
Diphen. I	6	121	5.4×10^{-5}	5.73	7	10×10^{-8}	120	7.00	4.4×10^{-6}	7.86
	8	118	4.2×10^{-5}	5.62	7	10×10^{-8}	120	7.00	2.7×10^{-6}	7.73
	8	118	4.2×10^{-5}	5.62	9	30×10^{-8}	116	6.50	9.4×10^{-6}	7.83
	10	119	3.2×10^{-5}	5.50	9	30×10^{-8}	116	6.50	5.5×10^{-6}	7.71
	1	113	3.4×10^{-5}	5.53	2	3×10^{-8}	115	7.50	4.8×10^{-5}	7.56
	3	109	4.2×10^{-5}	5.62	2	3×10^{-8}	115	7.50	1.1×10^{-6}	7.71
Diphen. II	5	130	5.3×10^{-5}	5.72	6	3×10^{-8}	130	7.50	1.5×10^{-6}	7.76
	7	129	4.8×10^{-5}	5.68	6	3×10^{-8}	130	7.50	1.3×10^{-6}	7.71
	7	129	4.8×10^{-5}	5.68	8	10×10^{-8}	128	7.00	3.4×10^{-6}	7.78
	9	122	5.4×10^{-5}	5.73	8	10×10^{-8}	128	7.00	4.3×10^{-6}	7.84
	9	122	5.4×10^{-5}	5.73	10	30×10^{-8}	122	6.50	2.6×10^{-6}	8.17
	11	112	4.5×10^{-5}	5.65	10	30×10^{-8}	122	6.50	1.8×10^{-7}	8.09

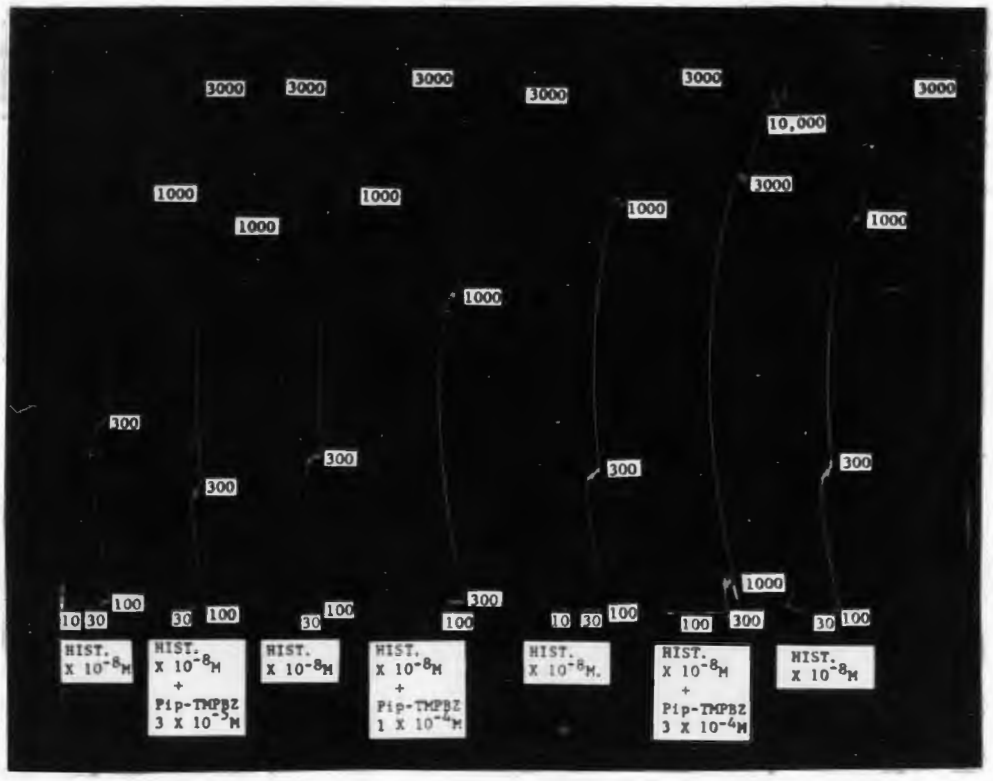
TABLE 3 - Continued

EXPERIMENT	RESPONSE TO AGONIST				RESPONSE TO AGONIST PLUS ANTAGONIST					
	NO.	MAX. RESP. (mm)	A_o	pD_2	NO.	B. 10^{-q}	MAX. RESP. (mm)	pA_x	A_B	pA_2
Diphen. III	6	144	5.4×10^{-5}	5.73	7	3×10^{-8}	147	7.50	1.4×10^{-6}	7.68
	8	146	5.4×10^{-5}	5.73	7	3×10^{-8}	147	7.50	1.4×10^{-6}	7.68
	8	146	5.4×10^{-5}	5.73	9	10×10^{-8}	148	7.00	3.1×10^{-6}	7.67
	10	147	5.0×10^{-5}	5.70	9	10×10^{-8}	148	7.00	2.6×10^{-6}	7.62
	10	147	5.0×10^{-5}	5.70	11	30×10^{-8}	145	6.50	2.0×10^{-7}	7.56
	12	142	5.3×10^{-5}	5.72	11	30×10^{-8}	145	6.50	7.5×10^{-6}	7.62

NO. = The number of the curve being measured.
A_o = The molar dose of the agonist to produce a 50% response.
pD_2 = The negative (-) log of the dose necessary to produce a 50% response.
B. 10^{-q} = The molar concentration of the antagonist.
pA_x = The negative (-) log of the molar dose of the antagonist needed to cause a shift x;
$pA_x = (q - \log B)$.
A_B = The molar dose of the agonist which produces a 50% response in the presence of the antagonist.
pA_2 = The negative (-) log of the molar concentration of antagonist which causes a shift of a factor 2;
$pA_2 = pA_x + \log(x-1)$; $x = A_o/A_B$.
Diphen. = Diphenhydramine hydrochloride.

FIGURE 6

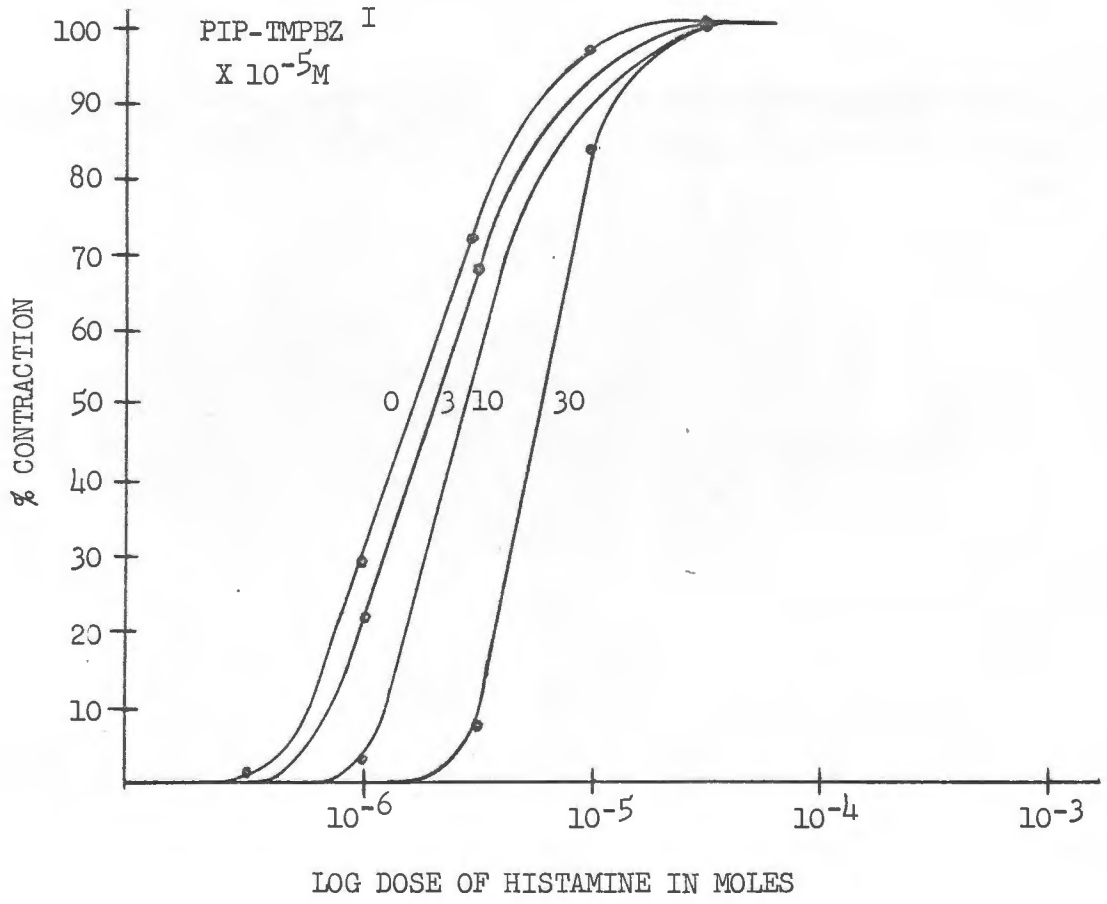
CUMULATIVE DOSE-RESPONSE CURVES OF HISTAMINE ON THE GUINEA PIG ILEUM IN THE PRESENCE AND ABSENCE OF PIP-TMPBZ



Note that as the concentration of Pip-TMPBZ is increased, a higher dose of histamine is needed to reach the same level.

FIGURE 7

CUMULATIVE DOSE-RESPONSE CURVES AS CALCULATED FROM FIGURE 6



Note the progressive shift of the dose-response curves as induced by the competitive antagonist. From this shift, the drug's affinity toward the receptor site can be calculated as a pA_2 value.

