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COMPARISONS OF VARIOUS HISTAMINE ANTAGONISTS

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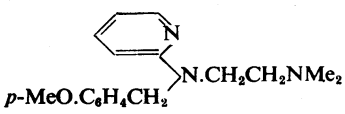
The experiments described below were undertaken in order to obtain quantitative comparisons of the actions of various histamine antagonists on various different tissues. Consideration has also been given to methods of carrying out two special types of experiment: the estimation of histamine antagonists by biological assay, and their use to aid the identification of histamine in tissue extracts and other fluids (Pellerat and Murat, 1945).

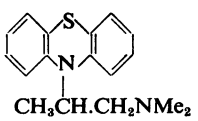
In order to test the specificity of the various antagonisms a number of drugs have been used to cause effects which were subsequently suppressed by the different antagonists. These active drugs included histamine, acetylcholine, potassium, nicotine, and adrenaline. In discussing these experiments the need has been felt for a collective term to describe these active drugs, and the word "agonist" has been adopted with some hesitation for this purpose. It may perhaps also be convenient in discussing other forms of antagonism to use this word to describe the active drug (acetylcholine, adrenaline, p-amino-benzoic acid, etc.) and to use the word antagonist to describe the drug (atropine, ergotoxine, sulphanilamide, etc.) which suppresses the action of the agonist.

METHODS

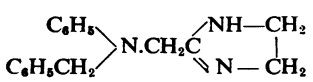
The formulae of the various antagonists used are shown below.



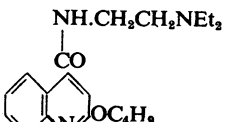
Neoantergan



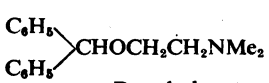
3277 RP



Antistin



Nupercaine



Benadryl

Concentrations of histamine are calculated in terms of histamine base and those of the other drugs either as molar concentrations or in terms of the salt used (nicotine tartrate, neoantergan maleate, antistin methane sulphonate and hydrochlorides of the other drugs).

Pieces of guinea-pig's ileum, 2–3 cm. long, were aerated in a 2-c.c. bath containing Tyrode's solution. The bath was connected with two flasks so that it could be filled either with ordinary Tyrode's solution or with a similar solution containing the antagonist.

Pieces of uterus from rabbits, cats, and guinea-pigs were suspended in a 40-c.c. bath filled with Dale's solution. Some of these uteri had been stored overnight at 4° C.

Isolated hearts from rabbits and cats were perfused with thoroughly oxygenated Locke's solution by Langendorff's method. The drugs were injected through the rubber tubing, close to the aorta. In all these experiments the temperature was 37° C.

Experiments on frog's plexus anaesthesia were carried out at room temperature by Sollmann's method as described by Bülbring and Wajda (1945).

RESULTS

Guinea-pig's ileum

Much previous work on the action of antihistamine compounds on isolated intestine has been devoted to antergan, benadryl and pyribenzamine, which are not included in this study (Halpern, 1942; Loew *et al.*, 1946; Winder *et al.*, 1946; Halpern and Mauric, 1946); Bovet (1944) and his collaborators give data for neoantergan. Meyer and Bucher (1946) found that antistin was more active against histamine than against acetylcholine. According to Halpern and Ducrot (1946) 3277 RP is not much more active against histamine than antergan, but Winter (1947) reports that 3277 RP is less active than neoantergan and more active than benadryl.

The results of such investigations are likely to depend to some exent on the design of the experiment. The method proposed by Schild (1947) appears to be particularly satisfactory, and has been adopted here. The action of the agonist is

first tested in ordinary Tyrode's solution, and a number of equal submaximal effects obtained at regular intervals. A similar solution containing the antagonist is then used to fill the bath and the dose of the agonist is doubled. The response may be increased at first, but it gradually diminishes and usually reaches a steady level in about 15 minutes. The object of the experiment is to find a concentration of the antagonist such that these final responses after 15 min. are equal to the original responses to half the dose in the absence of the antagonist. The whole experiment is repeated until this concentration is found. The negative logarithm of the molar concentration which has this effect is defined as the pA_2. Two tests with different drugs are shown in Fig. 1. In the first test the concentration of the antagonist was too low and in the second it was too high.

