and incomplete adaptation of strain B in our work would support this idea. Also, adaptation of yellow fever by Theiler and Smith,[4] dengue by Sabin and Schlesinger,[5] and perhaps other viruses to animals has not been obtained regularly.

The fact that strain A had been kept under laboratory conditions for a long period and had been cultured outside the body of swine may have influenced its adaptability to the rabbit, although it is possible that strain A was naturally more adaptable. On the other hand, strain B had been isolated recently. It showed poor adaptability and required 6 alternating passages before virus increase occurred in the rabbit spleens and it could be transferred consecutively in the rabbit.

Since neither strain A nor B produces definite signs of illness in rabbits, a study of other strains and perhaps in other laboratory animals should be made especially by the alternating method which, as was shown with strain B and rinderpest, allows opportunity for development of any latent pathogenic potentiality. Such a study may give results similar to those obtained with rinderpest and supply the laboratory animal that has been needed in hog cholera research.

Summary. Two strains of hog cholera virus were inoculated into rabbits. One strain (A) continued in serial passage, became attenuated for swine and, after 15 rabbit passages, produced a febrile reaction of short duration as the only sign of illness. This attenuated strain fully immunized swine to the virulent hog cholera virus. The other strain (B) was present in rabbits for 1 but not 2 consecutive passages. With 6 continued transfers alternately between swine and rabbits, the amount of virus in the rabbit's spleen attained 100 times that found in the initial rabbit transfer. Serial passage in the rabbit was then successful for 2 but not 3 transfers. Evidence of attenuation of this strain was the lengthened incubation period in swine inoculated with the rabbit-passed material. Possible explanations for the difference in adaptability of these strains are discussed.

[4] Theiler, M., and Smith, H. H., *J. Exp. Med.*, 1937, **65**, 767.

[5] Sabin, A. B., and Schlesinger, R. W., *Science*, 1945, **101**, 640.

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Antihistaminic Substances in Histamine Poisoning and Anaphylaxis of Mice.

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The conditions under which mice can be sensitized and the manifestations of sensitization in mice closely simulate those of anaphylaxis in guinea pigs and other animals (Braun,[1] Schultz and Jordan,[2] Ritz,[3] Sarnowski,[4] Bourden,[5] Weiser, Golub and Hamre[6]). Active sensitizations can be produced with various protein substances, horse, sheep, cow, guinea pig serum, or egg white; passive sensitizations with immune rabbit serum, antihorse guinea pig serum, antihorse rabbit serum, antipneumococcus Type I rabbit serum, etc. The sensitizations are specific and their duration varies from several weeks to several months. Refractoriness occurs after recovery from shock and active desensitization is obtained by

[1] Braun, H., *Münch. med. Wschr.*, 1909, **37**, 1880; *Z. Immunf.*, 1910, **4**, 590.

[2] Schultz, W. H., and Jordan, H. E., *J. Pharm. and Exp. Therap.*, 1911, **2**, 375.

[3] Ritz, H., *Z. Immunf.*, 1911, **9**, 321.

[4] Sarnowski, V., *Z. Immunf.*, 1913, **17**, 577.

[5] Bourdon, K. L., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 340.

[6] Weiser, R. S., Golub, O. J., and Hamre, D. M., *J. Inf. Dis.*, 1941, **68**, 97.

the usual desensitization methods. Specific precipitins are formed in mice actively sensitized against egg white with a titer varying from 1:100 to 1:400.

From all these facts it has been concluded that the "protein shock" in mice is a true anaphylaxis; they fulfill indeed Doerr's[7] criteria of allergy.

The mechanism which ultimately leads to the various anaphylactic manifestations in the different animal species is certainly not the same. The tissue which becomes the principal site of sensitization in guinea pigs is that of the lungs; in rabbits, that of the vascular system; in dogs, that of the liver; etc. The site of sensitization in mice is unknown.

The similarity of the symptoms of anaphylactic shock in guinea pigs or dogs to those of histamine poisoning was first recognized by Dale and Laidlaw.[8] The significant increases of the histamine level in the blood during anaphylactic shock in guinea pigs and dogs are strong support for the "histamine theory" of anaphylaxis. It is today commonly accepted that at least in these animal species the antigen-antibody reaction leads to a liberation of histamine or a histamine-like substance, which is the ultimate cause of the anaphylactic shock. These findings have been interpreted in a more general way, and histamine is considered to be the principal offender in all cases where antigen-antibody reactions in allergy lead to anaphylactic symptoms or to reactions of a similar nature.

