Antihistaminic Action of 'Pyronil'.* (19656)

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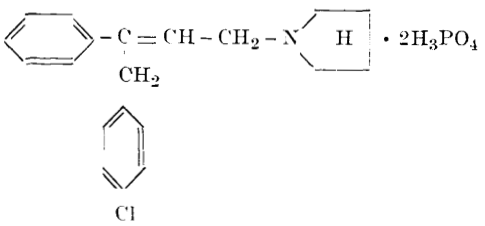
Continuous research for synthetic substances capable of inhibiting the physiological action of histamine has led to the preparation and study of numerous compounds possessing this property. The present goal in antihistaminic investigation appears to be the synthesis of a drug possessing no unpleasant clinical side reactions common to most antihistaminic substances, yet having a duration of activity extending at least 12 hours.

During the course of an antihistaminic screening program in these labortories, more than 50 compounds of the general series 1,2-diaryl-4-amino-2-butene were studied. These have been prepared by Drs. Mills and Rohrmann of the Organic Chemistry Division. One compound in particular was found to be singularly outstanding with regard to activity and length of action. This substance was the diphosphate salt of 1-*p*-chlorophenyl-2-phenyl-4-pyrrolidino-2-butene. This report deals with the pharmacology and toxicity of this compound.

General properties. The diphosphate salt of 'Pyronil' is a white crystalline substance with a melting point of 129.5-130°C (corrected). It is soluble in warm water to the extent of 10%. Most of the experimental data were obtained with the diphosphate salt, however, some experiments were performed with the hydrochloride. The structural formula of the drug appears below:



It will be noted that it bears a *p*-substituted chlorine in one of the benzene rings. In this respect it resembles structurally 2 other long-acting antihistaminic agents, namely, chlorcyclizine and chlorprophenpyridamine.

Antihistaminic properties. Isolated guinea pig ileum. An automatic device similar to one described by Gaddum(1) was employed to evaluate the relative activity of 'Pyronil.' This apparatus automatically stimulated at regular intervals a small segment of guinea pig ileum in a constant temperature bath containing histamine 0.5 μg per ml in Tyrode's solution. The contractile responses were recorded by the usual method on a kymograph. 'Pyronil', when applied to the muscle strip in sufficient quantities 2.4 minutes before the regular histamine stimulus, depressed the next few succeeding responses. The extent of depression depended upon the dose. A concentration of 'Pyronil' in the muscle bath of 0.005-0.01 μg per ml inhibited the histamine response about 50%. A comparison of the drug with diphenhydramine under these conditions indicated that the new antihistaminic exceeded diphenhydramine in activity 14.0 $\pm$ 1.65 times. The precision of this ratio was determined by the method of Loewe(2). The persistent activity of 'Pyronil' in contrast to diphenhydramine made itself apparent in these tests. After a single treatment with the new drug, many repeated treatments with histamine were necessary before the strip recovered its normal response.

Protection of normal guinea pigs in an aerosol of histamine. Antihistaminic activity in normal guinea pigs was studied with a modification of a method described by Halpern (3). A histamine aerosol was generated at a constant rate from 1.0% solution of histamine acid phosphate. This was passed through a chamber wherein 3 animals at a time could be observed. Only those animals showing definite signs of asphyxia within 3 minutes were chosen for test. Since the animals were not exposed long enough to be killed, they could be used repeatedly. The aerosol generator was pat-

* 'Pyronil' is a trade mark identifying Eli Lilly and Co. brand of Pyrrobutamine.

TABLE I. Antihistaminic Activity in Guinea Pigs Subjected to a Histamine Aerosol.

Drug	Route of administration	No. of animals used	$ED_{50} \pm$ S.E., mg/kg	Minimal ED_{100}, mg/kg	$ET_{50} \pm$ S.E., hr
'Pyronil'	Oral	47	.79 ± .125	2.75	13.3 ± .55
	Subcut.	35	.059 ± .007	.2	4.8 ± .54
	Intramusc.	68	.063 ± .009	.14	5.3 ± .35
'Pyronil' hydrochloride salt		126	.78 ± .096		
'Histadyl'		166	5.5 ± .99	36.5	3.5 ± .57
Chlorcyclizine HCl	Oral	64	2.31 ± .53	10	6.5 ± .22
Tripelennamine "		37	3.23 ± 1.26	20	4.1 ± .51
Diphenhydramine "		46	16.48 ± 2.2	70	8.1 ± .44