The pA_2 can be determined by plotting the results by the method shown in Fig. 2, in which

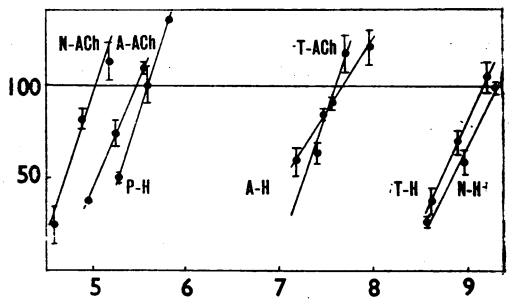


FIG. 2.—Guinea-pig's intestine. Ordinates: mean response (4–10 tests) to a double dose as percentage of the response to a single dose without antagonist. Abscissae: negative log. molar concentration of antagonist. ACh = acetylcholine. H = histamine. N = neoantergan. T = 3277 RP. A = antistin. P = nupercaine. Vertical lines show ± the estimated standard error.

the final response is calculated as a percentage of the initial response and plotted against the logarithm of the concentration of the antagonist. The figure corresponding to 100 per cent on this graph is the pA_2, and is determined by interpolation; the points can be satisfactorily fitted by straight lines. The points plotted in Fig. 2 are mostly means obtained from several experiments and the Figure shows standard errors calculated from the individual results; these show that the results are reasonably reliable, but more satisfactory evidence of the error of the method can be obtained by comparing independent estimates of the pA_2 using different pieces of gut. A few such estimates are shown in the Table; these results confirm the value of the method, and show the high potency of neoantergan and 3277 RP. The mean relative molar activities of the various drugs against histamine were nupercaine 1, antistin 125, 3277 RP 4,000, and neoantergan 5,500. Neoantergan is also much the most specific of these drugs. The ratio of its activity against histamine to its activity against acetylcholine was 19,500. The corresponding figure for antistin was 159, and for 3277 RP it was 36.5.

The procedure involves the assumption that after 15 min. the response to the agonist would remain unchanged. The fact that this is not certain may account for some of the variations in the results. Duplicate tests on the same piece of gut were sometimes done when the effect of the antagonist was quickly reversible and the inhibitions in such experiments usually agreed within 10–15 per cent. The time for recovery after 3277 RP was longer than after the other drugs.

The biological assay of neoantergan.—The above procedure would be a slow way of making biological assays of antihistamines. These can be carried out by giving a series of equal doses of histamine and observing the depression of the responses due to a brief addition of the antagonist to the bath. Fig. 3 shows that the effect of 0.002 μg. of neoantergan could be distingushed in this way from that of 0.001 μg. in the 2-c.c. bath. The effect was not always of this type, since the maximum depression sometimes occurred in the second response after the action of the antagonist. In judging the magnitude of the whole effect it is probably best to consider not only the maximum depression, but also the duration of the depression, since this was also increased when larger doses were used. It is possible in this way to make a rough assay of very small amounts of neoantergan, but care is needed, since the inhibition sometimes becomes weaker on successive additions of neoantergan (cf. Ackermann and Maurer, 1944).

The use of neoantergan as a specific test for histamine.—When the response of a piece of gut to a tissue extract is abolished by neoantergan this fact may be taken as evidence that the effect of the tissue extract was due to histamine, but the evidence is only convincing if the concentration of neoantergan is very low, since high concentrations abolish the responses to most drugs. A satisfactory method of carrying out tests of this kind is first to find doses of the extract and of histamine which cause equal effects, and then to continue giving these doses alternately and to study the effect, in the series of responses, of a brief addition (1 min.) of a small dose of neoantergan to the bath. The dose of neoantergan is chosen so as to pro-

TABLE

Values of pA_2 for guinea-pig's ileum—the negative logarithm of the molar concentration of antagonist which halves the sensitivity. Individual values, followed by value from Fig. 2