TABLE 4

PIP - TMPBZ ANTAGONISM TO HISTAMINE-INDUCED CONTRACTIONS OF ISOLATED GUINEA PIG ILEUM

EXPERIMENT	RESPONSE TO AGONIST				RESPONSE TO AGONIST PLUS ANTAGONIST					
	NO.	MAX. RESP. (mm)	A_o	pD_2	NO.	B. 10^{-q}	MAX. RESP. (mm)	pA_x	A_B	pA_2
Pip-TMPBZ I	1	132	6.3×10^{-5}	5.80	2	3×10^{-5}	132	4.50	8.2×10^{-5}	3.91
	3	134	5.9×10^{-5}	5.77	2	3×10^{-5}	132	4.50	7.1×10^{-5}	3.72
	3	134	5.9×10^{-5}	5.77	4	10×10^{-5}	131	4.00	1.0×10^{-6}	3.85
	5	134	5.6×10^{-5}	5.75	4	10×10^{-5}	131	4.00	9.5×10^{-5}	3.81
	5	134	5.6×10^{-5}	5.75	6	30×10^{-5}	122	3.50	2.0×10^{-6}	3.91
	7	132	5.4×10^{-5}	5.73	6	30×10^{-5}	122	3.50	1.8×10^{-6}	3.86
Pip-TMPBZ II	1	119	4.0×10^{-5}	5.60	2	10×10^{-5}	114	4.00	4.0×10^{-5}	2.59
	3	110	4.5×10^{-5}	5.65	2	10×10^{-5}	114	4.00	5.4×10^{-5}	3.22
	3	110	4.5×10^{-5}	5.65	4	30×10^{-5}	101	3.50	1.1×10^{-6}	3.68
	5	104	4.7×10^{-5}	5.67	4	30×10^{-5}	101	3.50	1.1×10^{-6}	3.65

TABLE 4 - Continued

EXPERIMENT	RESPONSE TO AGONIST				RESPONSE TO AGONIST PLUS ANTAGONIST					
	NO.	MAX. RESP. (mm)	A_o	pD_2	NO.	B. 10^{-q}	MAX. RESP. (mm)	pA_x	A_B	pA_2
Pip-TMPBZ III	9	134	3.0×10^{-5}	5.48	10	10×10^{-5}	130	4.00	3.3×10^{-5}	3.04
	11	134	3.4×10^{-5}	5.53	10	10×10^{-5}	130	4.00	4.4×10^{-5}	3.41
	11	134	3.4×10^{-5}	5.53	12	30×10^{-5}	118	3.50	6.8×10^{-5}	3.50
	13	130	3.0×10^{-5}	5.48	12	30×10^{-5}	118	3.50	5.4×10^{-5}	3.39

NO. = The number of the curve being measured.
A_o = The molar dose of the agonist to produce a 50% response.
pD_2 = The negative (-) log of the dose necessary to produce a 50% response.
B. 10^{-q} = The molar concentration of the antagonist.
pA_x = The negative (-) log of the molar dose of the antagonist needed to cause a shift x;
$pA_x = (q - \log B)$.
A_B = The molar dose of the agonist which produces a 50% response in the presence of the antagonist.
pA_2 = The negative (-) log of the molar concentration of antagonist which causes a shift of a factor 2;
$pA_2 = pA_x + \log(x-1)$; $x = A_o/A_B$.

TABLE 5

TRIPELENNAMINE HYDRCHLORIDE ANTAGONISM TO HISTAMINE-INDUCED

CONTRACTIONS OF ISOLATED GUINEA PIG ILEUM

	RESPONSE TO AGONIST				RESPONSE TO AGONIST PLUS ANTAGONIST					
EXPERIMENT	NO.	MAX. RESP. (mm)	A_o	pD_2	NO.	B. 10^{-q}	MAX. RESP. (mm)	pA_x	A_B	pA_2
Pyri. I	3	109	4.2×10^{-5}	5.62	4	10×10^{-9}	116	8.00	2.1×10^{-6}	8.60
	5	112	3.6×10^{-5}	5.55	4	10×10^{-9}	116	8.00	1.5×10^{-6}	8.52
	5	112	3.6×10^{-5}	5.55	6	30×10^{-9}	115	7.50	3.0×10^{-6}	8.36
	7	115	4.7×10^{-5}	5.67	6	30×10^{-9}	115	7.50	5.1×10^{-5}	8.49
	7	115	4.7×10^{-5}	5.67	8	3×10^{-9}	115	8.50	8.0×10^{-5}	8.31
	9	117	4.7×10^{-5}	5.67	8	3×10^{-9}	115	8.50	8.0×10^{-5}	8.31
Pyri. III	1	116	4.3×10^{-5}	5.63	2	3×10^{-9}	119	8.50	8.6×10^{-5}	8.50
	3	120	5.9×10^{-5}	5.77	2	3×10^{-9}	119	8.50	1.6×10^{-6}	8.27
	3	120	5.9×10^{-5}	5.77	4	10×10^{-9}	123	8.00	2.8×10^{-6}	8.58
	5	109	5.3×10^{-5}	5.72	4	10×10^{-9}	123	8.00	2.3×10^{-6}	8.52
	5	109	5.3×10^{-5}	5.72	6	30×10^{-9}	114	7.50	8.7×10^{-6}	8.69
	7	109	3.4×10^{-5}	5.53	6	30×10^{-9}	114	7.50	3.7×10^{-6}	8.49

TABLE 5 - Continued

EXPERIMENT	RESPONSE TO AGONIST				RESPONSE TO AGONIST PLUS ANTAGONIST					
	NO.	MAX. RESP. (mm)	A_o	pD_2	NO.	B. 10^{-q}	MAX. RESP. (mm)	pA_x	A_B	pA_2
Pyri. III	1	145	5.3×10^{-5}	5.72	2	3×10^{-9}	145	8.50	7.4×10^{-5}	8.12
	3	143	4.8×10^{-5}	5.68	2	3×10^{-9}	145	8.50	6.2×10^{-5}	7.99
	3	143	4.8×10^{-5}	5.68	4	10×10^{-9}	138	8.00	1.7×10^{-6}	8.41
	5	144	5.4×10^{-5}	5.73	4	10×10^{-9}	138	8.00	2.2×10^{-6}	8.47
	5	144	5.4×10^{-5}	5.73	6	30×10^{-9}	137	7.50	4.3×10^{-6}	8.34
	7	137	4.5×10^{-5}	5.65	6	30×10^{-9}	137	7.50	2.9×10^{-6}	8.24

NO. = The number of the curve being measured.
A_o = The molar dose of the agonist to produce a 50% response.
pD_2 = The negative (-) log of the dose necessary to produce a 50% response.
B. 10^{-q} = The molar concentration of the antagonist.
pA_x = The negative (-) log of the molar dose of the antagonist needed to cause a shift x;
$pA_x = (q - \log B)$.
A_B = The molar dose of the agonist which produces a 50% response in the presence of the antagonist.
pA_2 = The negative (-) log of the molar concentration of antagonist which causes a shift of a factor 2;
$pA_2 = pA_x + \log(x-1)$; $x = A_o/A_B$.
Pyri. = Tripelennamine hydrochloride.

TABLE 6

PHENIRAMINE MALEATE ANTAGONISM TO HISTAMINE-INDUCED CONTRACTIONS

OF ISOLATED GUINEA PIG ILEUM

EXPERIMENT	RESPONSE TO AGONIST				RESPONSE TO AGONIST PLUS ANTAGONIST					
	NO.	MAX. RESP. (mm)	A_o	pD_2	NO.	B. 10^{-q}	MAX. RESP. (mm)	pA_x	A_B	pA_2
Phen. I	1	110	6.7×10^{-5}	5.82	2	1×10^{-7}	111	7.00	1.5×10^{-6}	7.12
	3	109	7.6×10^{-5}	5.88	2	1×10^{-7}	111	7.00	2.1×10^{-6}	7.23
	3	109	7.6×10^{-5}	5.88	4	3×10^{-7}	112	6.50	3.6×10^{-6}	7.08
	5	109	8.9×10^{-5}	5.95	4	3×10^{-7}	112	6.50	5.1×10^{-6}	7.17
	5	109	8.9×10^{-5}	5.95	6	10×10^{-7}	113	6.00	1.2×10^{-7}	7.08
	7	109	6.3×10^{-5}	5.80	6	10×10^{-7}	113	6.00	5.9×10^{-6}	6.92
Phen. II	1	132	4.8×10^{-5}	5.68	2	1×10^{-7}	135	7.00	5.3×10^{-5}	7.06
	3	136	5.6×10^{-5}	5.75	2	1×10^{-7}	135	7.00	1.4×10^{-6}	7.18
	3	136	5.6×10^{-5}	5.75	4	3×10^{-7}	140	6.50	2.8×10^{-6}	7.10
	5	135	5.4×10^{-5}	5.73	4	3×10^{-7}	140	6.50	2.6×10^{-6}	7.08
	5	135	5.4×10^{-5}	5.73	6	10×10^{-7}	139	6.00	7.0×10^{-6}	7.08
	7	137	3.8×10^{-5}	5.58	6	10×10^{-7}	139	6.00	3.5×10^{-6}	6.92

TABLE 6 - Continued

EXPERIMENT	RESPONSE TO AGONIST				RESPONSE TO AGONIST PLUS ANTAGONIST					
	NO.	MAX. RESP. (mm)	A_o	pD_2	NO.	B. 10^{-q}	MAX. RESP. (mm)	pA_x	A_B	pA_2
Phen. III	1	156	5.4×10^{-5}	5.73	2	1×10^{-7}	158	7.00	1.8×10^{-6}	7.38
	3	157	5.6×10^{-5}	5.75	2	1×10^{-7}	158	7.00	2.1×10^{-6}	7.41
	3	157	5.6×10^{-5}	5.75	4	3×10^{-7}	158	6.50	2.5×10^{-6}	7.04
	5	161	5.3×10^{-5}	5.72	4	3×10^{-7}	158	6.50	2.2×10^{-6}	7.00
	5	161	5.3×10^{-5}	5.72	6	10×10^{-7}	158	6.00	5.5×10^{-6}	6.97
	7	155	4.8×10^{-5}	5.68	6	10×10^{-7}	158	6.00	4.7×10^{-6}	6.94

NO. = The number of the curve being measured.
A_o = The molar dose of the agonist to produce a50% response.
pD_2 = The negative (-) log of the dose necessary to produce a 50% response.
B. 10^{-q} = The molar concentration of the antagonist.
pA_x = The negative (-) log of the molar dose of the antagonist needed to cause a shift x;
$pA_x = (q - \log B)$.
A_B = The molar dose of the agonist which produces a 50% response in the presence of the antagonist.
pA_2 = The negative (-) log of the molar concentration of antagonist which causes a shift of a factor 2;
$pA_2 = pA_x + \log(x-1)$; $x = A_o/A_B$.
Phen. = Pheniramine maleate.

