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Histamine Antagonists. V. Comparison of Benadryl and Pyribenzamine in Histamine and Anaphylactic Shock.

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(Introduced by C. A. Dragstedt).

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Following the lead of French investigators, several new antihistaminic compounds have recently been synthesized in this country and made available for experimental and clinical trial. B-dimethylaminoethyl benzhydryl ether (Benadryl) and pyridil-N'-benzyl-N-dimethylethylenediamine (Pyribenzamine), an analogue of the later French compounds, have proven effective in histamine,[1-3] and anaphylactic[3-5] shock and in the management of some allergic conditions in man.[6-10]

In order to have a basis for a comparative activity of these and similar compounds it was felt that an experimental study of these substances by the same technic and in the same laboratory was required. The present experiments deal with the comparative efficacy of Benadryl* and Pyribenzamine* in fatal histamine and anaphylactic shock in guinea pigs.

Histamine Shock. Adult male guinea pigs were given injections of histamine in the dorsal vein of the penis. Histamine phosphate was employed in increasing doses in a series of control animals to determine the 100% lethal dose. (All values of histamine are expressed in terms of the base). Another group of guinea pigs received 3 mg/kg of Benadryl intraperitoneally 15 minutes before the administration of histamine. A third group of animals was similarly prepared with 3 mg/kg of Pyribenzamine (Table I). In the untreated control group, 0.4 mg/kg of histamine resulted in the death of all animals within 5 minutes. At lower doses varying degrees of shock were encountered in the surviving animals. In the Benadryl-treated group significant protection was afforded, in that 2.0 mg/kg of histamine, 5 times the amount necessary to kill all unprotected animals, were required to produce 100% mortality. Some degree of shock was encountered in practically all animals which survived lesser doses. The animals receiving Pyribenzamine showed a considerably higher degree of protection against the lethal effects of histamine. Little evidence of shock and no deaths were observed up to 2.0 mg/kg, while 15.0 mg/kg of histamine were required to kill all animals. The data obtained would indicate that Pyribenzamine is approximately 6 to 7 times more active than Benadryl in preventing fatal histamine shock in guinea pigs.

Anaphylactic Shock. Seventy-two male guinea pigs weighing from 300 to 400 g were passively sensitized by the subcutaneous injection of 0.5 cc of rabbit anti-horse serum

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* Benadryl was supplied by Parke-Davis and Co., Detroit, Mich.; the Pyribenzamine was furnished by Ciba Pharmaceutical Products, Inc., Summit, N.J.

TABLE I.
Protective Effect of Benadryl and Pyribenzamine Against Histamine Shock in Guinea Pigs.

Histamine I.V. mg (base)/kg	Control group Mortality		Group receiving Benadryl 3 mg/kg Mortality		Group receiving Pyribenzamine 3 mg/kg Mortality	
	Total deaths / Total used	%	Total deaths / Total used	%	Total deaths / Total used	%
.03 - 0.1	0/3	0				
0.2	2/6	33				
0.3	3/6	50	0/5	0		
0.4	10/10	100	2/7	29	0/6	0
0.8 - 1.6			2/6	33	0/5	0
2.0			6/6	100	0/4	0
2.4 - 3.2					1/6	16
3.6 - 6.8					2/10	20
7 -10					3/9	33
11 -13					5/8	62
15					10/10	100

TABLE II.
Protective Effect of Benadryl and Pyribenzamine Against Anaphylactic Shock in Guinea Pigs.