Antagonist	Histamine		Acetylcholine		Difference
Neoantergan	9.21, 9.41, 9.49 9.29, 9.29	9.32	4.95, 5.04 5.19	5.03	4.29
RP 3277	9.07, 9.09 9.21	9.18	7.67, 7.68	7.62	1.56
Antistin	7.55, 7.66 7.95, 7.95, 7.95	7.67	5.46, 5.57 5.58, 5.55	5.47	2.20
Nupercaine	5.52, 5.7	5.58			
	Results by Schild (1947)				
Neoantergan		9.46		4.86	4.6
Benadryl		8.02		6.57	1.45
Pethidine		6.13		5.84	0.29
Atropine		5.64		8.61	−3.03

duce 50–70 per cent inhibition of the subsequent response to histamine. The concentration of neoantergan for this effect is usually about 1/10 of the concentration of histamine.

Such an experiment is illustrated in Fig. 4, which shows that the action of neoantergan on the responses to the extract and to histamine were about equal both in magnitude and in duration. Such results provide evidence that the effect of the tissue extract was due to histamine. This is further illustrated in Fig. 5. In the first experiment shown there the extract was first compared both with histamine and with acetylcholine. The addition of neoantergan to the bath for 1 min. abolished the response to the extract and to histamine, but did not diminish the response to acetylcholine. In the second experiment neoantergan almost abolished the response to histamine but had little effect on the response to nicotine. In another experiment the response to nicotine tartrate (1 mg./l.) was unaffected by concentrations up to 400 μg./l. of neoantergan, but partially inhibited by a concentration of 4,000 μg./l., while a concentration of 1 μg./l. was sufficient to produce a marked and prolonged inhibition of equivalent responses to histamine. In another experiment the agonists were histamine (10 μg./l.) and KCl (800 mg./l.). The effect of histamine was completely abolished by neoantergan in a concentration of 10 μg./l. The effect of KCl was unaffected even when the concentration of neoantergan was increased to 1 mg./l. and was only partly inhibited when the concentration was 5 mg./l. The last result is shown in Fig. 5C.

Isolated uterus

Certain antihistamines themselves cause a contraction of the uterus which complicates experiments on their antagonism to histamine (Halpern, 1942). This antagonism has, however, been shown when the direct effect was abolished by leaving the antagonist in the bath for 1–2 hours (Bovet and Walthert, 1944), or by reducing the calcium concentration of the fluid (Halpern and Walthert, 1945), or by using small doses of the antagonist (Dews and Graham, 1946). Thiodiphenylamine compounds, such as 3277 RP, have been found to have no effect by themselves, but to antagonize histamine (Halpern and Ducrot, 1946).

Non-pregnant cat.—Neoantergan did not cause contraction of these uteri in concentrations up to 4 mg./l. Histamine (15–150 μg./l.) caused contractions which were abolished by equal concentrations of neoantergan. Lower concentrations of neoantergan (1/10 that of the histamine) still reduced the response by about 50 per cent. Higher concentrations were needed to interrupt an established histamine-response. Responses due to acetylcholine were unaffected by neoantergan in concentrations which abolished equivalent responses to histamine.

3277 RP (3 mg./l.) had no effect by itself, and did not interrupt an established response to histamine. If added 1–2 min. before histamine in equal concentration, it had little effect on the first subsequent response, but inhibited the second response completely. Recovery of the response to histamine was slow.

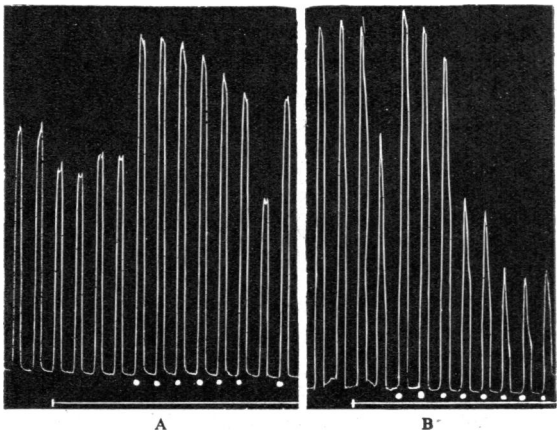


FIG. 1.—Guinea-pig's intestine. Schild's method of testing antagonists. Responses to acetylcholine, 12.5 μg./l. Dose doubled at times marked with a dot. Bath contained the antagonist at the times marked with a line. The effect of (A) neoantergan (2,500 μg./l.) was less than that of (B) 3277 RP (25 μg./l.).

