The Journal of PHARMACOLOGY And Experimental Therapeutics

PHARMACOLOGICAL PROPERTIES OF ANTIHISTAMINE DRUGS, BENADRYL, PYRIBENZAMINE AND NEOANTERGAN

T. R. SHERROD, EARL R. LOEW AND H. F. SCHLOEMER

From the Department of Pharmacology, University of Illinois College of Medicine, Chicago

Received for publication December 16, 1946

Comparisons have been made of the effects of antihistamine drugs on the depressor and spasmogenic actions of histamine in dogs since the resultant data would aid in evaluating such drugs relative to their use as pharmacological tools for determining the role of histamine in physiological and pathological processes. Furthermore, a study of these and other pharmacological and toxicological data with relation to therapeutic effectiveness of the various antihistamine drugs and to side-actions and toxicity should aid in selection of even more useful antihistamine drugs. The antagonism of the vascular effects of histamine assumes added significance in view of the recent reports that antihistamine drugs are therapeutically effective in allergic diseases (for references cf. 1–7) such as dermatoses, urticaria, and hay fever. Vascular reactions are undoubtedly fundamentally related to the major symptoms of allergy (8). The antihistamine drugs were also studied relative to their influence on the actions of acetylcholine and epinephrine on blood pressure, and their action on smooth muscle activity of the uterus and duodenum, since earlier studies (9–13) and our preliminary comparative studies (14) had shown that these drugs altered some actions of acetylcholine and epinephrine, both of which, like histamine, normally occur in the animal organism.

The antihistamine drugs employed[1] were β-dimethylaminoethyl benzhydryl ether hydrochloride (Benadryl) (12, 15–18), N-p-methoxybenzyl-N-dimethylaminoethyl α-aminopyridine·H_3PO_4 (2786 R.P. or Neoantergan) (9–11), and a homologue of the latter, N-benzyl-N-dimethylaminoethyl α-aminopyridine hydrochloride (Pyribenzamine) (13, 19, 20). Each of these potent antihistamine agents was recently studied almost concurrently by separate groups of investigators, which fact accounts for the almost complete absence of desirable, direct comparisons of their pharmacological properties. It is regrettable that the commendable, extensive studies made with Neoantergan by Bovet and coworkers (9, 10, 11) have received so little consideration (7, 13).

Evidence now available indicates that the drugs employed possess some similar antihistamine actions. Bronchospasm induced in guinea pigs and depression of blood pressure elicited in dogs by the injection of histamine, or by histamine liberated during anaphylaxis, is readily prevented or diminished by Benadryl (12, 14–18), Neoantergan (9–11, 14) and Pyribenzamine (13, 19, 21, 22). Each drug prevents histamine from exerting its spasmogenic action

[1] Benadryl and Pyribenzamine were supplied through the courtesy of Parke, Davis and Company and Ciba Pharmaceutical Products, Inc., respectively. Neoantergan was kindly furnished by Dr. Daniel Bovet, Institut Pasteur, 26 Rue de Docteur Roux, Paris, France.

Downloaded from jpet.aspetjournals.org at ASPET Journals on September 16, 2016

on isolated guinea pig ileum (9, 12, 13), and all exert some anti-acetylcholine action on isolated intestinal musculature. The effect of Benadryl on spontaneous or induced activity of uterine tissue has not been determined. Since Neoantergan is capable of stimulating isolated uterine tissue of the guinea pig (9), its homologue, Pyribenzamine may possess the same property even though both drugs antagonize the spasmogenic action of histamine on uterine tissue (9, 13, 14).

EXPERIMENTAL. Healthy female dogs were anesthetized with pentobarbital sodium (30 mgm./kgm., I.P.) and prepared for recording arterial blood pressure from the carotid artery by means of a mercury manometer. Respiration was recorded by a Marey tambour connected to a pneumograph fixed securely to the side of the chest. Duodenal and uterine activity were recorded by inserting a small balloon filled with water into the respective organ. The balloon was connected to a water manometer at a level of approximately 30 cm. above the organ. The contractions were relayed to a Marey tambour and recorded on a kymograph.