However, objections have been raised against such a generalization. For instance, unlike anaphylaxis in guinea pigs and dogs, the histamine blood level decreases in anaphylaxis of rabbits, horses and calves (Code and Hester,[9] Rose and Weil[10]). The connection between allergic manifestations and histamine is especially debatable in mouse anaphylaxis. Whereas the very small dose of 0.6-0.8 mg per kg body weight of intravenously-injected histamine phosphate kills guinea pigs almost instantly, mice are very resistant to histamine, and many animals survive the intravenous injection of 500 mg per kg body weight. In our own experiments, the dose of 750 mg per kg body weight was lethal in all cases. According to these figures, the mouse is about 1,000 times more resistant to histamine than the guinea pig.

The histamine content of the normal mouse averages about 10 mg of histamine per kg body weight. Since mice present the first signs of histamine shock only when 100 mg or more of free base are injected, it is rather improbable that histamine is involved in anaphylactic shock in mice.

In an endeavor to study this question a new approach was used, which was made possible by the recent introduction of the so-called antihistaminic substances supposed to counteract histamine specifically, and considered by many investigators as biologic reagents for histamine. It was of interest to investigate whether histamine poisoning in mice and mouse anaphylaxis responded to these antihistaminic substances in the same way as anaphylaxis of the guinea pig and the dog.

Experimental. I. Influence of Antihistaminic Substances Upon Histamine Poisoning in Mice. Since the toxicity of histamine for mice depends largely upon the concentration of histamine and the rapidity of injection, 2% solutions of histamine phosphate were uniformly used in all experiments; the solutions were injected at the rate of 1 ml per minute. Under these conditions 500 mg of histamine phosphate per kg body weight killed 50% of the mice; 375 mg per kg, 44%. 250 mg per kg body weight occasionally produced a brief period of muscular incoordination and convulsions, but after a few minutes almost all animals regained their normal state.

If antihistaminic substances such as Pyribenzamine* or Benadryl† were given in subcutaneous injections of 10 or 25 mg per kg body weight 15 minutes before the histamine injection, the toxicity of histamine was not

[7] Doerr, R., *Arch. f. Dermat.*, 1926, **151**, 3.

[8] Dale, H., and Laidlaw, P., *J. Physiol.*, 1911, **43**, 182.

[9] Code, C. F., and Hester, H. R., *J. Physiol.*, 1939, **127**, 71.

[10] Rose, B., Braun, H., and Weil, P., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 494.

* Trade name for N'-pyridyl-N'-benzyl-N-dimethyl ethylene diamine monohydrochloride (Ciba).

† Trade name for dimethyl amino ethyl benzhydryl ether hydrochloride (Parke, Davis & Co.).

TABLE I.
Toxicity for Mice of Histamine Phosphate Alone and in Combination with "Antihistaminic Substances."

Histamine doses mg/kg i.v.	No antihistaminic substance	Pyribenzamine		Benadryl	
		10 mg/kg	25 mg/kg	10 mg/kg	25 mg/kg
750	10/10*	3/3		6/6	
500	10/20	11/11	11/11	10/12	14/14
375	10/23	9/11	16/16	11/12	10/10
250	1/13	3/8	11/15	0/5	0/5

Histamine phosphate, 2% solutions, intravenous injection, 1 ml per minute.
Pyribenzamine and Benadryl given 15 minutes before histamine by subcutaneous injection.
* Denotes animals dead/animals tested.

decreased, as is usually the case with guinea pigs or dogs, but, on the contrary, was strongly enhanced. Histamine phosphate alone in a dose of 375 mg per kg body weight was lethal for 10 out of 23 mice (43%). In combination with 25 mg per kg Pyribenzamine, the same dose of 375 mg of histamine killed 16 out of 16 mice (100%) and in combination with 25 mg per kg Benadryl, 10 out of 10 mice (100%). A similar significant increase in toxicity occurred with a combination of 500 mg per kg histamine phosphate with 25 mg per kg Pyribenzamine or Benadryl (from 50% mortality in controls, to 100% with either substance). The dose of 25 mg per kg Pyribenzamine, or Benadryl is nontoxic for mice.

The results (shown in Table I) indicate that the increase in toxicity of histamine is proportional to the dose of Pyribenzamine or Benadryl previously injected. These surprising results indicate that Pyribenzamine or Benadryl, which strongly and specifically counteract and neutralize histamine in guinea pigs and dogs, are definite synergists of histamine in mice.

