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Pharmacological Characteristics of Neohetramine, a New Antihistaminic Drug.* I.

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The symptomatic relief afforded by antihistaminic agents in the treatment of various allergic phenomena has stimulated the search for superior drugs. This search has led to the synthesis of Neohetramine,† 2-(N-dimethylaminoethyl-N-p-methoxybenzyl) amino pyrimidine monohydrochloride. Comparison with other antihistaminic drugs reveals that Neohetramine is relatively non-toxic, and is efficient both in counteracting the effects of histamine and in preventing anaphylactic shock. Preliminary details of this study are set forth below.

Experimental. Acute Toxicity. Acute toxicities of Neohetramine and other antihistaminic drugs were determined in mice (17 to 22 g males, C.F. 1 strain) and guinea pigs (300 to 400 g males). In both species signs of acute toxicity consisted of convulsions, complete extension of fore and hind limbs followed by respiratory paralysis and cardiac arrest. Necropsies performed daily for one week on surviving mice disclosed no gross abnormalities. The LD_{50} doses and associated limits of error, computed graphically,[1] are summarized in Table I. Neohetramine is the least toxic member of the series. In mice, the oral LD_{50} of Neohetramine is approximately twice as high as the intraperitoneal LD_{50}, and in guinea pigs, it is about 5 times as high.

Chronic Toxicity. Neohetramine was administered to 105 weanling rats‡ (approximately 20 per group) either by way of the diet (50, 100, 200 mg/kg daily) or subcutaneously (10, 20 mg/kg twice daily) for a period of 91 days. The experimental animals and 21 (untreated) controls were weighed frequently and complete blood counts were taken at regular intervals. At the end of the test period the rats receiving Neohetramine were indistinguishable from their controls. Treated animals grew at a normal rate, exhibited no abnormalities in blood morphology,

TABLE I.
Acute Toxicity of Neohetramine.

Drug	LD_{50} mg/kg (base)	Limits of error (%)
Benadryl*	74.6	91-110
Pyribenzamine*	65.3	95-106
Hetramine*	61.0	91-110
Neohetramine*	119.0	96-105
" †	245.0	91-110
" † (guinea pig)	493.0	73-137

* Dose given intraperitoneally.
† Dose given orally.

TABLE II.
Protection Against Intravenous Histamine.

Drug	TD_{50}* mg/kg (base)	Limits of error (%)
Hetramine	2.72	85-118
Neohetramine	1.15	64-147
Benadryl	1.00	74-135
Pyribenzamine	0.055	64-148

* Dose protecting 50% of the animals.

TABLE III.
Protection Against Nebulized Histamine.

Drug	TD_{50} mg/kg i.p.	Limits of error (%)
Hetramine	6.4	74-136
Benadryl	3.5	70-143
Neohetramine	3.5	73-140
Pyribenzamine	0.2	65-155
Neohetramine*	3.6	76-132
Pyribenzamine*	0.8	78-128

*Histamine and drug nebulized from same solution.

* We are indebted to Mr. Bernard S. Rubin for his assistance.

† Neohetramine was formerly known as NH-188.

[1] de Beer, *J. Pharmacol.*, 1945, **85**, 1.

‡ Rats were maintained on a stock diet of Purina Dog Chow and guinea pigs were maintained on Rockland Rabbit diet.

TABLE IV.
Protection Against Anaphylactic Shock in the Guinea Pig.

Antihistaminic drug*	Dosage (mg/kg i.p.)	No. survivals/ No. animals injected	% survival
None (controls)	—	4/31	12.9
Neohetramine†	5	8/19	42.1
	10	12/20	60.0
	25	10/20	50
Pyribenzamine†	10	13/20	65
	25	14/20	70
Benadryl	25	3/10	30

* Administered intraperitoneally 30 minutes before the shock injection of horse serum.
† The differences between Neohetramine and Pyribenzamine at the 10 and 25 mg dose levels, tested by the Chi Square Method, are not significant.

and developed no organ pathology. We are indebted to Professor George K. Higgins of the New York Medical College for the examination of all microscopic sections.

Protection against intravenously administered histamine. Groups of 10 male guinea pigs (300 to 400 g) were given graded intraperitoneal doses of the various antihistaminics. Thirty minutes later, a uniformly fatal dose (LD_{100}) of histamine diphosphate (0.5 mg per kg histamine) was injected intravenously with the results shown in Table II. In this test Hetramine was the least active, Neohetramine and Benadryl were about equally active and Pyribenzamine was the most active antagonist.

