protein was fed.

The possibility that young rats might exhibit a more pronounced response to a diet containing 78% lactalbumin than did the adults prompted an investigation of this point. Eight rats, 45-49 days old, were fed the 78% lactalbumin diet for an average of 23 days and the data were compared with littermate controls fed fox chow. Despite *ad libitum* feeding, the rats consuming the high protein diet gained an average of only 62 g as compared to the average 83 gain exhibited by rats eating fox chow (Table II). Despite the retardation in body weight gain, the adrenal, kidney and liver weights from rats fed a 78% lactalbumin diet exceeded those from rats fed stock diet. These organ weight differences are more apparent when organ weight is considered in terms of 100 g body weight (Table III). The weight of the thyroid, testis, seminal vesicles and pituitary was not influenced.

Examination of the plasma protein concentrations for total protein, albumin and globulin suggested that rats fed fox chow had the higher plasma protein levels but the differences are not clearly significant. NPN, however, was significantly higher in rats fed the high protein diet.

Since liver protein can be readily influenced by a dietary deficiency in protein it was of interest to examine the livers from some of these young rats under the opposite conditions or that of protein excess. The livers from 4 pairs of rats were dried to constant weight at 95° C and analyzed for total protein. Water content of the livers from rats fed 78% lactalbumin was 69.9% and from rats fed fox chow was 70.0%. Liver protein was found to be greater in the lactalbumin-fed rats than in stock-diet animals. Following the feeding of the high protein diet the livers contained an average 1.15 (0.98-1.26) g protein/100 g body weight as compared with an average 0.83 (0.76-0.89) g protein/100 g body weight. Furthermore, the percentage protein of the dry liver increased from 64.0% in rats fed fox chow to 72.3% in rats fed the high lactalbumin diet and therefore the increased liver protein was not alone due to increase in liver size.

Summary. The effect of a high protein diet (78% casein or lactalbumin) was studied in male rats. The rate of body weight increase was retarded over a 3-week period by a 78% lactalbumin diet in young and adult rats. A modest increase in adrenal weight was observed but no indication of increased functional activity was suggested by alterations in plasma albumin or globulin concentrations since the plasma protein levels remained unchanged. Non-protein nitrogen was elevated by the excess dietary protein. Thyroid, pituitary, testis and seminal vesicle weights remained normal but kidney and liver weights were increased. Liver protein was increased in young rats fed the high lactalbumin diet.

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Pyribenzamine Aerosol Inhalation and its Influence on Histamine Poisoning and Anaphylaxis.

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The lethal dose of histamine for guinea pigs by intravenous injection is 0.3 to 0.4 mg/kg body weight,[1] but the amounts required to kill guinea pigs when introduced into the lungs by aerosol inhalation are fractions of these doses.[2,3] Indeed, many guinea pigs breathing air containing less than 0.1 γ his-

[1] Guggenheim, M., *Die Biogenen Amine*, Basle, 1940.

[2] Halpern, B. N., *Arch. internat. de pharmacodyn. et de Therap.*, 1942, **68**, 339.

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

tamine per ml die within 2 to 5 minutes. The high activity of histamine introduced directly into the lungs is explained by two facts, namely, that the lungs of guinea pigs contain tissues whose cells are exceedingly susceptible to this substance, and that the marked response of these cells in histamine poisoning is the primary cause of death.

Substances such as Pyribenzamine (PBZ) for example, which specifically counteract histamine, are believed to prevent histamine from acting upon organs susceptible to histamine. According to a theory accepted by many investigators, these antihistaminic substances compete with histamine, blocking the receptive sites within the susceptible cells which normally respond readily to histamine. If this theory is correct it would be expected that a specific antihistaminic substance, like PBZ, introduced directly into the lungs by aerosol inhalation would provide considerably more protection against subsequent histamine poisoning than when injected or given by mouth. Moreover, it might also be found that PBZ, as an aerosol, would counteract histamine or protect against anaphylaxis in such sufficiently small doses that side effects, common to the usual therapeutic administration, could be avoided.

Experiments with PBZ aerosol could therefore provide us with valuable information concerning the mechanism of action of antihistaminic substances. On the other hand, it seemed to us probable that a comparison of the effect of PBZ aerosols upon histamine poisoning and anaphylaxis might lead to further insight into the relationship between histamine poisoning and anaphylaxis. This question is especially important since there are many authors who consider histamine to be identical with the anaphylactic poison, while others reject the histamine theory of anaphylaxis.

In the following experiments we therefore investigated in guinea pigs the influence of PBZ aerosols upon the tolerance toward histamine and upon active and passive anaphylaxis.

Methods. 173 guinea pigs weighing from 150 to 200 g were subjected to inhalation of PBZ aerosols in the same manner as previously described for histamine aerosols.[2-6] In the various experiments performed, we vaporized solutions containing 0.5, 1, and 2% PBZ (hydrochloride), the air flow at the outlet of the vaporizers being maintained at approximately 20 liters per minute.