All drugs were administered intravenously, and unless otherwise specified, the stated doses represent amounts given per kgm. of body weight. Following the control responses to 5 to 10 micrograms of histamine diphosphate, 2.5 to 5 micrograms of acetylcholine hydrobromide and 2 to 4 doses of epinephrine hydrochloride (10 to 20 micrograms, total dose), one of the antihistamine drugs was administered in a single dose of 3 mgm. over a period of one minute. After 10 minutes the previous doses of histamine, acetylcholine and epinephrine were repeated and the responses compared with those obtained prior to treatment with the antihistamine drug.

In view of the fact that the pressor response to epinephrine was usually agumented by each of the antihistamine compounds it appeared possible that such enhanced responses might be due to vagal blocking. In order to test this hypothesis the effect of each of the antihistamine compounds on the pressor response to epinephrine (10 to 20 micrograms, total dose) was tested on three or four dogs previously atropinized (1 mgm./kgm.) and on another group of three dogs previously vagotomized. Epinephrine was usually administred three to four times before and following the antihistamine compound. The recording of duodenal and uterine activity was omitted in these experiments.

RESULTS. *A. Altered vascular responses.* Table 1 contains quantitative data relating to the effects of Benadryl, Pyribenzamine and Neoantergan on the depressor effects of histamine and acetylcholine. The average depressor response to histamine was diminished by 67 per cent under these experimental conditions following the administration of 3 mgm. of Benadryl. At this dose level the α-aminopyridine compounds were approximately as effective as Benadryl in antagonizing the depressor response to histamine.

Benadryl diminished the depressor response to acetylcholine by 41 per cent. In contrast to Benadryl, Pyribenzamine and Neoantergan did not diminish the acetylcholine-induced hypotension (table 1 and figure 1).

Following the intravenous administration of any one of the three antihistamine compounds the magnitude of the mean pressor response to epinephrine was definitely enhanced; 42 per cent for Benadryl, 45 per cent for Pyribenzamine and 28 per cent for Neoantergan (table 2, and figure 1). The pressor responses were prolonged by 50 to 75 per cent. This enhanced response to epinephrine was a consistent finding in all experiments with each of the drugs, although more variable and less in degree after treatment with Neoantergan.

Downloaded from jpet.aspetjournals.org at ASPET Journals on September 16, 2016

Experiments were next made in four vagotomized and four atropinized dogs to determine whether epinephrine potentiation still obtained when cardiac vagal action was already eliminated in animals to be treated with antihistamine drugs. A comparison of the degree of the enhanced pressor response to epinephrine caused by antihistamine drugs in normal, non-atropinized animals with that which occurred in atropinized animals (table 2) revealed that the phenomenon still persisted and was in no instance significantly diminished in atropinized dogs. An equal number of experiments were made in vagotomized animals and the data (not included in table 2) were almost identical to, and thus in support of, that obtained in atropinized dogs. Other strong support for the belief that the potentiation of epinephrine was not referable to vagal blocking caused by antihistamine drugs is apparent in the data. Before injection of

TABLE 1

Effect of antihistamine drugs on smooth muscle activity and on depressor actions of histamine and acetylcholine

TREATMENT	HISTAMINE DIPHOSPHATE, 5 TO 10 MICROGRAMS/KGM., I.V., DEPRESSOR RESPONSES*				ACETYLCHOLINE HYDROBROMIDE. 2.5 TO 5 MICROGRAMS/KGM., I.V., DEPRESSOR RESPONSES*				DUODENUM‡	UTERUS‡	RESPIRATION
	Before	After	Difference† $M_1 - M_2 \pm$ P. E.		Before	After	Difference† $M_1 - M_2 \pm$ P. E.				
	mm. Hg	*mm. Hg*	*mm. Hg*	*per cent*	*mm. Hg*	*mm. Hg*	*mm. Hg*	*Per cent*			
Benadryl	61	20	-41 ± 5.44	−67	42	25	-17 ± 5.52	−41	Decreased tone and motility	No effect	Stimulation
Pyribenzamine	59	27	-32 ± 4.12	−53	41	42	$+1 \pm 2.12$	+2	Spasmogenic	Spasmogenic	Stimulation
Neoantergan	55	27	-28 ± 4.82	−51	32	45	-13 ± 5.08	+40	Spasmogenic	Spasmogenic	Stimulation

* Average of 8 depressor responses caused by histamine or acetylcholine before and after treatment of 4 dogs with each antihistamine drug.