TABLE 7

MOR - TMPBZ ANTAGONISM TO HISTAMINE-INDUCED CONTRACTIONS OF ISOLATED GUINEA PIG ILEUM

EXPERIMENT	RESPONSE TO AGONIST				RESPONSE TO AGONIST PLUS ANTAGONIST					
	NO.	MAX. RESP. (mm)	A_o	pD_2	NO.	B. 10^{-9}	MAX. RESP. (mm)	pA_x	A_B	pA_2
Mor. I	7	132	5.4×10^{-5}	5.73	8	1×10^{-4}	132	4.00	-	-
	9	134	5.9×10^{-5}	5.77	8	1×10^{-4}	132	4.00	-	-
	9	134	5.9×10^{-5}	5.77	10	3×10^{-4}	130	3.50	9.4×10^{-5}	3.27
	11	134	5.0×10^{-5}	5.70	10	3×10^{-4}	130	3.50	6.8×10^{-5}	3.05
Mor. II	9	121	4.2×10^{-5}	5.62	10	1×10^{-4}	115	4.00	-	-
	11	122	4.2×10^{-5}	5.62	10	1×10^{-4}	115	4.00	-	-
	11	122	4.2×10^{-5}	5.62	12	3×10^{-4}	114	3.50	6.0×10^{-5}	3.12
	13	120	3.4×10^{-5}	5.53	12	3×10^{-4}	114	3.50	4.0×10^{-5}	2.72

TABLE 7 - Continued

EXPERIMENT	RESPONSE TO AGONIST				RESPONSE TO AGONIST PLUS ANTAGONIST					
	NO.	MAX. RESP. (mm)	A_o	pD_2	NO.	B. 10^{-q}	MAX. RESP. (mm)	pA_x	A_B	pA_2
Mor. III	1	124	3.8×10^{-5}	5.58	2	1×10^{-4}	133	4.00	-	-
	3	137	3.6×10^{-5}	5.55	2	1×10^{-4}	133	4.00	-	-
	3	137	3.6×10^{-5}	5.55	4	3×10^{-4}	131	3.50	-	-
	5	135	3.0×10^{-5}	5.47	4	3×10^{-4}	131	3.50	-	-

NO. = The number of the curve being measured.
A_o = The molar dose of the agonist to produce a 50% response.
pD_2 = The negative (-) log of the dose necessary to produse a 50% response.
B. 10^{-q} = the molar concentration of the antagonist.
pA_x = The negative (-) log of the molar dose of the antagonist needed to cause a shift x;
$pA_x = (q - \log B)$.
A_B = The molar dose of the agonist which produces a 50% response in the presence of the antagonist.
pA_2 = The negative (-) log of the molar concentration of antagonist which causes a shift of a factor 2;
$pA_2 = pA_x + \log(x-1)$; $x = A_o/A_B$.
Mor. = Mor - TMPBZ.

TABLE 8

TMT ANTAGONISM TO HISTAMINE-INDUCED CONTRACTIONS OF ISOLATED GUINEA PIG ILEUM

EXPERIMENT	RESPONSE TO AGONIST				RESPONSE TO AGONIST PLUS ANTAGONIST					
	NO.	MAX. RESP. (mm)	A_o	pD_2	NO.	B. 10^{-q}	MAX. RESP. (mm)	pA_x	A_B	pA_2
TMT I	1	114	3.4×10^{-5}	5.53	2	1×10^{-4}	117	4.00	4.8×10^{-5}	3.55
	3	118	4.2×10^{-5}	5.62	2	1×10^{-4}	117	4.00	7.1×10^{-5}	3.81
	3	118	4.2×10^{-5}	5.62	4	3×10^{-4}	122	3.50	9.2×10^{-5}	3.41
	5	121	4.7×10^{-5}	5.67	4	3×10^{-4}	122	3.50	1.2×10^{-5}	3.32
TMT II	1	130	5.0×10^{-5}	5.70	2	1×10^{-4}	129	4.00	5.0×10^{-5}	2.59
	3	125	5.0×10^{-5}	5.70	2	1×10^{-4}	129	4.00	5.0×10^{-5}	2.59
	3	125	5.0×10^{-5}	5.70	4	3×10^{-4}	122	3.50	6.0×10^{-5}	2.72
	5	130	5.3×10^{-5}	5.72	4	3×10^{-4}	122	3.50	6.0×10^{-5}	2.83

TABLE 8 - Continued

EXPERIMENT	RESPONSE TO AGONIST				RESPONSE TO AGONIST PLUS ANTAGONIST					
	NO.	MAX. RESP. (mm)	A_o	pD_2	NO.	B. 10^{-q}	MAX. RESP. (mm)	pA_x	A_B	pA_2
TMT III	2	143	4.7×10^{-5}	5.67	3	1×10^{-4}	143	4.00	-	-
	4	144	5.0×10^{-5}	5.70	3	1×10^{-4}	143	4.00	5.5×10^{-5}	2.90
	4	144	5.0×10^{-5}	5.70	5	3×10^{-4}	143	3.50	8.5×10^{-5}	3.35
	6	144	5.6×10^{-5}	5.75	5	3×10^{-4}	143	3.50	1.1×10^{-6}	3.41

NO. = The number of the curve being measured.
A_o = The molar dose of the agonist to produce a 50% response.
pD_2 = The negative (-) log of the dose necessary to produce a 50% response.
B. 10^{-q} = The molar concentration of the antagonist.
pA_x = The negative (-) log of the molar dose of the antagonist needed to cause a shift x;
$pA_x = (q - \log B)$.
A_B = The molar dose of the agonist which produces a 50% response in the presence of the antagonist.
pA_2 = The negative (-) log of the molar concentration of antagonist which causes a shift of a factor 2;
$pA_2 = pA_x + \log(x-1)$; $x = A_o/A_B$.

TABLE 9

PYL - TMPBZ ANTAGONISM TO HISTAMINE-INDUCED CONTRACTIONS OF ISOLATED GUINEA PIG ILEUM

EXPERIMENT	RESPONSE TO AGONIST				RESPONSE TO AGONIST PLUS ANTAGONIST					
	NO.	MAX. RESP. (mm)	A_o	pD_2	NO.	B. 10^{-q}	MAX. RESP. (mm)	pA_x	A_B	pA_2
Pyl-TMPBZ I	1	128	5.0×10^{-5}	5.70	2	10×10^{-5}	127	4.00	6.5×10^{-5}	3.49
	3	130	5.6×10^{-5}	5.75	2	10×10^{-5}	127	4.00	8.4×10^{-5}	3.67
	3	130	5.6×10^{-5}	5.75	4	30×10^{-5}	125	3.50	1.6×10^{-6}	3.71
	5	124	4.3×10^{-5}	5.63	4	30×10^{-5}	125	3.50	8.6×10^{-5}	3.50
	5	124	4.3×10^{-5}	5.63	6	3×10^{-5}	127	4.50	-	-
	7	129	5.4×10^{-5}	5.73	6	3×10^{-5}	127	4.50	-	-
Pyl-TMPBZ II	5	119	4.5×10^{-5}	5.65	6	10×10^{-5}	118	4.00	4.5×10^{-5}	2.59
	7	123	4.8×10^{-5}	5.68	6	10×10^{-5}	118	4.00	5.3×10^{-5}	3.04
	7	123	4.8×10^{-5}	5.68	8	30×10^{-5}	113	3.50	8.2×10^{-5}	3.31
	9	121	4.2×10^{-5}	5.62	8	30×10^{-5}	113	3.50	5.9×10^{-5}	3.12

TABLE 9 - Continued

EXPERIMENT	RESPONSE TO AGONIST				RESPONSE TO AGONIST PLUS ANTAGONIST					
	NO.	MAX. RESP. (mm)	A_o	pD_2	NO.	B. 10^{-q}	MAX. RESP. (mm)	pA_x	A_B	pA_2
Pyl-TMPBZ III	5	135	3.0×10^{-5}	5.47	6	10×10^{-5}	133	4.00	3.9×10^{-5}	3.41
	7	131	2.5×10^{-5}	5.40	6	10×10^{-5}	133	4.00	2.8×10^{-5}	2.90
	7	131	2.5×10^{-5}	5.40	8	30×10^{-5}	123	3.50	3.3×10^{-5}	2.91
	9	134	3.2×10^{-5}	5.50	8	30×10^{-5}	123	3.50	5.1×10^{-5}	3.27

NO. = The number of the curve being measured.
A_o = The molar dose of the agonist to produce a 50% response.
pD_2 = The negative (-) log of the dose necessary to produce a 50% response.
B. 10^{-q} = The molar concentration of the antagonist.
pA_x = The negative (-) log of the molar dose of the antagonist needed to cause a shift x;
$pA_x = (q - \log B)$.
A_B = The molar dose of the agonist which produces a 50% response in the presence of the antagonist.
pA_2 = The negative (-) log of the molar concentration of antagonist which causes a shift of a factor 2;
$pA_2 = pA_x + \log(x-1)$; $x = A_o/A_B$.

TABLE 10

TMBD ANTAGONISM TO HISTAMINE-INDUCED CONTRACTIONS OF ISOLATED GUINEA PIG ILEUM

	RESPONSE TO AGONIST				RESPONSE TO AGONIST PLUS ANTAGONIST					
EXPERIMENT	NO.	MAX. RESP. (mm)	A_o	pD_2	NO.	B. 10^{-q}	MAX. RESP. (mm)	pA_x	A_B	pA_2
TMBD I	2	108	7.4×10^{-5}	5.87	3	3×10^{-5}	107	4.50	8.9×10^{-5}	3.72
	4	107	7.9×10^{-5}	5.90	3	3×10^{-5}	107	4.50	1.0×10^{-6}	3.91
	4	107	7.9×10^{-5}	5.90	5	10×10^{-5}	103	4.00	1.5×10^{-6}	3.93
	6	108	7.6×10^{-5}	5.88	5	10×10^{-5}	103	4.00	1.4×10^{-6}	3.89
	6	108	7.6×10^{-5}	5.88	7	30×10^{-5}	102	3.50	4.3×10^{-6}	4.17
	8	108	1.2×10^{-6}	6.07	7	30×10^{-5}	102	3.50	1.0×10^{-7}	4.38
TMBD II	1	132	5.9×10^{-5}	5.77	2	3×10^{-5}	129	4.50	6.5×10^{-5}	3.54
	3	132	6.7×10^{-5}	5.82	2	3×10^{-5}	129	4.50	8.7×10^{-5}	3.91
	3	132	6.7×10^{-5}	5.82	4	10×10^{-5}	128	4.00	1.9×10^{-6}	4.26
	5	134	6.8×10^{-5}	5.83	4	10×10^{-5}	128	4.00	1.4×10^{-6}	4.29
	5	134	6.8×10^{-5}	5.83	6	30×10^{-5}	133	3.50	3.7×10^{-6}	4.14
	7	128	6.7×10^{-5}	5.82	6	30×10^{-5}	133	3.50	3.5×10^{-6}	4.12