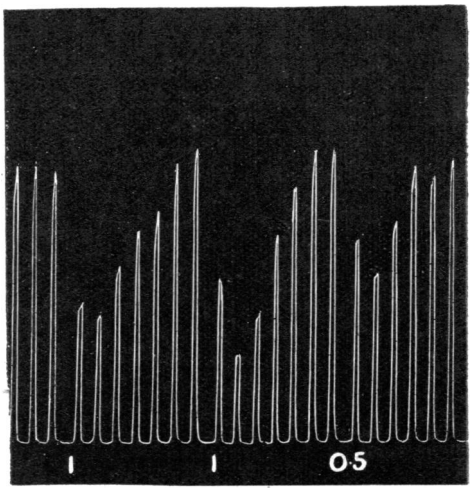


FIG. 3.—Guinea-pig's intestine. Assay of neoantergan. Responses to histamine (10 μg./l.) at intervals of 1 min. Inhibitory effects of neoantergan for 1 min. Effect of 1 μg./l. greater than that of 0.5 μg./l.

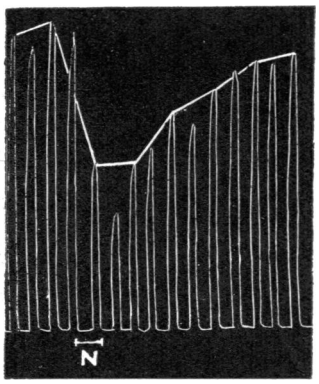
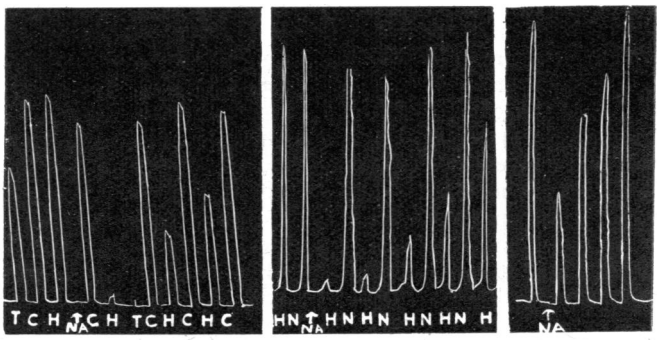
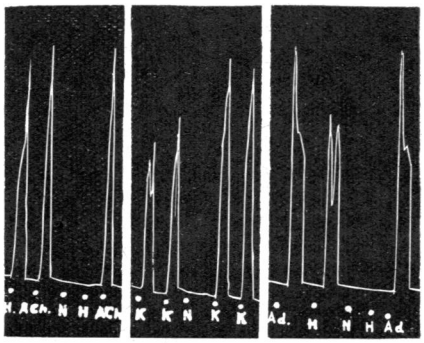


FIG. 4.—Guinea-pig's intestine. Test to identify histamine in an extract. Responses at intervals of 1 min. The line joins responses due to 0.2 c.c. of urine extract. Other effects are due to histamine (7.5 μg./l.) except the first (7 μg./l.). N = neoantergan (0.8 μg./l.) for 1 min. This causes roughly equal inhibitions of the effects of the extract and of the histamine given in alternate doses.



A B C

FIG. 5.—Guinea-pig's intestine. NA indicates neoantergan administration. A.—Neoantergan (0.5 μg./l. for 1 min.) inhibited the response to histamine (5 μg./l., H) and 0.2 c.c. of the extract (T), but not that to acetylcholine (25 μg./l., C). B.—Neoantergan (10 μg./l. for 1 min.) inhibited the response to histamine (10 μg./l., H) much more than that to nicotine (1 mg./l., N). C.—Neoantergan (5 mg./l. for 1 min.) inhibited the response to KCl (0.8 mg./ml.).