II. *Influence of Pyribenzamine Upon Active Anaphylaxis in Mice.* The picture of a fully developed mouse anaphylaxis is the following: Five to 10 minutes after the shocking dose is injected, the animals show some excitement, but rapidly become quiet. After a short time, increasing difficulty in breathing develops. This state is followed by loss of coordination, and about 20 minutes later a progressive paralysis of the body starts, beginning with the hind legs. In those instances in which the animal dies, this paralytic state is soon succeeded by a short period of violent convulsions and coma, in which the animals succumb in about half an hour. The entire sequence is sometimes over in 15 minutes, but may last an hour or more. The symptoms are quite similar to those seen in anaphylaxis in guinea pigs, rabbits, or dogs—they merely extend over a much longer period of time.

210 mice were sensitized, in 2 separate groups, by 4 consecutive intraperitoneal injections of 1 ml each of undiluted horse serum every other day. Twenty-one days later a challenging dose of 1 ml of undiluted horse serum, warmed to 37°C, was injected intravenously into 169 mice still alive at that time. Preliminary experiments had shown that this amount, representing in sensitized mice about 2 shocking doses, did not affect normal mice.

Fifty-eight out of the 169 surviving sensitized mice served as controls and received the challenging injection without any other treatment. All of them went into severe shock with a mortality of 89%. The other 111 mice were divided into 4 groups of 20 to 30 animals each and received subcutaneous injections of Pyribenzamine in doses of 10, 25, 30 or 50 mg per kg body weight respectively, 15 minutes before the challenging dose of horse serum was injected intravenously. The challenging injection of horse serum produced in animals, without exception, the depressive stage of shock with varying degrees of prostration. Nevertheless, about one-half of the mice treated with 10 to 30 mg of Pyribenzamine remained free from convulsions and recovered. Thus, the morbidity picture, compared to the controls, was somewhat improved and the mortality rate definitely

TABLE II.
Effect of Pyribenzamine upon Mouse Anaphylaxis.

	Controls No treatment	Treatment with Pyribenzamine (mg/kg) 10	25	30	50
No. of animals dead in shock over No. of animals used	53/58	18/29	13/31	12/24	19/27
% of *deaths in shock*	89%	65%	42%	50%	70%

decreased. In contrast to a mortality rate of 89% for the controls, only 65% of the mice pretreated with 10 mg of Pyribenzamine per kg body weight died (18 out of 29); 42% of those pretreated with 25 mg of Pyribenzamine (13 out of 31); 50% after pretreatment with 30 mg of Pyribenzamine (12 out of 24); and 70% after pretreatment with 50 mg of Pyribenzamine. This last dose of Pyribenzamine is definitely toxic for mice and it is probable that a number of the deaths included in the 70% are due to Pyribenzamine. (LD_{50} of Pyribenzamine by subcutaneous injection for mice is 75 mg per kg body weight). Table II shows the results obtained.

It is significant that in no case was complete freedom of all shock symptoms ever obtained, even with high doses of Pyribenzamine, although significant protection against convulsions and death was afforded. In any event, protection conferred on the sensitized mice was much less spectacular, as compared to the certainly more complete protection obtained in sensitized guinea pigs with doses of Pyribenzamine only 1/50 as great.

Discussion. It has often been suggested that a positive correlation exists between a species sensitivity to histamine and the ease with which one may induce anaphylaxis in this species. Indeed, guinea pigs, dogs, rabbits, or man which are highly sensitive to histamine, become sensitized quite easily. In these same species, Pyribenzamine and other antihistaminic substances have been shown to be most powerful antagonists of histamine, as well as of anaphylaxis (Dekanski,[11] Mayer, Huttrer and Scholz,[12] Mayer,[13] Yonkman, Hays and Rennick,[14] Arbesman, Koepf and Miller[15]).

In vitro, small doses of Pyribenzamine prevent several typical histamine effects on isolated guinea pig intestines, uterus, or lungs; *in vivo,* 5 mg per kg protect guinea pigs against 100 and more lethal doses of histamine. Anaphylactic shock in guinea pigs can be prevented by doses as small as 0.1 to 0.5 mg per kg body weight; somewhat higher doses are active against histamine poisoning or anaphylactic shock of dogs (3-3.5 mg per kg body weight).

Contrary to guinea pigs and dogs, the mouse is highly resistant to histamine; it tolerates about 1,000 times more histamine than the guinea pig. Correspondingly, the mouse is very resistant to any sensitizing procedures.