† Where $M_1 - M_2 = 3 \times$ P.E., the difference was considered significant (Pearl; Medical Biometry and Statistics, 1930).

‡ Each of the antihistamine drugs usually blocked the spasmogenic action of histamine on the duodenum and uterus.

antihistamine drugs in atropinized dogs the pressor responses to epinephrine never exceeded the corresponding responses in normal dogs. Thus, if atropine itself failed to augment the pressor action of small doses of epinephrine in dogs anesthetized with pentobarbital it is apparent that the phenomenon noted after treatment with antihistamine drugs is not due to an atropine-like action.

B. Activity of the duodenum and uterus. Benadryl reduced the tone and inhibited the spontaneously active duodenum (figure 2) for 10 to 30 minutes and also prevented the spasmogenic effects of histamine on this organ. Neoantergan and Pyribenzamine also regularly antagonized the spasmogenic effects induced by the intravenous administration of histamine, but these compounds differed from Benadryl in that they induced or increased duodenal tone for 3 to 30 minutes when administered intravenously (table 1 and figure 2). This effect usually began within 2 to 3 minutes following injection. The small dose

Downloaded from jpet.aspetjournals.org at ASPET Journals on September 16, 2016

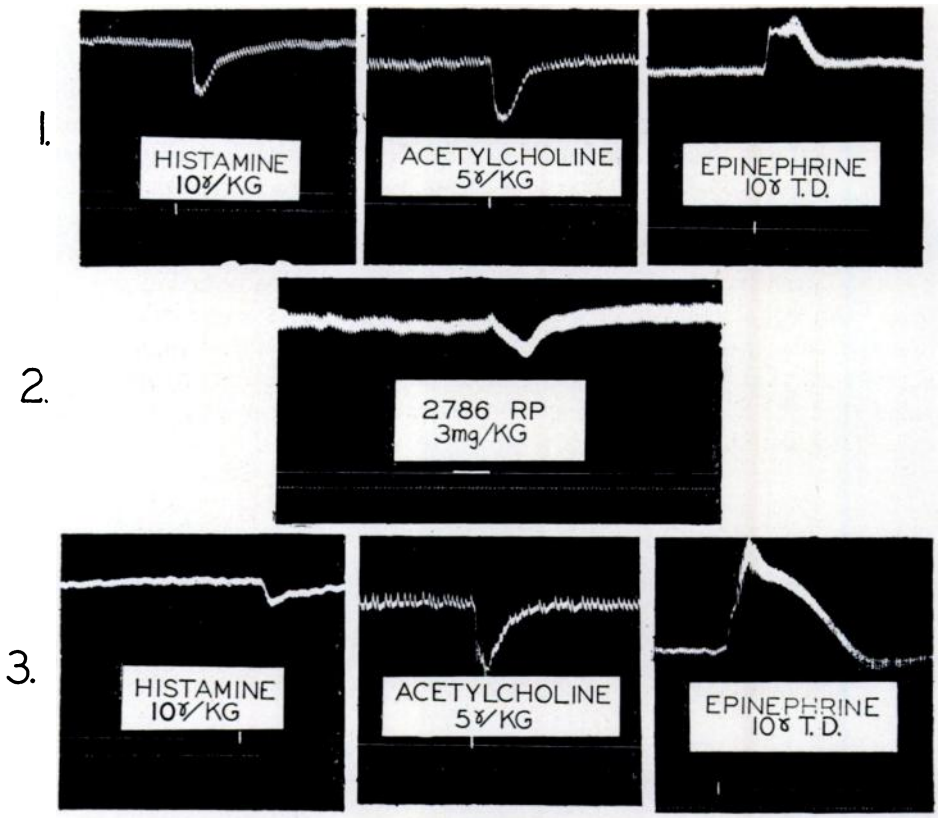

FIG. 1. BLOOD PRESSURE RECORD SHOWING THE DEPRESSOR ACTION OF HISTAMINE AND ACETYLCHOLINE AND THE PRESSOR ACTION OF EPINEPHRINE BEFORE AND AFTER 2786 R. P. (NEOANTERGAN)

Time = 5 second intervals. 1. Upper: Depressor response to histamine diphosphate and acetylcholine hydrobromide, and pressor response to epinephrine hydrochloride before 2786 R. P. 2. Center: Injection of 2786 R. P. 3. Lower: Depressor responses to histamine diphosphate and acetylcholine hydrobromide, and pressor response to epinephrine hydrochloride 10 to 30 minutes following the administration of 2786 R. P.