A B C

FIG. 6.—Uteri in 40 ml. bath. A.—Guinea-pig. Neoantergan (0.5 μg. for 1 min.) inhibited the response to histamine (10 μg.) but not that to acetylcholine (10 μg.). B.—Guinea-pig. Neoantergan (100 μg.) increased the response to KCl (60 mg.). C.—Rabbit. Neoantergan (15 μg.) inhibited the response to histamine (25 μg.), but not that to adrenaline (25 μg.).

The antihistamine activity of benadryl was clearly shown, though less than that of neoantergan. On the other hand, benadryl showed pronounced antagonism to acetylcholine.

Rabbit.—Both pregnant and non-pregnant uteri were used and no difference was seen between them. Neither neoantergan nor 3277 RP had any direct effect in concentrations up to 3 mg./l. The response to histamine (1.7 mg./l.) was completely inhibited by neoantergan (0.35 mg./l.) added 1 min. previously. Recovery occurred after several washings and the inhibition could be repeated. In another experiment, neoantergan (0.7 mg./l.) inhibited an established response to histamine (1.7 mg./l.).

Antistin was a less powerful histamine-antagonist than neoantergan on this preparation as it was on other preparations.

Rabbit uterus differs from the intestine and the other uteri used in that it is stimulated by adrenaline, so that it provides an opportunity for studying adrenaline-antagonisms. Fig. 6C shows that the response to adrenaline was unaffected by a dose of neoantergan which abolished the response to histamine. Neoantergan (1,700 μg./l.) had no effect on the response of this tissue to acetylcholine (70 μg./l.), but 3277 RP in the same concentration completely abolished the response to acetylcholine as well as that to histamine. Benadryl (350 μg./l.) was also active against acetylcholine.

Non-pregnant guinea-pig.—Neoantergan had no direct effect in concentrations of 1 mg./l. or less, but a concentration of 5 mg./l. caused a small contraction. In lower concentrations (16–50 μg./l. for 1 min.) it abolished the response to histamine (300 μg./l.) but not that to acetylcholine (cf. Fig. 6A). It did not inhibit the response to KCl, but increased it when high concentrations (2.5 mg./l.) were used (see Fig. 6B). 3,277 RP also antagonized the response to histamine.

Isolated heart

Experiments on the heart were in progress when the paper of Dews and Graham (1946) appeared. These authors found that neoantergan antagonized the action of histamine on the rabbit's auricle and on the coronary flow in isolated hearts from cats and dogs.

Cat's heart.—The injection of 0.3–0.5 μg. of histamine increased the coronary flow and larger doses (5–10 μg.) increased the amplitude and rate of the beat. All the antihistamine drugs tested (antergan, neoantergan, benadryl, and 3277 RP) increased the coronary flow and depressed the force and rate of the beat. They all had some antihistamine action, but this action was less than in the experiments on the intestine and uterus. The action of neoantergan was more than 3 times that of benadryl.

Rabbit's heart.—In some of the earlier experiments the hearts did not beat, apparently because of deficient oxygenation. These experiments provided an opportunity for studying effects on the coronary flow uncomplicated by the secondary effects of changes in the beat which have been discussed by various authors (see Hammouda and Kinosita, 1926). The experiments may be compared with those of Wiggers (1909), who deliberately stopped the beat in order to avoid such complications.

Histamine (20 μg.) always decreased the flow. Antergan (50 μg.) increased the flow slightly, and when given 1 min. before histamine it abolished and sometimes reversed the effect of the latter drug. In one experiment 3277 RP (50 μg.) increased the flow and caused a small diminution of the effect of histamine.

When the heart was beating the antihistamine drugs depressed the amplitude of the beat to various degrees and they all decreased the stimulation due to histamine, but normally did not abolish it entirely. Neoantergan was more active than 3277 RP and more than 3 times as active as antistin. In a dose of 25 μg. it had a large effect against 4 μg. of histamine, but was almost inactive against 20 μg. Similar doses of benadryl abolished the response to 0.5 μg. of histamine, but only slightly depressed the response to 1 μg.