A similar difference existed in the response to antihistaminic substances. Pyribenzamine and Benadryl not only failed to protect mice from histamine poisoning, but definitely acted as synergists to histamine. After an injection of 25 mg per kg body weight of Pyribenzamine, for instance, the toxicity of histamine in mice was doubled.

That the antihistaminic substances act quite differently in mice than they do in guinea pigs seems not to be a fortuitous occurrence, but rather seems to be in agreement with the theory that antihistaminic substances are competitors of histamine.

An analysis of the general and quite uncharacteristic picture of acute histamine poisoning in mice does not give any answer to the question of whether histamine acts in mice in the same manner, that is, on the same organs and by identical mechanisms as in guinea pigs and dogs. The absence of any protection by antihistaminic substances in the

[11] Dekanski, J., *J. Physiol.*, 1945, **104**, 151.

[12] Mayer, R. L., Huttrer, C. P., and Scholz, C. R., *Science*, 1945, **102**, 93; *Fed. Proc.*, 1945, **4**, 129.

[13] Mayer, R. L., *J. Allergy*, 1946, **17**, 153.

[14] Yonkman, F. F., Hays, H. W., and Rennick, B., *Fed. Proc.*, 1945, **4**, 144.

[15] Arbesman, C. E., Koepf, G. F., and Miller, G. E., *J. Allergy*, **1946, 17, 203.**

case of histamine poisoning of mice seems to indicate that different mechanisms are involved. On the other hand, the synergism of histamine and "antihistaminic" substances cannot be explained by a possible histamine-like activity of these "antihistaminic substances." There is no such action in mice. We rather conclude that in the mouse, histamine affects organ systems other than those affected in histamine-sensitive animals, and that in these points of attack, antihistaminic substances are unable to compete with histamine. On the contrary, each poison acts on its own, both toxicities being additive in effect.

It seems quite inconsistent with the synergistic action of histamine and Pyribenzamine that the latter should have a certain protective action in mouse anaphylaxis. The very fact that Pyribenzamine in the mouse does not act as an antihistaminic agent, leads to the belief that this "protection" against mice anaphylaxis is not due to a specific antianaphylactic effect, comparable to that which Pyribenzamine exercises in anaphylaxis of guinea pigs or dogs. It is rather probable that some pharmacologic properties of Pyribenzamine other than its antihistaminic power are responsible for this protection. It may be that it acts here through its local anesthetic power, since it is known that general anesthetics, as well as local anesthetics, have a definite influence upon anaphylaxis (Wolfsohn[16]). The same problem has been discussed in a study concerning the influence of Pyribenzamine upon contact dermatitis in guinea pigs (Mayer[17]). Other experiments by Yonkman, Hays, Chess and Rennick[18] seem to indicate that the general pharmacological activity of an antihistaminic agent such as Pyribenzamine may well include other properties than those associated with antihistaminic power.

Further experiments are necessary to correlate allergy in mice with that of other animals and to identify the "anaphylactic poison" of mouse anaphylaxis, which quite probably is not histamine.

Conclusions. (1) "Antihistaminic substances" act as synergists in the histamine poisoning of the mouse; (2) contrary to the action on histamine poisoning, a certain protective power in mouse anaphylaxis has been observed; (3) the possible conclusions following from these observations are discussed.

16 Wolfsohn, G., *The Palestine and Near East Med. J.*, 1944, **3**, 11.

17 Mayer, R. L., *Ann. Allergy*, in press.

18 Yonkman, F. F., Hays, H. W., Chess, D., and Rennick, B., *J. Pharm. and Exp. Therap.*, in press.

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Concentration and Properties of the Adrenocorticotrophic Substance in Female Human Urine.

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Adrenocorticotrophin has been demonstrated to be present in the blood serum[1] and in the anterior pituitary gland. Its possible presence in the urine has been suggested by the effects of injecting urine from pregnant[2] and nonpregnant[3] women on the adrenal cortex of young guinea pigs. No attempt has been made to identify the substance which may have caused the adrenal hyperplasia. Since the corpus luteum hormone[3] and estrogens[4,5] can induce hyperplasia of the

1 Golla, M. L., and Reiss, M., *J. Endocrinology*, 1942, **3**, 5.

2 de Boissezon, P., *Bull. histol. appliq. physiol.*, 1936, **13**, 129.

3 Blumenthal, H. T., *J. Lab. Clin. Med.*, 1945, **30**, 428.

4 Janes, R. G., and Nelson, W. O., *Am. J. Physiol.*, 1942, **136**, 136.

5 Uotilla, U. Y., *Endocrinology*, 1940, **26**, 123.