TABLE 2

Influence of antihistamine drugs on the pressor action of epinephrine in normal and atropinized dogs

Average pressor responses to epinephrine hydrochloride, 10 to 20 micrograms, total dose

TREATMENT 3.0 MGM./KGM., I.V.	NORMAL (4 DOGS)					ATROPINIZED‡ (4 DOGS)				
	No. of injections*	Before	After	Difference† $M_1 - M_2 \pm$ P.E.		No. of injections*	Before	After	Difference† $M_1 - M_2 \pm$ P.E.	
		mm. Hg	*mm. Hg*	*mm. Hg*	*per cent*		*mm. Hg*	*mm. Hg*	*mm. Hg*	*per cent*
Benadryl	12	45	64	+19 ±4.88	+42	34	38	52	+14 ±2.48	+33
Pyribenzamine	12	40	58	+18 ±5.22	+45	18	40	67	+27 ±4.66	+68
Neoantergan	21	39	50	+11 ±1.19	+28	26	38	55	+17 ±5.48	+45

* Number of injections before and again after injection of antihistamine drugs.

† Where $M_1 - M_2 = 3 \times$ P.E., the difference was considered significant (Pearl; Medical Biometry and Statistics, 1930).

‡ Essentially identical data were obtained from an equal number of vagotomized dogs.