TABLE 10 - Continued

EXPERIMENT	RESPONSE TO AGONIST				RESPONSE TO AGONIST PLUS ANTAGONIST					
	NO.	MAX. RESP. (mm)	A_o	pD_2	NO.	B. 10^{-q}	MAX. RESP. (mm)	pA_x	A_B	pA_2
TMBD III	1	108	7.9×10^{-5}	5.90	2	3×10^{-5}	110	4.50	1.4×10^{-6}	4.39
	3	112	6.3×10^{-5}	5.80	2	3×10^{-5}	110	4.50	8.8×10^{-5}	4.12
	3	112	6.3×10^{-5}	5.80	4	10×10^{-5}	110	4.00	1.6×10^{-6}	4.21
	5	111	7.9×10^{-5}	5.90	4	10×10^{-5}	110	4.00	2.6×10^{-6}	4.36
	5	111	7.9×10^{-5}	5.90	6	30×10^{-5}	100	3.50	7.3×10^{-6}	4.42
	7	109	6.8×10^{-5}	5.83	6	30×10^{-5}	100	3.50	5.8×10^{-6}	4.34

NO. = The number of the curve being measured.
A_o = The molar dose of the agonist to produce a 50% response.
pD_2 = The negative (-) log of the dose necessary to produce a 50% response.
B. 10^{-q} = The molar concentration of the antagonist.
pA_x = The negative (-) log of the molar dose of the antagonist needed to cause a shift x;
$pA_x = (q - \log B)$.
A_B = The molar dose of the agonist which produces a 50% response in the presence of the antagonist.
pA_2 = The negative (-) log of the molar concentration of antagonist which causes a shift of a factor 2;
$pA_2 = pA_x + \log(x-1)$; $x = A_o/A_B$.

TABLE 11

DMA - TMPP ANTAGONISM TO HISTAMINE-INDUCED CONTRACTIONS OF ISOLATED GUINEA PIG ILEUM

EXPERIMENT	RESPONSE TO AGONIST				RESPONSE TO AGONIST PLUS ANTAGONIST					
	NO.	MAX. RESP. (mm)	A_o	pD_2	NO.	B. 10^{-q}	MAX. RESP. (mm)	pA_x	A_B	pA_2
Dma-TMPP I	7	129	5.6×10^{-5}	5.75	8	1×10^{-4}	127	4.00	-	-
	9	131	7.9×10^{-5}	5.90	8	1×10^{-4}	127	4.00	-	-
	9	131	7.9×10^{-5}	5.90	10	3×10^{-4}	117	3.50	3.2×10^{-6}	3.97
	11	127	5.3×10^{-5}	5.72	10	3×10^{-4}	117	3.50	1.4×10^{-6}	3.71
Dma-TMPP II	1	115	4.0×10^{-5}	5.60	2	1×10^{-4}	115	4.00	-	-
	3	118	4.5×10^{-5}	5.65	2	1×10^{-4}	115	4.00	-	-
	3	118	4.5×10^{-5}	5.65	4	3×10^{-4}	98	3.50	8.6×10^{-5}	3.46
	5	119	4.2×10^{-5}	5.62	4	3×10^{-4}	98	3.50	7.6×10^{-5}	3.39

TABLE 11 - Continued

EXPERIMENT	RESPONSE TO AGONIST				RESPONSE TO AGONIST PLUS ANTAGONIST					
	NO.	MAX. RESP. (mm)	A_o	pD_2	NO.	B. 10^{-q}	MAX. RESP. (mm)	pA_x	A_B	pA_2
Dma-TMPP III	1	132	3.4×10^{-5}	5.53	2	1×10^{-4}	135	4.00	-	-
	3	129	3.8×10^{-5}	5.58	2	1×10^{-4}	135	4.00	-	-
	3	129	3.8×10^{-5}	5.58	4	3×10^{-4}	131	3.50	7.2×10^{-5}	3.43
	5	131	3.0×10^{-5}	5.47	4	3×10^{-4}	131	3.50	4.2×10^{-5}	3.12

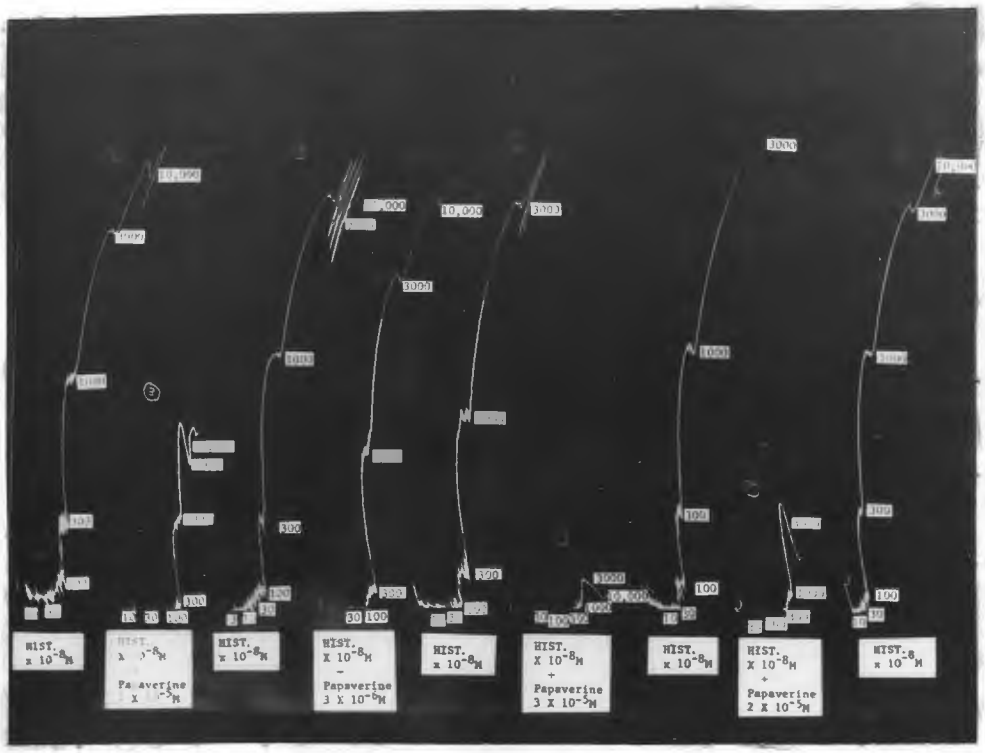
NO. = The number of the curve being measured.
A_o = The molar dose of the agonist to produce a 50% response.
pD_2 = The negative (-) log of the dose necessary to produce a 50% response.
B. 10^{-q} = The molar concentration of the antagonist.
pA_x = The negative (-) log of the molar dose of the antagonist needed to cause a shift x;
$pA_x = (q - \log B)$.
A_B = The molar dose of the agonist which produces a 50% response in the presence of the antagonist.
pA_2 = The negative (-) log of the molar concentration of antagonist which causes a shift of a factor 2;
$pA_2 = pA_x + \log(x-1)$; $x = A_o/A_B$.

FIGURE 8

CUMULATIVE DOSE-RESPONSE CURVES OF HISTAMINE

ON THE GUINEA PIG ILEUM IN THE PRESENCE

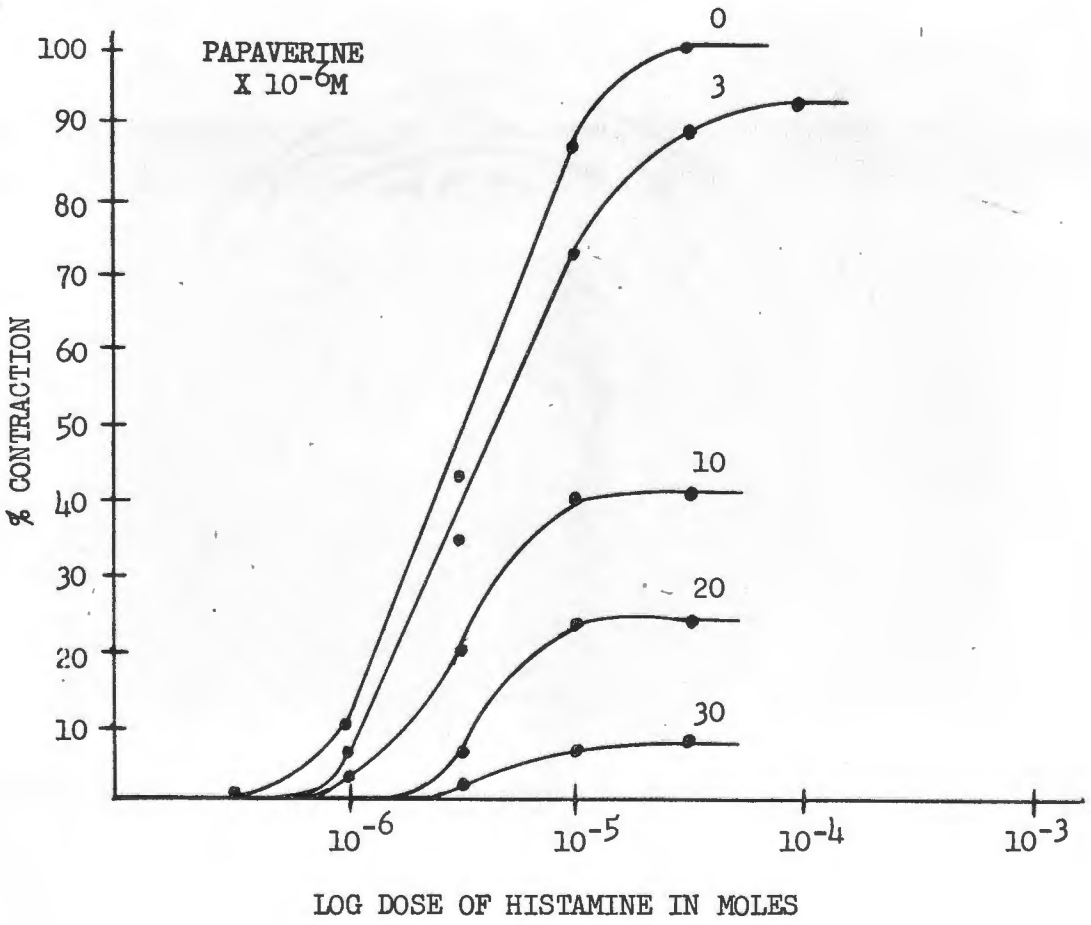
AND ABSENCE OF PAPAVERINE



Note that the maximum response of histamine diminishes as the concentration of papaverine (a known non-competitive antagonist) is increased.