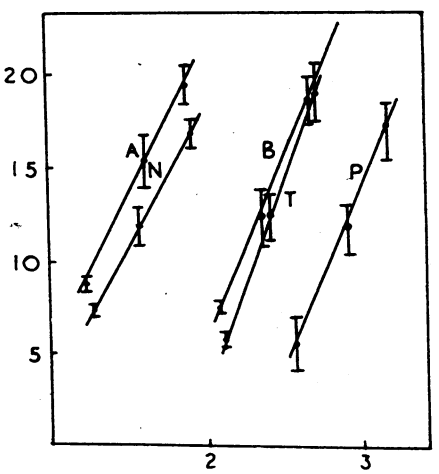


FIG. 7.—Frog's plexus anaesthesia. Ordinates: time to onset of anaesthesia in minutes. Abscissae: negative log. molar concentration. A = Antistin. N = Neoantergan. B = Benadryl. T = 3277 RP. P = Nupercaine. Each point is the mean of 4–8 tests ± the estimated standard error.

Local anaesthesia

Halpern (1942) states that derivatives of the antergan series are potent local anaesthetics, but gives no details. Benadryl (Friedländer and Feinberg, 1946) and antistin (Meyer and Bucher, 1946) have been shown to be local anaesthetics. Using intracutaneous injections in guinea-pigs, Dews and Graham (1946) found that neoantergan was 3.1 times as potent as procaine, and Leavitt and Code (1947) found that benadryl was 2.5–6 times as active as procaine in human beings.

Fig. 7 shows the results of a comparison of four antihistamine drugs with nupercaine, by their effect on the frog's lumbar plexus. The time to the onset of anaesthesia is plotted against the logarithm of the concentration and the results fitted by straight lines. As judged by the concentrations needed to cause anaesthesia in 15 min. the relative molar activities of the drugs tested were as follows —nupercaine 100, 3277 RP 30, benadryl 26, neoantergan 5.5, antistin 3.

Discussion

The experiments with guinea-pig ileum gave the best estimates of the relative activity of the various drugs against histamine (see Table). Neoantergan was the most active, 3277 RP was slightly less active, but its effect was slower in onset and lasted longer. Antistin was much less active.

Experiments on the uteri and isolated hearts were less complete but gave similar results. Whenever they were tested the above drugs showed antihistamine activity and the order of their activities was as given above.

The activity of the thiodiphenylamine drugs was discovered by Halpern and Ducrot (1946), who found that when 10–20 mg. of 3277 RP was injected subcutaneously in guinea-pigs it protected them against the immediate effects of 1,400–1,500 lethal doses of histamine given intravenously 20 min. later, while neoantergan only protected them against 100 lethal doses. It was therefore surprising that 3277 RP was slightly less active than neoantergan on isolated organs. The meaning of this discrepancy is obscure, but may perhaps be related to the fact that the effects of 3277 RP are more prolonged. The results described above are more easily reconcilable with those of Winter (1947), who found that neoantergan was more active than 3277 RP in antagonizing bronchospasm, contraction of the intestine and the lethal effect of small doses of histamine in guinea-pigs.

Besides being the most active of these drugs against histamine, neoantergan is, so far, also the most specific. Benadryl and 3277 RP are both much more active than neoantergan against acetylcholine not only on the intestine but also on the uterus. Antistin has little action against acetylcholine on the intestine, but neoantergan has less still. The specificity of neoantergan was also shown in experiments with other agonists. In concentrations which suppressed the action of histamine it has little or no effect on the actions of potassium or nicotine on the guinea-pig's intestine or on the action of adrenaline on the rabbit's uterus. Higher concentrations did, however, antagonize the actions of all the drugs used in experiments on the intestine, and according to Bovet and Walthert (1944) neoantergan also antagonizes the response of the rabbit's uterus to adrenaline. These results probably explain the conclusions of Danielopolu *et al.* (1941–5), who deny any specificity to antergan. They certainly used very large quantities of the drug, although the exact dose is not mentioned. Schild (1947) also found that antihistamines in sufficient concentrations antagonize the actions of other drugs besides histamine. Neoantergan is clearly the best of the drugs studied to use in specific tests for histamine, but it is useless if high concentrations are used. Experiments of the type shown in Fig. 4 are particularly suitable for tests of this kind, since they provide a quantitative comparison of the antagonism of the drug to histamine and to the unknown solution.