terned after a design published by Dautrebande(4). The animal weight ranged between 175 and 350 g. The drug 'Pyronil' was then administered orally, subcutaneously or intramuscularly to the histamine sensitive animals. Thirty to 60 minutes later, and hourly thereafter, the animals were tested in the chamber for their ability to withstand the histamine. An animal was considered to be protected if it remained in the aerosol for 3 minutes without showing any signs of asphyxia. The number of animals per group varied from 5 to 10, and the dosage schedule was altered sufficiently so that it ranged from no protection to complete protection of animals in a group. From the percentage of the animals protected in a group and the dose administered, a median protective dose or ED_{50} was calculated by the method of Bliss (5). These data for drug 'Pyronil' are shown in Table I. In a similar manner the oral ED_{50}'s for diphenhydramine, 'Histadyl',† tripelennamine and chlorcyclizine were estimated. They are listed in Table I. An estimate of the duration of action of this antihistaminic drug was obtained from calculation of the ET_{50} for the minimal dose which protected all of the animals. The ET_{50} represents the median time for recovery of histamine sensitivity of a group of animals. This value was calculated by the graphic method of Litchfield(6). The ET_{50}'s for 'Pyronil' as well as the other antihistaminics mentioned are listed in the last column of Table I. It will be observed that 'Pyronil' was more potent and its effect more persistent than any of the other antihistaminics with which it was compared.

Antihistaminic activity on anesthetized animals. The intravenous injection of 1.0 mg per kg of 'Pyronil' into an anesthetized cat abolished the vasodepressor response of 0.8 μg of histamine similarly administered. The vascular reactions of 0.2 μg dose of methacholine iodide and 5 μg of epinephrine hydrochloride were unaltered by the antihistaminic.

Other pharmacological effects. Several types of smooth muscle tissue suspended in Tyrode's solution by the usual Magnus method were used to study the reactions of the antihistaminic. The motility of an isolated segment of rabbit ileum was depressed by the addition of this drug, 2.5-5.0 μg per ml final concentration, in the bath fluid. The movements of an isolated portion of a virgin rabbit's uterine horn were depressed when the concentration of the antihistaminic drug in the bath fluid reached 25 μg per ml. On the isolated guinea pig ileum this compound inhibited the contractions of methacholine iodide 0.5 μg per ml about 1/50 to 1/100 as effectively as atropine sulfate. The intravenous injection of doses of 1.6 to 6.4 mg per kg of 'Pyronil' (hydrochloride) into an anesthetized dog depressed the mean blood pressure for several minutes. Respiratory rate increased during the hypotension. In a second dog, both the increased secretion of saliva and the spasm of a section of the duodenum provoked by the intravenous injection of small doses of methacholine iodide 10 μg per kg were only very slightly diminished by doses of 'Pyronil' (hydrochloride salt), 1.0-3.0 mg per kg given by vein. By contrast, a small

† 'Histadyl' is a trade mark identifying Eli Lilly and Co. brand of Thenylpyramine.

TABLE II. Acute Toxicity Studies—'Pyronil' and 'Histadyl.'

Drug	Animal species	Route of administration	No. of animals used	$LD_{50} \pm$ S.E., mg/kg
'Pyronil'	Mouse	Intrav.	150	53.53 ± 1.61
	"	Subcut.	150	1270 ± 156
	"	Intramuse.	21	836.8 ± 95
	"	Oral	48	1116 ± 73
	Guinea pig	Subcut.	37	1241 ± 165
	"	Intramuse.	29	625.6 ± 41.9
	"	Oral	24	992.6 ± 107
'Histadyl'	Mouse	Intrav.	80	19.85 ± .69
	"	Oral	45	182.2 ± 12.8
	Guinea pig	"	20	374.9 ± 34.5

dose of an anticholinergic agent completely nullified these responses. Apparently the drug has little anticholinergic activity.