Downloaded from jpet.aspetjournals.org at ASPET Journals on September 16, 2016

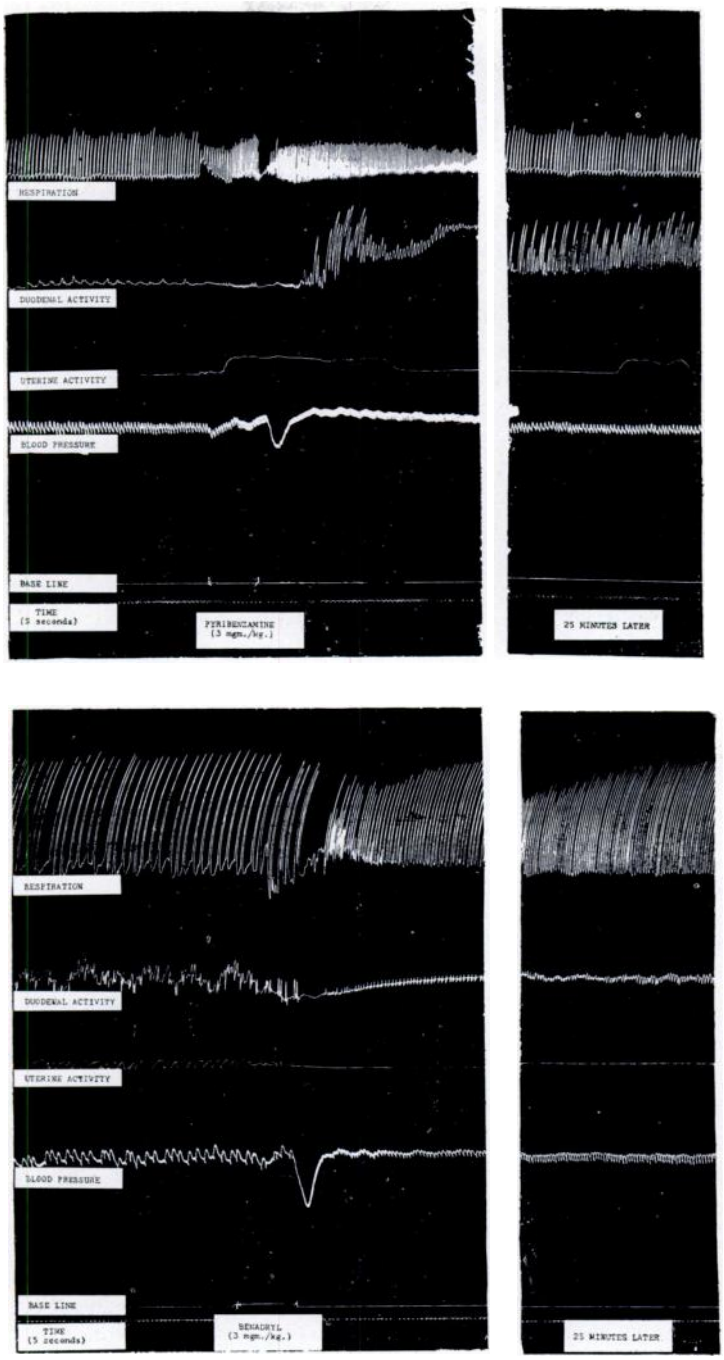

FIG. 2. 1. Upper: Effects of Pyribenzamine on respiration, duodenal and uterine activity, and on blood pressure. 2. Lower: Effects of Benadryl on respiration, duodenal and uterine activity, and on blood pressure. (Note: The small excursions in the uterine record are respiratory movements; not uterine contractions.)

Downloaded from jpet.aspetjournals.org at ASPET Journals on September 16, 2016

of acetylcholine chosen to induce the desired depressor response only occasionally stimulated the duodenum and uterus, thus precluding the possibility of demonstrating any anti-acetylcholine action of these drugs on these organs.

With regard to the uterus, Benadryl had no regular effect on spontaneous activity, but the activity induced or augmented by histamine was blocked in three of the four experiments. In no instance did Pyribenzamine or Neoantergan reduce the spontaneous activity of the uterus, although they did oppose the spasmogenic action of histamine. The α-aminopyridine compounds differed from Benadryl in that they quite regularly induced uterine contraction when injected intravenously (figure 2). These induced contractions were equal in magnitude to those occurring spontaneously at intervals during the experiments, and persisted for approximately three minutes.

C. Bronchospasm in guinea pigs. The approximate minimal doses of Benadryl, Pyribenzamine and Neoantergan which were capable of diminishing

TABLE 3

The anti-asthmatic action and acute toxicity of antihistamine drugs

TREATMENT OF GUINEA PIGS			MORTALITY		DECREASE IN PERCENTAGE MORTALITY	P*	ACUTE TOXICITY IN MICE, $LD_{50} \pm$ S.E.
Drug	Mol. wt.	Dose	Ratio	Per cent			
		mgm./kgm., I.P.					*mgm./kgm., I.P.*
Controls (untreated) ...			20/20	100			
Benadryl.....	291.45	1.5	16/20	80	20	0.04	75 ±5.2
Pyribenzamine.....	203.65	0.3	7/20	35	65	<0.001	80 ±5.8
		0.1	19/20	95	5		
Neoantergan.....	267.22	0.075	14/20	70	30	0.01	90 ±6.9

* P value (from Fisher's table) less than 0.05 indicates a significant difference from mortality in control group.

the incidence of mortality in guinea pigs subjected to atomized histamine were determined. Data presented in table 3, including the LD_{50} for each of the antihistamine drugs, were supplied to us through the cooperation of Dr. A. C. Bratton and Margaret E. Kaiser of Parke, Davis and Company Research Laboratories. The experimental conditions were identical to those previously employed to determine the relative potency of benzhydryl alkamine ethers (15), alkyloxytriazines (23) and other drugs (24).

On a dosage basis, Benadryl proved to be about one-fifth as potent as Pyribenzamine. These data are in general agreement with other data which indicate that Pyribenzamine is more potent than Benadryl in counteracting histamine bronchoconstriction (25), although it was demonstrated that the two drugs were equally effective in diminishing the mortality rate in anaphylactic shock. In the present experiment, the potency of Neoantergan was found to exceed that of Pyribenzamine and Benadryl four and twenty times, respectively.

Downloaded from jpet.aspetjournals.org at ASPET Journals on September 16, 2016

There exists a close similarity in the acute toxicity of the three antihistamine drugs in mice (table 3). Five doses of each drug were injected intraperitoneally in twelve mice of both sexes, each weighing 20 grams. Deaths were recorded for five days and the LD_{50} determined from dose-mortality data graphed on log probit paper.