Protection against nebulized histamine. Groups of 10 guinea pigs (300 to 400 g males) were exposed, 4 at a time, to an atmosphere containing histamine aerosol.[2] In these experiments, 0.35 cc of a solution containing 12.5 mg histamine per cc was nebulized in 10 minutes into an 18 liter chamber. Untreated animals invariably died of typical histamine poisoning, but animals exposed 30 minutes after the intraperitoneal injection of increasing doses of the antihistaminic agents were protected as shown in Table III. When tested in this manner Neohetramine has the same potency as Benadryl. Pyribenzamine appears to be 17 times more active than Neohetramine but when, by a slight modification of procedure, histamine and antihistaminic drug were nebulized from the same solution, Pyribenzamine was only 4-5 times as potent as Neohetramine.

Influence on Capillary Permeability. The ability of the various drugs to prevent histamine wheals was determined according to a modification of the method of Last and Loew.[3] Two concentrations of antihistaminic drug (1 and 4 micromols per cc) were combined with equal volumes of histamine in each of 7 concentrations (1, 2, 4, 8, 16, 32, and 64 micromols per cc). Randomized intracutaneous injections of 0.2 cc of each solution were made into the shaved abdominal skin of the rabbit and immediately afterward, 10 cc of 1% aqueous trypan blue was injected intravenously. Histamine antagonism was indicated by failure of the injected site to become blue. The end-point was taken as the highest concentration of histamine antagonized by each dose of antihistaminic drug. An analysis of variance performed on the data of quadruplicate assays indicated that differences attributable to animal variation were nonsignificant. Mean values are, therefore, presented. The data show that the effect of a solution containing 1.9 micromols of histamine per cc (*i.e.*, 0.21 mg) was completely nullified by the incorporation of one micromol of Neohetramine per cc (0.26 mg). In other words, one molecule of Neohetramine antagonized approximately 2 molecules of histamine. Under similar conditions one mole-

[2] Loew, E. R., Kaiser, M. E., and Moore, *J. Pharmacol.*, 1945, **83**, 120.

[3] Last, M. R., and Loew, E. R., *J. Pharmacol.*, 1946, **89**, 81.

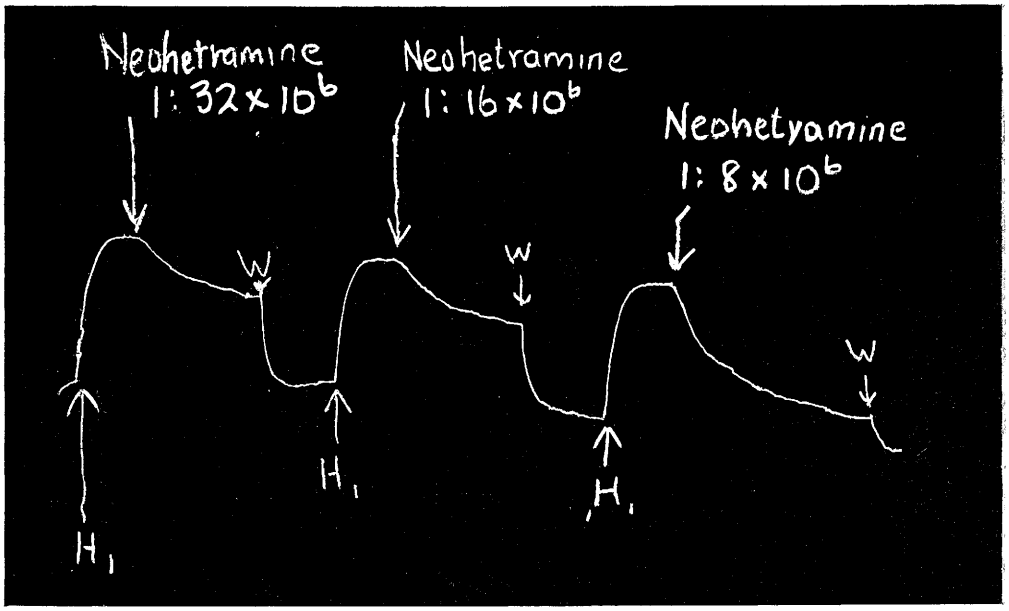

FIG. 1.

cule of Benadryl inhibited 1.5 molecules of histamine and one of Pyribenzamine nullified the action of nearly 9 molecules of histamine.

Excised tracheal tissue. Excised guinea pig tracheal tissue was studied according to the method of Castillo and de Beer.[4] In a 25 cc bath using Hastings-van Dyke solution with glucose, uniform contractions of the tracheal 'chains" produced by 0.72 μg of histamine per cc of bath fluid were reduced by incorporating in the histamine solution graded concentrations of Neohetramine (.029 to 1.876 μg per cc of bath fluid), or Pyribenzamine (.026 to 1.664 μg per cc). As shown in Figure 1, a good linear response was obtained with increasing concentrations of Neohetramine, whereas with Pyribenzamine the response was more erratic. Since the highest drug concentrations were equimolar and reduced the histamine contraction to the same extent (55%), Neohetramine and Pyribenzamine exhibited equal activity against this test object.