FIGURE 9

CUMULATIVE DOSE-RESPONSE CURVES AS CALCULATED FROM FIGURE 8



The affinity of papaverine (a non-competitive antagonist) for the receptor site can be calculated as a pD'_2 value from the reduction in the maximum-induced histamine response.

TABLE 12

PAPAVERINE HYDROCHLORIDE ANTAGONISM TO HISTAMINE-INDUCED

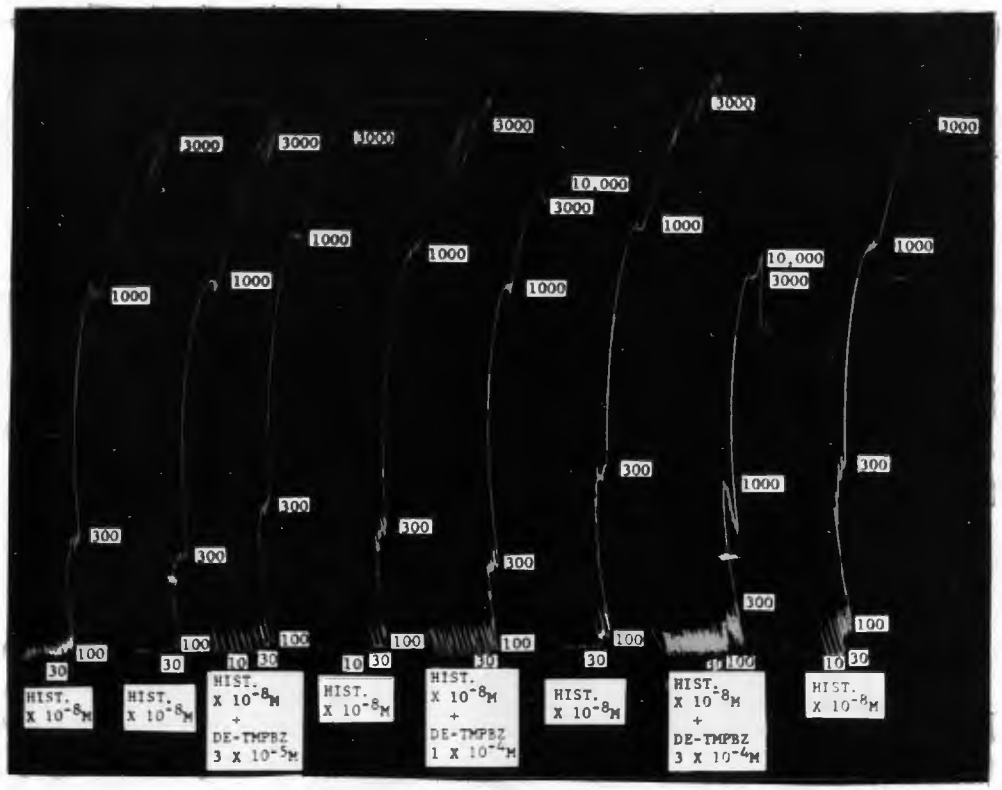
CONTRACTIONS OF ISOLATED GUINEA PIG ILEUM

	RESPONSE TO AGONIST				RESPONSE TO AGONIST PLUS ANTAGONIST					
EXPERIMENT	NO.	MAX. RESP. (mm)	A_o	pD_2	NO.	B. 10^{-p}	MAX. RESP. (mm)	pD'_x	%	pD'_2
PAPAVERINE	2	141	3.4×10^{-5}	5.53	3	10×10^{-6}	58	5.00	41	5.16
	4	144	3.7×10^{-5}	5.57	3	10×10^{-6}	58	5.00	40	5.18
	4	144	3.7×10^{-5}	5.57	5	3×10^{-6}	133	5.50	91	-
	6	144	2.7×10^{-5}	5.43	5	3×10^{-6}	133	5.50	91	-
	6	144	2.7×10^{-5}	5.43	7	30×10^{-6}	10	4.50	7	-
	8	149	3.7×10^{-5}	5.57	7	30×10^{-6}	10	4.50	7	-
	8	149	3.7×10^{-5}	5.57	9	20×10^{-6}	34	4.70	23	5.23
	10	143	3.7×10^{-5}	5.57	9	20×10^{-6}	34	4.70	24	5.20

NO. = The number of the curve being measured.
A_o = The molar concentration of agonist to produce a 50% response.
pD_2 = The negative (-) log of the dose necessary to produce a 50% response.
B. 10^{-P} = The molar concentration of the antagonist.
pD'_x = The negative (-) log of the molar dose of the antagonist needed to cause a shift x; $pD'_x = (p - \log B)$.
% = The maximum effect of the agonist in the presence of the antagonist is expressed as a percentage of the maximum effect of the agonist alone; % = 100/x.
pD'_2 = The negative log of the molar concentration which causes a 50% depression; $pD'_2 = pD'_x + \log(x-1)$.

FIGURE 10

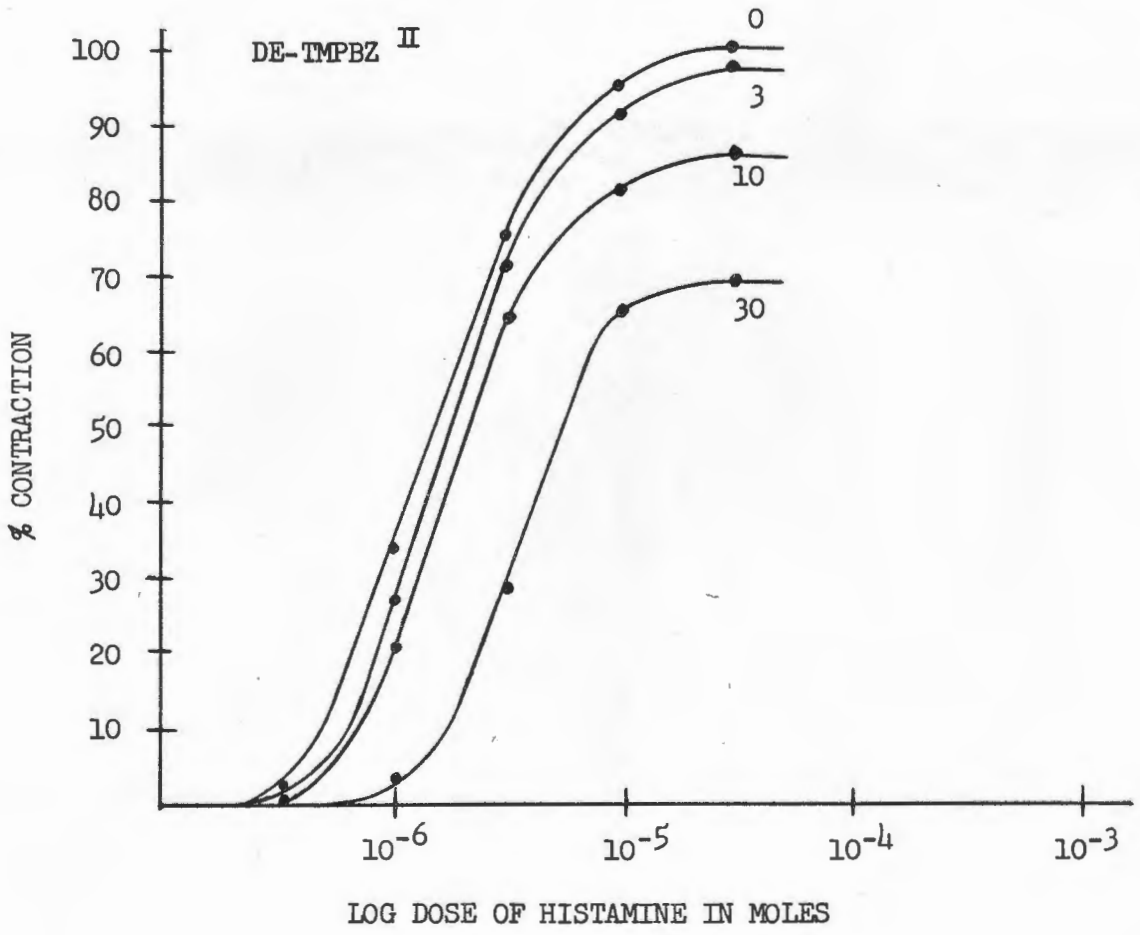
CUMULATIVE DOSE-RESPONSE CURVES OF HISTAMINE ON THE GUINEA PIG ILEUM IN THE PRESENCE AND ABSENCE OF DE-TMPBZ



Note that the maximum response of histamine diminishes as the concentration of De-TMPBZ is increased.

FIGURE 11

CUMULATIVE DOSE-RESPONSE CURVES AS CALCULATED FROM FIGURE 10



The affinity of De-TMPBZ for the receptor site can be calculated as a pD'_2 value from the reduction in the maximum induced histamine response.