DISCUSSION. Hypotension induced in dogs by the intravenous administration of histamine was definitely diminished after treatment with antihistamine drugs, thus confirming earlier findings concerning Neoantergan (9–11), Benadryl (12, 17) and Pyribenzamine (26). The fixed intravenous dose of 3 mgm./kgm. for each drug resulted in essentially the same degree of antagonism of the histamine-induced hypotension. This does not permit the conclusion, however, that equal potency has been demonstrated for the three antihistamine compounds with respect to the depressor effects of histamine, since the dose of Benadryl, and possibly the α-aminopyridines, must be varied several fold in order to elicit an appreciable change in the percentage inhibition of the depressor action of histamine (12, 17). The ability of the antihistamine drugs to diminish the vascular response to histamine is of importance because of the major role of vascular reactions in anaphylaxis (27) and allergy (8).

Neoantergan and Pyribenzamine were definitely more potent than Benadryl in preventing fatal bronchospasm in guinea pigs. Variations in absorption, distribution and excretion would influence the degree of action. Relative potencies based upon a given pharmacological response may diverge widely when based upon response of a different tissue or organ. However, it is of interest to note that Pyribenzamine has recently been shown to be more effective than Benadryl in reducing the severity of histamine shock, yet no more effective in preventing fatal anaphylactic shock in guinea pigs (25) even though much evidence indicates that the severe bronchospasm occurring during anaphylaxis in this species is due to histamine (cf. 18 for refs.). In anaphylaxis, rate of histamine release and site of action may differ materially from conditions prevailing when histamine is injected or inhaled.

As previously reported (11, 12, 14, 26) each of these antihistamine compounds augment the pressor response to epinephrine. This phenomenon could be referable to a sensitization of the myocardium or arterioles to epinephrine or to the fact that the antihistamine drugs diminished or blocked vagal, cardioinhibitory impulses. The latter seemed most likely in view of the atropine-like properties of Benadryl and even possible with Pyribenzamine and Neoantergan since these compounds, like p-N-alkoxyphenylethylamines (28), could block the cardiac vagus even though failing to diminish the depressor action of acetylcholine. However, the data presented appear to rule out the possibility that the enhanced pressor response to epinephrine could be due to blocking of the cardiac vagal fibers. Yonkman *et al.* (20) sometimes noted evidence of adrenergic (and cholinergic!) potentiation relative to salivary secretion and response of the nictitating membrane in cats when injected intra-arterially with small doses of Pyribenzamine. Antergan also enhances certain responses to epinephrine (29). An obvious need exists for quantitative data and extended

Downloaded from jpet.aspetjournals.org at ASPET Journals on September 16, 2016

studies referable to augmentation of adrenergic responses after treatment with antihistamine drugs.

Since Neoantergan and Pyribenzamine are homologous compounds one would expect a close similarity in their pharmacological properties as reported, respectively, by Bovet and co-workers (9–11) and by Mayer and his collaborators (13, 19, 20, 26). The present study revealed no qualitative differences between Neoantergan and Pyribenzamine. However, Neoantergan was more effective in preventing fatal, histamine-induced bronchospasm in guinea pigs. Compared on a weight basis the acute toxicity in mice was essentially the same for each compound.

Unlike Neoantergan and Pyribenzamine, Benadryl antagonized the depressor action of acetylcholine as demonstrated in previous (12) and present experiments. Studies on isolated guinea pig ileum (12) revealed that the atropine-like property of Benadryl was much weaker than that of Trasentin and Papaverine. Similar studies with Neoantergan and Pyribenzamine (9, 13) indicated some degree of anti-acetylcholine action. This weak and restricted atropine-like action of the α-aminopyridine compounds in conjunction with their strong antihistamine action indicates that they may be more specific histamine antagonists than Benadryl. The latter compound possesses antispasmodic properties which contrast with the tendency of Neoantergan and Pyribenzamine to act as intestinal and uterine spasmogenic agents.

The similarities and differences in properties of Benadryl, Neoantergan and Pyribenzamine should be considered when choosing antihistamine compounds for use as pharmacological tools. Following extensive clinical use, consideration of the advantages and deficiencies of the individual antihistamine compounds in relation to pharmacological properties should render it possible to develop antihistamine compounds possessing a wider range of utility and a greater degree of clinical effectiveness and safety.

SUMMARY

1. In dogs, equal doses of Benadryl, Pyribenzamine and Neoantergan were of the same effectiveness in antagonizing the depressor action of histamine, whereas the depressor action of acetylcholine was diminished only by Benadryl.