Winter(7) has shown that there appears to be some correlation between clinical sedation observed with some antihistaminics and their ability to prolong barbiturate anesthesia in mice. The new compound was therefore compared with 'Histadyl' and diphenhydramine by Winter's method as regards its ability to prolong the sleeping times of mice lightly anesthetized with hexobarbital sodium. The dose level of the antihistaminics was 10 mg per kg by subcutaneous injection, whereas the barbiturate was given intraperitoneally, 100 mg per kg. The results of this comparison indicated that the new antihistaminic was more effective than 'Histadyl' but less potent than diphenhydramine. At this dose range, 'Histadyl' did not significantly prolong sleeping time.

One experimental condition which may be produced in certain animals and which closely resembles some of the clinical entities observed in allergy is the production of a high degree of sensitivity to some foreign protein. In guinea pigs it is relatively simple to sensitize the animal to some protein such as egg white and to bring on a condition of anaphylactic shock by subsequent injection of the same antigen at a later date. In order to demonstrate the effectiveness of the new antihistaminic in anaphylactic shock, 16 guinea pigs were sensitized by the intraperitoneal injection of 0.5 mg of crystalline egg white. Twenty-one days later the animals were divided into 2 groups of 8 animals each. One group served as a control while the animals in the other group were treated one hour in advance with 20 mg per kg by mouth of antihistaminic. All animals were then challenged with a shocking dose of the antigen by vein. Only 2 of the control animals survived, whereas 7 of the 8 treated animals were able to withstand the shock.

Toxicity studies. 1) *Acute toxicity.* The toxicity of 'Pyronil' was studied in several species and by various routes of administration. The median lethal doses as calculated by the method of Bliss in mice and guinea pigs are compiled in Table II. For the purpose of comparison, the LD_{50}'s of 'Histadyl' in the same species are also found in Table II. In the toxic dose range the compound produced convulsions followed by prostration and death, or recovery, usually within 72 hours irrespective of the mode of administration. In general it will be observed that 'Pyronil' in these 2 species was less toxic than 'Histadyl'. A comparison of the effective and the lethal doses of the new drug in guinea pigs by various routes of administration reveals that in these animals it has a high margin of safety. Antihistaminic substances appear to be more toxic in rabbits than in some of the other rodents (8). 'Pyronil' injected by vein in rabbits in toxic doses caused convulsions with collapse and death apparently due to respiratory failure. Although the number of animals was too small to determine a median lethal dose accurately, it appeared to be in the range of 15 to 20 mg per kg since one of 5 animals died on 15 mg per kg, while 4 of 5 animals died on the 20 mg per kg dose. Animals receiving doses of 8, 10, and 12 mg per kg all survived the treatment and did not appear to be ill

during a 72-hour observation period. These rabbits were sacrificed and their organs examined after microscopic section. One of the 5 animals receiving 8 mg per kg had a few red blood cells in the renal collecting tubules. The other 4 were normal. Those animals receiving 10 mg per kg all had a small amount of blood in the renal collecting tubules. Thrombi appeared in the pulmonary arteries of one animal and endothelial proliferation was observed in the pulmonary arteries of another. A dose of 12 mg per kg caused a small amount of blood to appear in the renal collecting tubules of 2 rabbits, while thrombi were found in the pulmonary arteries of 3.

2) *Chronic toxicity in rats.* Four groups of 5 female rats each were fed diets containing various concentrations of the drug for 4 weeks using the method previously published(9). An additional group of 5 rats was fed normal rat ration as a control. The concentrations used, daily food and drug intake and effect on body weight follow:

% drug in diet	Avg daily food intake, g	Avg daily drug intake, mg	Avg body wt change, g
.05	13.01	6.51	+36
.10	12.99	12.99	+40
.20	11.88	23.76	+20.8
.50	9.43	47.15	—13.8
Control	13.88	—	+38.4

Hemoglobin levels were determined and erythrocyte, leukocyte, and differential counts were made on the animals receiving 0.2 and 0.5% diets prior to and at intervals during the 4-week treatment period. One rat on the 0.05% diet died after 10 days with an infection characterized by necrosis of the liver and spleen. Another rat on the 0.1% diet died after 9 days. Pulmonary edema and atrophy of the thymus were present. All others survived, were sacrificed and submitted for necropsy. Gross and microscopic studies of the heart, lungs, liver, spleen, intestines, kidney, thymus, thyroid, and adrenals, as well as the hematological studies showed no abnormalities attributable to the treatment.