Anaphylaxis. The antianaphylactic activity of the various drugs was studied in 140 guinea pigs (300 to 400 g males). Approximately 2 weeks after intraperitoneal sensitization with one cc of 10% horse serum,[5] 27 of 31 untreated animals succumbed to an intravenous shocking dose of 0.5 cc of undiluted horse serum. Intraperitoneal injection of the antihistaminics 30 minutes before the shock dose resulted in survival as shown in Table IV. Protection was obtained with as little as 5 mg of Neohetramine per kg. When 10 mg per kg was injected 60% of the animals survived, but larger doses afforded no greater protection. Pyribenzamine exhibited the same order of activity as Neohetramine, and afforded no greater protection at higher dose levels. Benadryl afforded some protection (30%) at a dosage of 25 mg per kg but 50 mg per kg proved toxic to 3 out of 4 animals. Further experiments involving passive sensitization will be reported in the near future.

Discussion. Wherever possible, the foregoing data were recalculated in terms of the number of molecules of histamine antagonized by one molecule of Neohetramine; the ratios thus obtained varied from 0.5 to 9.0, indicating that the amount of histamine antagonized depended upon the test method employed. Depending on the type of tissue, the species

[4] Castillo, J. C., and de Beer, E. J., *J. Pharmacol.*, 1947, **89**, 104.

[5] Frank, D. E., *J. Immunol.*, 1946, **52**, 59.

of animal, the mode of administration, etc., Pyribenzamine may appear to be one or 20 times as active, and Benadryl may appear to be one-half or equally as active as Neohetramine. The significance of these comparisons is not clear because laboratory methods of testing are as yet unrelated to clinical effectiveness.

In a clinical comparison of 2 antihistaminic drugs, Loveless[6] recently reported that Benadryl was better suited to the treatment of Ménières syndrome and intrinsic allergic asthma while Pyribenzamine was more effective in the treatment of non-seasonal extrinsic asthma. It may be inferred from this report that different drugs will be required in the treatment of different forms of hypersensitivity. Since laboratory methods of testing do not give a reliable index of the clinical value of the antihistaminic drugs, it would appear that final evaluation of these drugs can be established only in human subjects, and then only with reference to the treatment of specific allergic states.

Summary. As judged by the intraperitoneal toxicity in mice, Neohetramine is about one-half as toxic as other antihistaminic agents. Weanling rats, receiving as much as 200 mg of Neohetramine per kg body weight per day over a period of 3 months, grew at a normal rate, exhibited no abnormalities in blood morphology and developed no organ pathology. Neohetramine showed marked activity against the bronchiolar and capillary actions of histamine. Quantitative estimates of the amount of histamine antagonized by Neohetramine varied with the method of testing. Neohetramine conferred protection against anaphylactic shock in guinea pigs actively sensitized to horse serum. The implications of these findings are discussed.

[6] Loveless, M. H., *Am. J. Med.*, 1947, **3**, 296.

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Pharmacological Characteristics of Neohetramine, a New Antihistaminic Drug. II.

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Several compounds which have anti-histaminic properties have been described recently.[1,2] Stress has been laid mainly on their ability to counteract histamine activity and certain allergic phenomena. Many of these compounds possess other properties as shown by their action on smooth muscle and glandular activity.[3,4] Neoantergan[3] is stated to have local anesthetic and quinidine-like properties. Benadryl[4] has some atropine-like activity. Pyribenzamine[5] and 01013[6] enhance epinephrine responses. Described below are some effects of a new anti-histamine compound, Neohetramine.

Its anti-histamine activity was tested on the isolated ileum of guinea pig; isolated uteri of guinea pig, cat, and rat; blood pressure of cats and dogs; the intestine of the cat *in situ*, and blood vessels of the rabbit ear perfused at room temperature. Flow of saliva from submaxillary gland and blood pressure of the cat and dog served to test the effects of Neohetramine on the autonomic nervous system. All excised organs were suspended in

[1] Feinberg, S. M., *J. A. M. A.*, 1946, **132**, 703.

[2] Winters, C. A., *J. Pharmacol. and Exp. Therap.*, 1946, **87**, 256.

[3] Dews, P. B., and Graham, J. D. P., *British J. Pharmacol.*, 1946, **1**, 278.

[4] Dreyer, N. B., and Denton, C., *Fed. Proc. Am. Soc. Exp. Biology*, 1947, **6**, 324.

[5] Yonkman, F. F., *et al.*, *J. Pharmacol. and Exp. Therap.*, 1946, **87**, 256.

[6] Lee, H. M., *et al.*, *ibid.*, 1947, **90**, 83.