TABLE 13

DE - TMPBZ ANTAGONISM TO HISTAMINE-INDUCED CONTRACTIONS OF ISOLATED GUINEA PIG ILEUM

EXPERIMENT	RESPONSE TO AGONIST				RESPONSE TO AGONIST PLUS ANTAGONIST					
	NO.	MAX. RESP. (mm)	A_o	pD_2	NO.	B. 10^{-p}	MAX. RESP. (mm)	pD'_x	%	pD'_2
De-TMPBZ I	1	117	5.9×10^{-5}	5.77	2	3×10^{-5}	115	4.50	98	-
	3	115	6.3×10^{-5}	5.80	2	3×10^{-5}	115	4.50	100	-
	3	115	6.3×10^{-5}	5.80	4	10×10^{-5}	106	4.00	92	-
	5	114	6.8×10^{-5}	5.83	4	10×10^{-5}	106	4.00	93	-
	5	114	6.8×10^{-5}	5.83	6	30×10^{-5}	84	3.50	74	3.04
	7	110	8.9×10^{-5}	5.95	6	30×10^{-5}	84	3.50	76	3.01
De-TMPBZ II	1	121	5.0×10^{-5}	5.70	2	3×10^{-5}	131	4.50	103	-
	3	131	5.6×10^{-5}	5.75	2	3×10^{-5}	131	4.50	100	-
	3	131	5.6×10^{-5}	5.75	4	10×10^{-5}	113	4.00	86	3.20
	5	134	6.7×10^{-5}	5.82	4	10×10^{-5}	113	4.00	84	3.23
	5	134	6.7×10^{-5}	5.82	6	30×10^{-5}	92	3.50	69	3.15
	7	136	6.0×10^{-5}	5.78	6	30×10^{-5}	92	3.50	68	3.17

TABLE 13 - Continued

EXPERIMENT	RESPONSE TO AGONIST				RESPONSE TO AGONIST PLUS ANTAGONIST					
	NO.	MAX. RESP. (mm)	A_o	pD_2	NO.	B. 10^{-p}	MAX. RESP. (mm)	pD'_x	%	pD'_2
De-TMPBZ III	1	154	5.9×10^{-5}	5.77	2	3×10^{-5}	153	4.50	99	-
	3	154	6.8×10^{-5}	5.83	2	3×10^{-5}	153	4.50	99	-
	3	154	6.8×10^{-5}	5.83	4	10×10^{-5}	144	4.00	93	-
	5	148	6.3×10^{-5}	5.80	4	10×10^{-5}	144	4.00	97	-
	5	148	6.3×10^{-5}	5.80	6	30×10^{-5}	114	3.50	77	2.98
	7	154	5.9×10^{-5}	5.77	6	30×10^{-5}	114	3.50	74	3.04

NO. = The number of the curve being measured.
A_8 = The molar concentration of agonist to produce a 50% response.
pD_2 = The negative (-) log of the dose necessary to produce a 50% response.
B. 10^{-p} = The molar concentration of the antagonist.
pD'_x = The negative (-) log of the molar dose of the antagonist needed to cause a shift x; $pD'_x = (p - \log B)$.
% = The maximum effect of the agonist in the presence of the antagonist is expressed as a percentage of the maximum effect of the agonist alone; % = 100/x.
pD'_2 = The negative log of the molar concentration which causes a 50% depression; $pD'_2 = pD'_x + \log (x-1)$.

TABLE 14

TMPBZ ANTAGONISM TO HISTAMINE-INDUCED CONTRACTIONS OF ISOLATED GUINEA PIG ILEUM

EXPERIMENT	RESPONSE TO AGONIST				RESPONSE TO AGONIST PLUS ANTAGONIST					
	NO.	MAX. RESP. (mm)	A_o	pD_2	NO.	B. 10^{-p}	MAX. RESP. (mm)	pD'_x	%	pD'_2
TMPBZ I	11	134	5.3×10^{-5}	5.72	12	10×10^{-5}	79	4.00	59	3.85
	13	130	5.9×10^{-5}	5.77	12	10×10^{-5}	79	4.00	61	3.81
	13	130	5.9×10^{-5}	5.77	14	3×10^{-5}	108	4.50	83	3.80
	15	131	5.6×10^{-5}	5.75	14	3×10^{-5}	108	4.50	82	3.84
	15	131	5.6×10^{-5}	5.75	16	30×10^{-5}	44	3.50	34	3.79
	17	126	5.6×10^{-5}	5.75	16	30×10^{-5}	44	3.50	35	3.77
TMPBZ II	1	129	2.8×10^{-5}	5.45	2	10×10^{-5}	62	4.00	48	4.03
	3	122	3.4×10^{-5}	5.53	2	10×10^{-5}	62	4.00	51	3.98
	3	122	3.4×10^{-5}	5.53	4	3×10^{-5}	82	4.50	67	4.19
	5	120	4.0×10^{-5}	5.60	4	3×10^{-5}	82	4.50	68	4.17
	5	120	4.0×10^{-5}	5.60	6	30×10^{-5}	11	3.50	9	-
	7	116	3.0×10^{-5}	5.48	6	30×10^{-5}	11	3.50	9	-

TABLE 14 - Continued

EXPERIMENT	RESPONSE TO AGONIST				RESPONSE TO AGONIST PLUS ANTAGONIST					
	NO.	MAX. RESP. (mm)	A_o	pD_2	NO.	B. 10^{-p}	MAX. RESP. (mm)	pD'_x	%	pD'_2
TMPBZ III	7	137	4.7×10^{-5}	5.67	8	3×10^{-5}	112	4.50	82	3.84
	9	132	4.8×10^{-5}	5.68	8	3×10^{-5}	112	4.50	85	3.73
	9	132	4.8×10^{-5}	5.68	10	10×10^{-5}	76	4.00	58	3.86
	11	133	6.3×10^{-5}	5.80	10	10×10^{-5}	76	4.00	57	3.88
	11	133	6.3×10^{-5}	5.80	12	30×10^{-5}	26	3.50	20	3.90
	13	129	5.0×10^{-5}	5.70	12	30×10^{-5}	26	3.50	20	3.90

NO. = The number of the curve being measured.
A_8 = The molar concentration of agonist to produce a 50% response.
pD_2 = The negative (-) log of the dose necessary to produce a 50% response.
B. 10^{-p} = The molar concentration of the antagonist.
pD'_x = The negative (-) log of the molar dose of the antagonist needed to cause a shift x; $pD'_x = (p - \log B)$.
% = The maximum effect of the agonist in the presence of the antagonist is expressed as a percentage of the maximum effect of the agonist alone; % = 100/x.
pD'_2 = The negative log of the molar concentration which causes a 50% depression; $pD'_2 = pD'_x + \log(x-1)$.

TABLE 15

A SUMMARY OF COMPETITIVE DRUG ANTAGONISM OF HISTAMINE-INDUCED CONTRACTIONS OF ISOLATED GUINEA PIG ILEUM

COMPOUND	MEAN pA_2	± S.D.
Tripelennamine Hydrochloride	8.40	0.18
Diphenhydramine Hydrochloride	7.75	0.16
Pheniramine Maleate	7.10	0.14
2-(3,4,5-trimethoxybenzhydryloxy)-N,N-dimethyl-ethylamine Succinate (TMBD)	4.12	0.25
-(1-Piperidino)-N-(2-pyridyl)-N-(3,4,5-trimethoxy-benzyl) ethylamine Dicyclamate (Pip-TMPBZ)	3.54	0.35
-Dimethylamino-3,4,5-trimethoxypropiophenone Hydrochloride (Dma-TMPP)	3.51	0.29
-(1-Pyrrolidino)-N-(2-pyridyl)-N-(3,4,5-trimethoxy-benzyl) ethylamine Dicyclamate (Pyl-TMPBZ)	3.24	0.35
1-(2-pyridyl)-1-(3,4,5-trimethoxyphenyl)-3-dimethyl-aminopropane Cyclamate (TMT)	3.13	0.42
-(4-Morpholino)-N-(2-pyridyl)-N-(3,4,5-trimethoxy-benzyl) ethylamine Dicyclamate (Mor-TMPBZ)	3.04	0.23

TABLE 16

A SUMMARY OF NON-COMPETITIVE DRUG ANTAGONISM OF HISTAMINE-INDUCED CONTRACTIONS OF ISOLATED GUINEA PIG ILEUM

COMPOUND	MEAN pD'_2	± S.D.
Papaverine Hydrochloride	5.19	–
N,N,-Dimethyl-N'-(2-pyridyl)-N'-(3,4,5-trimethoxybenzyl) ethylenediamine Disuccinate (TMPBZ)	3.90	0.13
N,N,-Diethyl-N'-(2-pyridyl)-N'-(3,4,5-trimethoxybenzyl) ethylenediamine Dicyclamate (DE-TMPBZ)	3.10	0.09

TABLE 17

EFFECT OF DIPHENHYDRAMINE HYDROCHLORIDE ON THE SYSTOLIC BLOOD PRESSURE OF MALE ALBINO RATS

DOSE Moles/Kg	DOSE Mg/Kg	NORMAL BLOOD PRESSURE (mmHg)	DEPRESSOR RESPONSE DURATION (min:sec)	DEPRESSOR RESPONSE %	PRESSOR RESPONSE DURATION (min:sec)	PRESSOR RESPONSE %
1.0×10^{-3}	0.29	94	3'10"	11	-	-
3.0×10^{-3}	0.87	114	5'00"	22	-	-
1.0×10^{-2}	2.92	90	1'00"	79	11'06"	24
3.0×10^{-2}	8.75	84	3'10"	63	41'10"	51

% = The maximum systolic pressor or maximum depressor response expressed as a percent of the normal blood pressure.

TABLE 18

EFFECT OF TMBD ON THE SYSTOLIC BLOOD PRESSURE OF MALE ALBINO RATS

DOSE Moles/Kg	DOSE Mg/Kg	NORMAL BLOOD PRESSURE (mmHg)	DEPRESSOR RESPONSE		PRESSOR RESPONSE	
			DURATION (min:sec)	%	DURATION (min:sec)	%
5.0×10^{-2}	23.20	100	2'20"	20	-	-
1.0×10^{-1}	46.40	86	2'10"	27	4'50"	2
2.0×10^{-1}	98.70	74	2'00"	66	12'50"	22

% = The maximum systolic pressor or maximum systolic depressor response expressed as a percent of the normal blood pressure.

TABLE 19

EFFECT OF PHENIRAMINE MALEATE ON THE SYSTOLIC BLOOD PRESSURE OF MALE ALBINO RATS

DOSE Moles/Kg	DOSE Mg/Kg	NORMAL BLOOD PRESSURE (mmHg)	DEPRESSOR RESPONSE		PRESSOR RESPONSE	
			DURATION (min:sec)	%	DURATION (min:sec)	%
3.0×10^{-3}	1.07	106	2'20"	13	29'30"	9
1.0×10^{-2}	3.56	99	3'30"	15	35'50"	19
3.0×10^{-2}	10.70	94	10'35"	15	20'10"	9
1.0×10^{-1}	35.60	76	20'00"	18	35'30"	30

% = The maximum systolic pressor or maximum systolic depressor response expressed as a percent of the normal blood pressure.

TABLE 20

EFFECT OF DMA - TMPP ON THE SYSTOLIC BLOOD PRESSURE

OF MALE ALBINO RATS

DOSE Moles/Kg	DOSE Mg/Kg	NORMAL BLOOD PRESSURE (mmHg)	DEPRESSOR RESPONSE DURATION (min:sec)	%
2.5×10^{-2}	7.60	94	10'05"	32
5.0×10^{-2}	15.20	84	8'30"	31
1.0×10^{-1}	30.40	112	14'10"	43
1.5×10^{-1}	45.60	96	27'25"	42

% = The maximum systolic depressor response expressed as a percent of the normal blood pressure.