3) *Chronic toxicity in dogs.* Three groups of 3 female dogs each were given daily doses of 'Pyronil' by mouth. The dose levels were 5, 10, and 15 mg per kg. Hemoglobin and non-protein nitrogen levels as well as erythrocyte, leukocyte, and differential counts were made weekly. Urinary tests for sugar and albumin were made at the same time. All animals survived a 4-week treatment period and were then sacrificed. Gross and microscopic examination of the heart, lungs, liver, spleen, stomach, intestines, kidneys, pancreas, thyroid, and adrenal glands were made.

With the exception of one animal there were no appreciable body weight changes observed during the 4-week period. This dog exhibited a considerable loss in weight, hemoglobin, and red cells, and on sacrifice was found to have a myocardial lesion. Since the changes in this animal were unique, they were not considered to be attributable to the drug. Among the other 8 dogs there were slight fluctuations in hemoglobin and red cells with over-all loss. These last observations might have been associated with the presence of erythrocytes found in the collecting tubules at the apices of the renal pyramids of the kidneys of 3 of the 9 dogs. One animal on the 5 mg per kg dose and 2 dogs on the 15 mg per kg dose showed this effect. The number of red cells was small and they were present in only a small number of tubules. That this finding is of no great significance is borne out by the fact that the benzidine test of urine obtained by needle aspiration of the bladder at necropsy was positive in only one instance. The lack of change in non-protein nitrogen levels in blood and the normal urinary findings were proof of the absence of any injury to kidney tissue. Although the leukocyte counts showed the usual fluctuations, they were never significantly depressed. All other tissues examined in these animals were normal.

Conclusions. 1. 'Pyronil', a new histamine antagonist, was found to possess an antihistaminic activity of remarkable degree and duration in 2 different test preparations. Its other pharmacological effects, aside from its ability to protect guinea pigs from anaphylactic shock, were unimportant. 2. In mice and guinea pigs 'Pyronil' had a relatively low toxicity when administered orally, subcutaneously or intramuscularly. In mice by vein it

was moderately toxic although apparently less toxic than many of the other antihistaminics in mice as judged from published lethal doses in the literature(8). In guinea pigs the ratio of toxic to therapeutic doses indicated a high margin of safety. In rabbits this compound had the same toxic dosage level as other antihistaminics. 3. Rats fed diets containing as high as 0.1% of the drug in the diet for 4 weeks gained weight comparable to the weight of control rats fed normal ration. Higher concentrations impaired growth but produced no visceral damage as shown by hematological and pathological studies. 4. Dogs given large daily doses of 'Pyronil' for 4 weeks, aside from the presence of occasional red cells in the collecting tubules of the kidney, showed no significant pathological changes attributable to the drug.

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Some Factors Involved in the Fibrogenesis of Collagen *in vitro*.* (19657)

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In solutions of collagen (ichthyocol) obtained by filtration of dilute acetic acid extracts of carp swim bladder tunic, the collagen is dispersed as extremely thin filaments, or highly asymmetric particles, less than 50 A in width, rather than as cross-striated fibrils characteristic of native collagen(1). By suitable adjustment of ionic strength (usually with NaCl, though many other salts are effective) and of pH, cross-striated fibrils typical of native collagen as seen in the electron microscope, are precipitated(1-5). The authors previously reported(2) that reconstitution of collagen-type fibrils could be effected by addition to the acid filtrate of serum acid glycoprotein(6,7) in a concentration of about 10^{-9} M (0.001%) followed by dialysis against water. If the concentration of glycoprotein (GP) is higher (ca 10^{-7} M, or 0.05%), the reconstituted fibrils have the long-spacing (LS) pattern, with an axial period of about 2100 A, previously observed in the "procollagen" obtained by dialysis of acid citrate extracts of connective tissue(8). Because GP is so effective in reconstitution, it was suggested(2) that GP, or a component thereof, may be involved in the fibrogenesis of collagen. This effectiveness in reconstituting collagen-type fibrils in the absence of inorganic ions does not, in itself, constitute evidence that GP plays any role in the natural process of collagen fibrogenesis. However, analytical evidence, too scanty to warrant description at the time, supported the view that certain of the components of glycoproteins and polysaccharides are present in the precipitated cross-striated fibrils, whether of the collagen- or LS-type.

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