The antihistamine drugs were found to depress the isolated heart and dilate the coronary vessels. They antagonized the actions of histamine on the force of the beat and on the coronaries, whether this was constrictor or dilator. The fact that this antihistamine action was comparatively feeble may perhaps be due to the conditions of administration, since the tissues were only exposed to the drugs for a very short time.

The local anaesthetic effects of these drugs were quite unrelated to their action against histamine, but may perhaps be related to their action against acetylcholine. Benadryl and 3277 RP were, in fact, much more potent than antistin and neoantergan, both as local anaesthetics and as acetylcholine-antagonists. It would, however, be unwise to lay stress on these facts until the experiments have been extended to other drugs and to other methods of testing for local anaesthesia.

Summary

1. The actions of a number of histamine-antagonists on isolated organs have been compared in various ways.

2. When tested by their power to antagonize histamine they were placed in the following order of descending activity—neoantergan, 3277 RP, benadryl, antistin, nupercaine.

3. Neoantergan was the most specific of the drugs used. Its action against histamine was greatest, and its action against acetylcholine was least. Its action against nicotine, potassium, and adrenaline was much smaller than its action against histamine.

4. Neoantergan is the best of these drugs to use in the identification of histamine in unknown solutions, but may give misleading results unless used in very low concentrations. A method of carrying out such tests is described.

5. A method for the rough biological assay of neoantergan is described which involves the use of only about 0.002 μg. of the drug per dose.

6. The antihistamines tested depressed the beat of isolated hearts and increased the coronary flow.

7. The activity of these drugs as local anaesthetics on the frog's lumbar plexus appeared to be more nearly related to their activity against acetylcholine than to their activity against histamine.

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References

Ackermann, D., and Maurer, H. (1944). *Pflüg. Arch. ges. Physiol.*, **247**, 450.
Bovet, D., and Walthert, F. (1944). *Ann. pharmaceut. franc.*, Suppl. No. 4.
Bülbring, E., and Wajda, I. (1945). *J. Pharmacol.*, **85**, 78.
Danielopolu, D., Popesco, M., and Mezinesco, E. (1941–5). *J. Physiol. Path. gén.*, **38**, 316.
Dews, P., and Graham, J. D. (1946). *Brit. J. Pharmacol.*, **1**, 278.
Friedländer, S., and Feinberg, S. M. (1946). *J. Allergy*, **17**, 129.
Halpern, B. N. (1942). *Arch. int. Pharmacodyn.*, **68**, 339.
Halpern, B. N., and Walthert, F. (1945). *C.r. Soc. Biol., Paris*, **139**, 402.
Halpern, B. N., and Ducrot, R. (1946). *C.r. Soc. Biol., Paris*, **140**, 361.
Halpern, B. N., and Mauric, G. (1946). *C.r. Soc. Biol., Paris*, **140**, 440.
Hammouda, H., and Kinosita, R. (1926). *J. Physiol.*, **61**, 615.
Leavitt, N. D., and Code, C. F. (1947). *J. Lab. clin. Med.*, **32**, 334.
Loew, E. R., McMillan, R., and Kaiser, M. E. (1946). *J. Pharmacol.*, **86**, 229.
Meyer, R., and Bucher, K. (1946). *Schweiz. med. Wschr.*, **14**, 294.
Pellerat, J., and Murat, M. (1945). *C.r. Soc. Biol., Paris*, **139**, 1139.
Schild, H. O. (1947). *Brit. J. Pharmacol.*, **2**, 189.
Wiggers (1909). *Amer. J. Physiol.*, **24**, 391.
Winder, C. R., Kaiser, M., Anderson, M., and Glassco, E. (1946). *J. Pharmacol.*, **87**, 121.
Winter, C. A. (1947). *Fed. Proc.*, **6**, No 1, Part II, 228.