TABLE 21

EFFECT OF TMT ON THE SYSTOLIC BLOOD PRESSURE

OF MALE ALBINO RATS

DOSE Moles/Kg	DOSE Mg/Kg	NORMAL BLOOD PRESSURE (mmHg)	PRESSOR RESPONSE DURATION (min:sec)	%
2.5×10^{-2}	12.7	104	20'00"	22
5.0×10^{-2}	25.5	98	32'50"	31
1.0×10^{-1}	51.0	104	6'15"	35
1.5×10^{-1}	76.4	106	30'20"	47

% = The maximum systolic pressor response expressed as a percent of the normal blood pressure.

TABLE 22

EFFECT OF TRIPELENNAMINE HYDROCHLORIDE ON THE SYSTOLIC BLOOD PRESSURE OF MALE ALBINO RATS

DOSE Moles/Kg	DOSE Mg/Kg	NORMAL BLOOD PRESSURE (mmHg)	DEPRESSOR RESPONSE DURATION (min:sec)	DEPRESSOR RESPONSE %	PRESSOR RESPONSE DURATION (min:sec)	PRESSOR RESPONSE %
1.0×10^{-2}	2.92	83	1'40"	54	7'10"	6
2.0×10^{-2}	5.84	80	3'15"	64	21'30"	20
3.0×10^{-2}	8.76	97	12'50"	45	31'00"	28
4.0×10^{-2}	11.68	90	2'20"	72	42'10"	22

% = The maximum systolic pressor or maximum depressor response expressed as a percent of the normal blood pressure.

TABLE 23

EFFECT OF TMPBZ ON THE SYSTOLIC BLOOD PRESSURE

OF MALE ALBINO RATS

DOSE Moles/Kg	DOSE Mg/Kg	NORMAL BLOOD PRESSURE (mmHg)	DEPRESSOR RESPONSE DURATION (min:sec)	%
1.0×10^{-2}	5.8	78	5'05"	23
2.0×10^{-2}	11.6	94	6'35"	36
3.0×10^{-2}	17.4	97	7'50"	51
4.0×10^{-2}	23.3	108	10'20"	55

% = The maximum systolic depressor response expressed as a percent of the normal blood pressure.

TABLE 24

EFFECT OF DE - TMPBZ ON THE SYSTOLIC BLOOD PRESSURE

OF MALE ALBINO RATS

DOSE Moles/Kg	DOSE Mg/Kg	NORMAL BLOOD PRESSURE (mmHg)	DEPRESSOR RESPONSE DURATION (min:sec)	DEPRESSOR RESPONSE %
1.0×10^{-2}	7.3	105	2'20"	28
2.0×10^{-2}	14.6	87	2'40"	43
3.0×10^{-2}	22.0	120	8'10"	47
4.0×10^{-2}	29.3	120	6'50"	18

% = The maximum systolic depressor response expressed as a percent of the normal blood pressure.

TABLE 25

EFFECT OF PIP - TMPBZ ON THE SYSTOLIC BLOOD PRESSURE

OF MALE ALBINO RATS

DOSE Moles/Kg	DOSE Mg/Kg	NORMAL BLOOD PRESSURE (mmHg)	PRESSOR RESPONSE DURATION (min:sec)	%
2.0×10^{-2}	14.9	108	1'00"	6
3.0×10^{-2}	22.3	88	2'40"	24
5.0×10^{-2}	37.2	104	6'35"	15
1.0×10^{-1}	74.4	88	23'04"	44

% = The maximum systolic pressor response expressed as a percent of the normal blood pressure.

TABLE 26

EFFECT OF PYL - TMPBZ ON THE SYSTOLIC BLOOD PRESSURE

OF MALE ALBINO RATS

DOSE Moles/Kg	DOSE Mg/Kg	NORMAL BLOOD PRESSURE (mmHg)	PRESSOR RESPONSE DURATION (min:sec)	%
5.0×10^{-2}	36.5	122	0'50"	7
7.5×10^{-2}	54.8	74	23'00"	53
1.0×10^{-1}	73.0	84	47'50"	40
1.5×10^{-1}	109.5	113	43'50"	31

% = The maximum systolic pressor response expressed as a percent of the normal blood pressure.

TABLE 27

EFFECT OF MOR - TMPBZ ON THE SYSTOLIC BLOOD PRESSURE OF MALE ALBINO RATS

DOSE Moles/Kg	DOSE Mg/Kg	NORMAL BLOOD PRESSURE (mmHg)	PRESSOR RESPONSE DURATION (min:sec)	PRESSOR RESPONSE %
5.0×10^{-2}	37.3	102	2'40"	29
7.5×10^{-2}	56.0	115	7'15"	23
1.0×10^{-1}	74.6	108	5'50"	22
1.5×10^{-1}	111.9	77	24'00"	53

% = The maximum systolic pressor response expressed as a percent of the normal blood pressure.

TABLE 28

THE EFFECT OF DMA-TMPP AND PHENIRAMINE ON THE EXPLORATORY ACTIVITY OF MALE ALBINO MICE

Time Minutes	CAGE I Vehicle-Treated (0.2 ml. water) $\bar{X}$ ± S.D.	C(%)	CAGE I Vehicle-Treated (0.2 ml. water) $\bar{X}$ ± S.D.	C(%)	"t" Test Evaluation
0-15	808 ± 203	25.1	843 ± 174	20.6	N.S.
0-30	1272 ± 395	31.1	1330 ± 498	37.4	N.S.
	CAGE II Vehicle-Treated (0.2 ml. water)		CAGE II Pheniramine-Treated ($1x10^{-1}$ M/Kg.)		
0-15	1239 ± 43	3.4	1484 ± 218	14.7	SIG.
0-30	2004 ± 188	9.2	2687 ± 506	18.8	SIG.
	CAGE III Vehicle-Treated (0.2 ml. water)		CAGE III Dma-TMPP'-Treated ($1x10^{-1}$ M/Kg.)		
0-15	1387 ± 165	11.9	1544 ± 227	14.7	N.S.
0-30	2217 ± 235	10.9	2495 ± 636	25.5	N.S.
	CAGE IV Vehicle-Treated (0.2 ml. water)		CAGE IV Dma-TMPP"-Treated ($2x10^{-1}$ M/Kg.)		
0-15	1382 ± 221	16.0	1281 ± 152	11.9	N.S.
0-30	2019 ± 447	22.2	2157 ± 256	12.1	N.S.

$\bar{X}$ ± S.D. = The mean counts generated per unit time ± the standard deviation.

C = Coefficient of variation, S.D./$\bar{X}$ x 100%.

Comparisons were evaluated by means of Student's "t" Test (Snedecor, 1956), with SIG. = Significant ($P \leq 0.05$) and N.S. = Not Significant ($P \leq 0.05$).

TABLE 29

THE EFFECT OF MOR-TMPBZ AND TRIPELENNAMINE ON THE EXPLORATORY ACTIVITY OF MALE ALBINO MICE

Time Minutes	CAGE I Vehicle-Treated (0.2 ml. water) $\bar{X}$ ± S.D.	C(%)	CAGE I Vehicle-Treated (0.2 ml. water) $\bar{X}$ ± S.D.	C(%)	"t" Test Evaluation
0-15	808 ± 203	25.1	923 ± 289	31.4	N.S.
0-30	1272 ± 395	31.1	1581 ± 647	40.9	N.S.
	CAGE II Vehicle-Treated (0.2 ml. water)		CAGE II Tripelennamine-Treated (7.6×10^{-2} M/Kg.)		
0-15	1239 ± 43	3.4	1297 ± 260	20.0	N.S.
0-30	2004 ± 188	9.2	2473 ± 491	19.8	N.S.
	CAGE III Vehicle-Treated (0.2 ml. water)		CAGE III Mor-TMPBZ'-Treated (7.6×10^{-2} M/Kg.)		
0-15	1387 ± 165	11.9	625 ± 197	31.7	SIG.
0-30	2217 ± 235	10.9	811 ± 184	22.7	SIG.
	CAGE IV Vehicle-Treated (0.2 ml. water)		CAGE IV Mor-TMPBZ"-Treated (1.5×10^{-1} M/Kg.)		
0-15	1383 ± 221	16.0	394 ± 90	22.8	SIG.
0-30	2019 ± 447	22.2	670 ± 194	28.9	SIG.