2. Pyribenzamine and Neoantergan may be more specific than Benadryl as regards antihistamine action, and are more potent relative to prevention of fatal histamine shock in guinea pigs. Benadryl exerted an antispasmodic action on the dog's intestine but not on the uterus and all the compounds diminished the spasmogenic action of histamine on the duodenum and uterus. Pyribenzamine and Neoantergan contracted the duodenum and uterus.

3. These antihistamine compounds enhanced the pressor response to epinephrine, the phenomenon being unrelated to a cardiac vagal blocking action.

REFERENCES

1. O'Leary, P. A., and Farber, E. M.: Proc. Staff Meet., Mayo Clin., **21:** 295, 1946.
2. Todd, L. C.: Ann. Allergy, **4:** 282, 1946.
3. Arbesman, C. E., Koepf, G. F., and Lenzner, A. R.: Jour. Allergy, **17:** 275, 1946.

Downloaded from jpet.aspetjournals.org at ASPET Journals on September 16, 2016

4. Baer, R. L., and Sulzberger, M. B.: Jour. Invest. Dermat., **7:** 147, 1946.
5. Decourt, P.: Bull. et mém. Soc. méd. hôp. de Paris, **59:** 3, 1943.
6. Sciclounoff, R., and Junet, R.: Rev. med. de la Suisse Rom., **63:** 570, 1943.
7. *Council on Pharmacy and Chemistry*, J. A. M. A., **132:** 702, 1946.
8. Harkavy, J.: Arch. Int. Med., **67:** 709, 1941.
9. Bovet, D., and Walthert, France,: Ann. pharm. franc., **2:** suppl. to no. 4, 1, 1944.
10. Bovet, D., Horclois, R., and Walthert, France,: Compt. rend. Soc. de biol., **138:** 99, 1944.
11. Bovet, D., Horclois, R., and Fournel, J.: *Ibid.*, 165, 1944.
12. Loew, E. R., MacMillan, R., and Kaiser, Margaret,: This Journal, **86:** 229, 1946.
13. Mayer, R. L.: Jour. Allergy, **17:** 153, 1946.
14. Sherrod, T. R., Schloemer, H. F., and Loew, E. R.: Fed. Proc., **5:** 202, 1946.
15. Loew, E. R., Kaiser, Margaret E., and Moore, V.: This Journal, **83:** 120, 1945.
16. Loew, E. R., and Kaiser, Margart E.: Proc. Soc. Exper. Biol. and Med., **58:** 235, 1945.
17. Wells, J. A., Morris, H. C., Bull, H. B., and Dragstedt, Carl A.: This Journal, **85:** 122, 1945.
18. Wells, J. A., Morris, H. C., and Dragstedt, Carl A.: Proc. Soc. Exper. Biol. and Med., **61:** 104, 1946.
19. Mayer, R. L., Huttrer, C. P., and Scholz, C. R.: Sci., **102:** 93, 1945.
20. Yonkman, F. F., Chess, Dorothy, Mathieson, D., and Hansen, Nicoline: This Journal, **87:** 256, 1946.
21. Yonkman, F. F., Hays, H. W., and Rennick, B.: Fed. Proc., **4:** 144, 1945.
22. Arbesman, C. E., Koepf, G. F., and Miller, G. E.: Jour. Allergy, **17:** 203, 1946.
23. Loew, E. R., Kaiser, Margaret E., and Anderson, Mona: This Journal, **86:** 7, 1946.
24. Loew, E. R., Kaiser, Margaret E., and Moore, V.: This Journal, **86:** 1, 1946.
25. Friedlaender, S., Feinberg, S. M., and Feinberg, A. R.: Proc. Soc. Exper. Biol. and Med., **62:** 65, 1946.
26. Yonkman, F. F., Chess, Dorothy, Hays, H. W., Rennick, Barbara, and Mayer, R.: Fed. Proc., **5:** 216, 1946.
27. Nolf, P., and Adant, M.: Arch. int. pharmacodyn. et therap., **72:** 93, 1946.
28. Broom, W. A., and Wayne, E. J.: This Journal, **86:** 83, 1946.
29. Parrot, J. L.: Compt. rend. Soc. biol., **137:** 378, 1943.

Downloaded from jpet.aspetjournals.org at ASPET Journals on September 16, 2016