Amount of drug used	mg/kg	No. of animals used	Survived	Died
None		12	1	11
Benadryl	1	10	5	5
	2	10	7	3
	3	10	10	0
Pyribenzamine	1	10	4	6
	2	10	7	3
	3	10	10	0

(Table II). After 48 hours, the intravenous injection of 1 cc of horse serum in the penile veins of 12 animals in this group resulted in typical fatal anaphylactic shock in 11. One animal manifested severe symptoms with recovery. The remaining animals were divided into 6 groups of 10 each, and given intraperitoneal injections of 1, 2, or 3 mg/kg of Benadryl or Pyribenzamine 15 minutes before the intravenous administration of 1 cc of normal horse serum. A significant degree of protection was afforded by 1 mg/kg of either drug. Two mg/kg gave somewhat increased protection, while 3 mg/kg of Benadryl or Pyribenzamine protected against fatal anaphylactic shock in all animals tested. Some manifestations of anaphylaxis were observed in the majority of the surviving guinea pigs. Subject to the limitations of the above experiment, this might indicate that there is no essential difference in the anti-anaphylactic activity of the 2 drugs under study.

Discussion. The protective effect of Benadryl and Pyribenzamine against histamine and anaphylactic shock is striking and in accord with the theory that histamine plays a role in anaphylaxis. Pyribenzamine on a weight basis has a greater protective effect against histamine than does Benadryl as manifested by the large increase in the LD_{100} of histamine in Pyribenzamine-treated animals. On a weight basis the 2 drugs appear to have an equal effectiveness against anaphylactic shock. This apparent discrepancy may be due to the fact that the maximum amount of histamine liberated during anaphylaxis in the guinea pig is of the order of 0.4 mg/kg, at which dose the 2 drugs are nearly equally effective. There is of course the consideration that phenomena other than the liberation of histamine may account for some difference between the protective effect of chemical agents against histamine shock on the one hand, and against anaphylactic shock on the other.

Summary. The LD_{100} of histamine was

first determined in a control group of guinea pigs. It was found that approximately 5 times this amount was required to kill all animals previously treated with 3 mg/kg of Benadryl, while 35 times the lethal dose of histamine was necessary to produce 100% mortality in animals receiving 3 mg/kg of Pyribenzamine. No apparent difference was discernible between the 2 drugs in preventing anaphylaxis in passively sensitized guinea pigs. One mg/kg of either compound gave significant protection against a shocking dose of antigen, while 3.0 mg/kg prevented fatal anaphylaxis in all animals tested.

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Bed-side Agglutination Test with Whole Blood for Rapid Diagnosis of Tularemia.*

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The agglutination test has been the most practical method for the laboratory diagnosis of tularemia. McCoy and Chapin[1] showed the presence of agglutinins in the serum of patients infected with *B. tularense* and Francis[2] applied the agglutination test to the serological diagnosis of the infection. Recently Damond and Johnson[3] described what they call the "shake method" of agglutination by which it is possible to accelerate the reaction and read the results in 3 minutes.

Considering the possibility of a further improvement by using the method recommended by Castaneda and collaborators for typhus fever[4] and brucellosis,[5] we prepared a concentrated antigen conveniently stained and used either with whole blood as a bed-side test or with serum as in the case of the so-called rapid antigens developed by Huddleson[6] and Welch.[7]

Preparation of the Antigen. The strain of *B. tularense* No. 408, obtained by courtesy of Dr. R. R. Parker from the Rocky Mountain Laboratory of Hamilton, Montana, was used for the preparation of the antigen. The culture medium, recently described,[8] consisted briefly in a concentrated liver infusion with cystine, glucose, sodium chloride, peptone and agar, without blood or hemoglobin and distributed in Roux's bottles. Each bottle was inoculated with a concentrated emulsion of *B. tularense* and after 72 hours of incubation at 37°C the organisms were emulsified with isotonic saline containing 10% formaline (40%), filtered through wet cotton and left at ordinary temperature for 72 hours. The emulsion was centrifuged and the supernatant fluid was discarded; the organisms were emulsified in a small amount of isotonic saline. The concentration of the emulsion was standardized in order that one-tenth of antigen diluted with 10 cc of saline gave a turbidity corresponding to No. 3 of McFarland's Nephelometer. When the concentration of the antigen was adequate, enough aqueous solution of methylene blue was added to stain the antigen to a deep blue color. After 24 hours the antigen was centrifuged at high speed and the supernatant fluid was discarded.

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