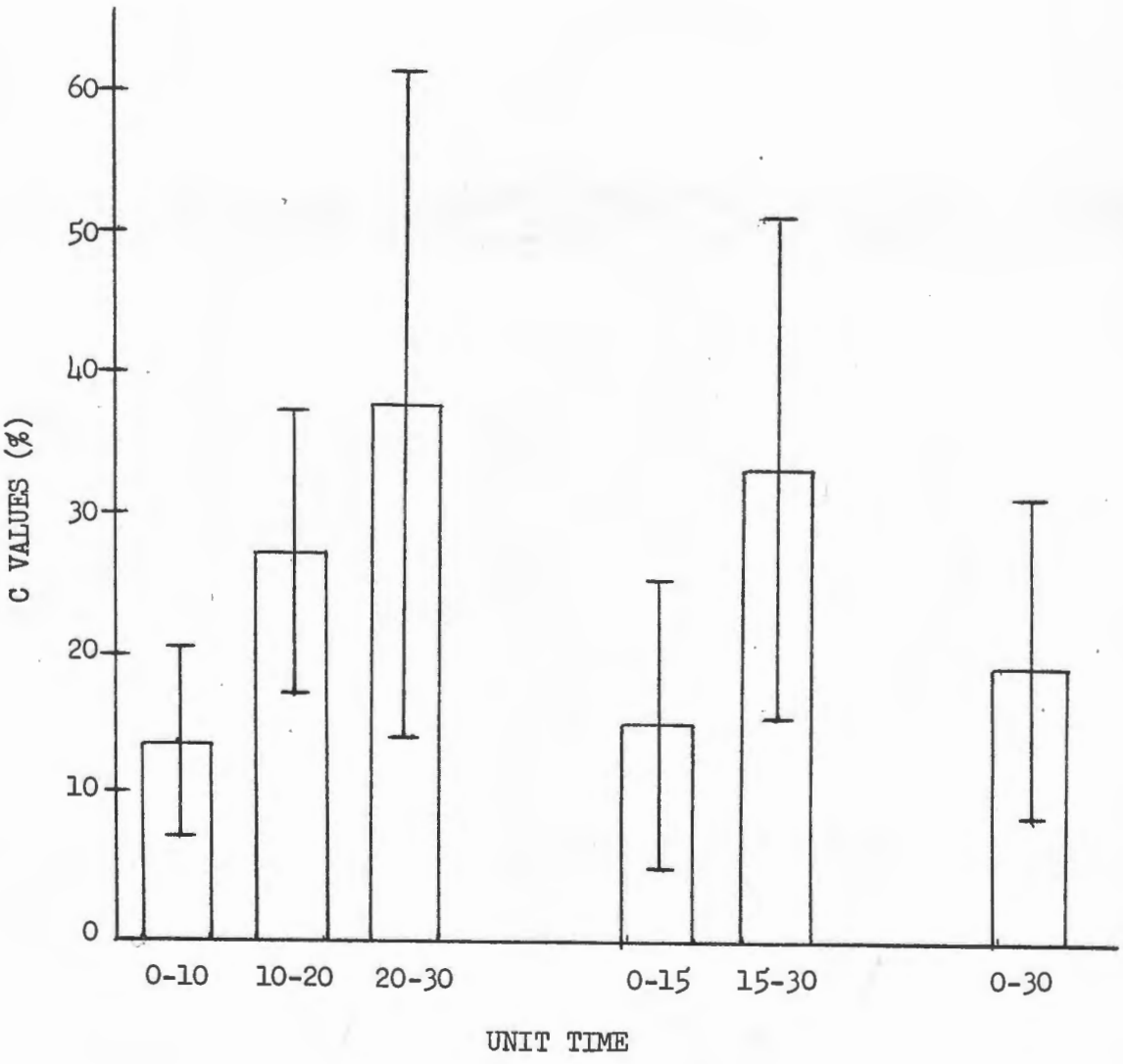
$\bar{X}$ ± S.D. = The mean counts generated per unit time ± the standard deviation.

C = Coefficient of variation, S.D./$\bar{X}$ x 100%.

Comparisons were evaluated by means of Student's "t" Test (Snedecor, 1956), with SIG. = Significant ($P \leq 0.05$) and N.S. = Not Significant ($P \leq 0.05$).

FIGURE 12

COMPARISON OF C VALUES PER UNIT TIME GENERATED BY VEHICLE-TREATED MALE ALBINO MICE IN THE ACTOPHOTOMETER



C = Coefficient of variation ($S.D./\bar{X} \times 100\%$)

V. DISCUSSION

A. ISOLATED TISSUE STUDY

The cumulative dose-response technique of Van Rossum and Van Den Brink (1963) proved to be a valid method for measuring the histamine antagonizing ability of the parent compounds and their analogs. As expected, the parent compounds were shown to be highly effective competitive antagonists of histamine-induced smooth muscle contractions. The pA_2 values in Table 15 quantify this effectiveness and serve as convenient standards for evaluating the antihistamine properties of the trimethoxy analogs.

The histamine-antagonizing abilities of the trimethoxy-substituted antihistamines were, in all cases, much weaker than the original parent compound, (Tables 15 and 16). All the analogs exhibited some competitive or non-competitive antagonism of histamine-induced smooth muscle spasms. De-TMPBZ and TMPBZ, which are analogs of tripelennamine, were shown to be non-competitive histamine antagonists. In terms of potency, De-TMPBZ and TMPBZ were about 10-100 times weaker than papaverine, which is a known non-competitive inhibitor of histamine, (Table 16). The remaining tripelennamine analogs were all competitive inhibitors, as was tripelennamine itself, and were approximately 10,000-100,000 times weaker than their parent antihistamine, (Table 15).

According to Van Rossum (1963), the difference in the type of inhibition shown by tripelennamine and the two non-competitive analogs, De-TMPBZ and TMPBZ, indicates a basic change in the activity of the experimental drugs from the parent compound. He stated that competitive

and non-competitive inhibitors elicit their different responses by means of independent receptor sites.

It seems that the changes in activity of the above tripelennamine analogs are dependent upon the presence of a 3,4,5-trimethoxyphenyl substitution and the presence of either a terminal dimethyl or diethyl group. The necessity of both types of substitutions can be seen by examining the structural formulas of tripelennamine, Mor-TMPBZ, Pyl-TMPBZ, and Pip-TMPBZ, (Figure 1). These compounds are competitive histamine antagonists and have the same structural formula as De-TMPBZ and TMPBZ except that they lack either the trimethoxy group or the terminal diethyl or dimethyl group. However, the presence of these two chemical groups on an antihistamine drug does not necessarily mean that the compound will be a non-competitive inhibitor. TMBD and TMT both have a trimethoxy group and a terminal dimethyl group and are competitive antagonists of histamine. It appears that the receptor sites exhibit a high degree of specificity as TMT and TMPBZ, which have very similar chemical structures, are different types of antagonists.

B. BLOOD PRESSURE STUDY

The results of the blood pressure study (Tables 17-27) show that the parent compounds and their trimethoxy analogs did not have a significant effect upon the systolic blood pressure of male albino rats. This is in agreement with the earlier works of LaBelle and Tislow (1955). Yonkman et al. (1946), and Loew et al. (1946), who reported that pheniramine, tripelennamine and diphenhydramine did not significantly alter the systolic blood pressure of dogs. All the compounds in this study did, however, produce minor fluctuations in

the blood pressure which are of some interest.

When pheniramine was injected intravenously, it produced a transient fall in blood pressure, which was followed by a slight rise in blood pressure, (Table 19). TMT, a trimethoxy analog of pheniramine, caused an immediate, transient rise in blood pressure but did not have the hypotensive effect of pheniramine, (Table 21). On the other hand, Dma-TMPP, when injected intravenously into rats, elicited an immediate fall in blood pressure but lacked the hypertensive effect of pheniramine, (Table 20). This compound also has a trimethoxy substitution, however, in addition, the pyridyl ring found on the parent compound has been replaced by a molecule of oxygen.

An interesting observation was made with the pheniramine analogs, TMT and Dma-TMPP, in regard to the blood pressure and tissue studies. While these compounds exhibited similar actions on histamine-induced contractions (i.e., they were both weak competitive antagonists of histamine), they produced opposite effects on the blood pressure. Dma-TMPP had a hypotensive effect and TMT produced a hypertensive effect, both of which were similar in degree to those induced by pheniramine. These changes were brought about by approximately the same doses of all drugs. It seems likely that the blood pressure effects of TMT, Dma-TMPP, and pheniramine are independent of their histamine-antagonizing abilities.

From Table 17, it can be seen that injections of diphenhydramine caused a transient decrease followed by a slight rise in blood pressure. This diphasic response was also seen by Loew et al. (1946) following intravenous injections of diphenhydramine into dogs. Injections of

TMBD, which is the trimethoxy analog of diphenhydramine, produced the same diphasic response in rats as the parent compound, (Table 18). The only noticeable difference between the two compounds was that a higher dose of the analog was needed to produce the same response.

Intravenous injections of tripelennamine induced a transient fall in blood pressure followed by a slight rise in pressure, (Table 22). Injections of equal concentrations of De-TMPBZ and TMPBZ resulted in a transient drop in blood pressure while injections of higher concentrations of the remaining trimethoxy analogs caused a temporary rise in the pressure, (Tables 23-27). From the results of the blood pressure and isolated tissue studies, it is evident that the actions of De-TMPBZ and TMPBZ are very different from the other trimethoxy analogs of tripelennamine.

C. ACTIVITY STUDIES

The actophotometer was used to evaluate the effects of tripelennamine, Mor-TMPBZ, pheniramine, and Dma-TMPP on the locomotor activity of mice. This has been shown to be an effective method for measuring locomotor activity (Mendillo, 1965). The experimental design used for the study was modified after principles outlined by Mendillo in his study of the chronic effects of drugs in rats.

As mice were used in this study and counts were taken for only thirty minutes, it was necessary to determine the best method of analyzing the data. The thirty minute counts for each cage were recorded as incremental ten minute and fifteen minute counts, and a C value (i.e., coefficient of variation, $S.D./\bar{X} \times 100\%$, Snedecor, 1956) was computed for the counts in each time interval. The C values supply a measure of the efficiency of each time interval by determining the

randomness of the data around the mean. Therefore, a low C value indicates little randomness in the data and greater efficiency than a higher C value.

The results in Figure 12 show that the counts generated at 0-10, 0-15, and 0-30 minutes have the lower C values and are therefore the most efficient time intervals. The counts generated in the 20-30 minute and 15-30 minute periods had the higher C values, indicating an increased randomness in the data. The mean counts at 0-15 and 0-30 minute intervals were reported in Tables 28 and 29, because they represented the time intervals which best estimated drug action.

The results in Table 28 show that pheniramine caused a significant increase in locomotor activity of mice while the trimethoxy analog, Dma-TMPP, had no effect. This stimulatory activity of pheniramine had been reported earlier by LaBelle and Tislow (1955), who administered pheniramine orally to dogs.

In Table 29, it is seen that tripelennamine did not have a significant effect on locomotor activity in mice, while Mor-TMPBZ significantly depressed locomotor activity. The depression induced by Mor-TMPBZ did not appear to be the result of somnolence, since the mice were found to be awake at the end of the run and their motor coordination appeared normal. Results from the Mor-TMPBZ activity experiments indicate that in future studies, this phase of the investigation should be expanded to determine if this derivative does, in fact, possess tranquilizing properties.

VI. SUMMARY AND CONCLUSIONS

A. The cumulative dose-response technique used in this study was a reliable method for determining the histamine-antagonizing abilities of experimental compounds.

B. The trimethoxy analogs of tripelennamine, pheniramine, and diphenhydramine were very weak antagonists of histamine-induced intestinal spasms. De-TMPBZ and TMPBZ were non-competitive inhibitors of histamine, while the remainder of the analogs were competitive inhibitors of histamine.

C. The trimethoxy analogs of the above antihistamines did not (with one exception, TMBD) elicit diphasic effects on the systolic blood pressure, as seen with the parent compounds. None of the compounds significantly affected the blood pressure.

D. Tripelennamine and Dma-TMPP, injected intraperitoneally, did not have a significant effect on locomotor activity of mice as measured by the actophotometer. Pheniramine, however, caused a significant increase in locomotor activity. Mor-TMPBZ caused a significant decrease in locomotor activity and warrants further consideration.

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