The influence of the aerosol inhalation upon histamine intoxication and anaphylaxis was tested as follows: In the first series of experiments, the duration of the PBZ aerosol treatment was varied and the influence of each time-treatment upon the resistance of the animals to histamine was measured. In the second series we kept the duration of aerosol-treatment constant and determined the duration of protection to histamine. In both series histamine was injected intracardially as histamine phosphate. In the third and fourth series of experiments we tested in an identical way the influence of PBZ aerosol inhalation upon active and passive anaphylaxis. Active anaphylaxis was produced with horse serum by the usual technic, the intracardial "challenge" injections being made three weeks after sensitization, and passive sensitization by injection with anti-horse rabbit serum, followed by the "challenge" injection 24 hours later.

Results. Histamine Shock. I. *Histamine Intoxication.* In preliminary trials, the sensitivity of guinea pigs to the histamine used for these experiments was determined. The minimal toxic dose producing more than 50% deaths was found to be between 0.7 to 0.8 mg histamine phosphate per kg, corresponding to 0.25 to 0.29 mg of histamine respectively. The MLD_{50} was about 25% higher in the present experiments than in our earlier work,[3] a fact which may have been due to variation among the histamine lots commercially available, differences in animal strains, minor variations in rates of injection, or to seasonal changes in the sensitivity of the guinea pigs.

II. *Influence of the Concentration and Duration of Pyribenzamine Aerosol Inhalation.*

[4] Kallos, P., and Pagel, W., *Acta Med. Scandinav.*, 1937, **91**, 292.

[5] Schaumann, O., *Arch. f. Exp. Path. u. Pharmakol.*, 1940, **196**, 109.

[6] von Issekutz, B., and Genersich, P., *Arch. f. Exp. Path. u. Pharmakol.*, 1943, **202**, 201.

Inhalation of aerosols produced by vaporizing 0.5% and 1.0% PBZ solutions for various periods of time conferred definite protection against histamine to a few animals. A number of animals, however, did not show any significant beneficial response when treated with these relatively low concentrations.

Consistent results were obtained in almost all animals when the concentration of PBZ in the vaporizing fluid was increased to 2%. 34 animals were submitted to inhalations of 2% PBZ aerosol of varying duration. The shortest time of inhalation producing a definite protective effect against 2-3 lethal doses of histamine was between 1 and 2 minutes. With 2 minutes of inhalation, the animals tolerated intracardial injection of 2 mg histamine phosphate per kg, constituting approximately 2.5 lethal doses, without any symptoms of shock. With 3 minutes of inhalation they tolerated approximately 3.75 mg histamine phosphate (5 MLD), while inhalation for 5 to 10 minutes produced a resistance to 5-11 mg histamine phosphate (7 to 15 MLD).

The guinea pigs so protected did not go into shock and survived; however, all animals which were protected from typical histamine shock and had received histamine doses ranging from 5 to 11 mg per kg body weight presented very unusual signs of histamine intoxication. A few minutes after histamine injection the animals became motionless and then rapidly fell into deep narcosis with regular respiration, although very superficial and slow; there were also occasional athetotic movements of all legs. This interesting form of histamine intoxication is not normally observed, since death occurs from bronchoconstriction and asphyxia before narcosis could develop.

III. *Duration of Protection with 2% Pyribenzamine Aerosols.* Three series of 10, 16, and 10 animals were submitted to PBZ aerosol inhalation of 15, 30, and 60 minutes duration respectively. At varying intervals after completion of the PBZ treatment 2 animals of each series received an intracardial injection of 3 mg per kg histamine phosphate (4 MLD).

In the first series of experiments in which the 2% PBZ aerosol was inhaled for 15 minutes, the protection afforded against subsequent injection of 3 mg of histamine phosphate (4 MLD) lasted for at least 1 hour (Table I). Inhalation of PBZ aerosol for 30 minutes gave protection lasting for 3½ hours, and inhalation for 60 minutes did not further increase the protection.

TABLE I.
Duration of Protection in Guinea Pigs Against 4 Lethal Doses of Histamine Phosphate After PBZ Aerosol Inhalation (2%) for 15, 30 and 6 Minutes.

Interval between end of PBZ inhalation and histamine injection	No. of animals protected		
	Duration of aerosol inhalation		
	15 min	30 min	60 min
0	2/2*		
15 min	2/2		
30 "	2/2	2/2	2/2
45 "	2/2	2/2	2/2
1 hr	2/2	2/2	1/2
2 "		2/2	2/2
3 "		1/2	2/2
3½ "		2/2	
4 "		0/4	
Controls, no PBZ		0/6	

* Number of animals protected and surviving over number of animals used.

Anaphylaxis. I. *Influence of the duration of Pyribenzamine Aerosol Inhalation.* Animals actively sensitized to horse serum or passively sensitized with anti-horse rabbit serum could be effectively protected by 2% PBZ aerosol inhalations. The shortest time of inhalation protecting animals against immediate anaphylactic shock was found to be 3 minutes. Animals challenged immediately after this time showed delayed symptoms of shock, but invariably died after 10 to 30 minutes. Complete protection against both shock and anaphylactic death was conferred after an inhalation period of at least 10 minutes.

Since the amounts of histamine or histamine-like substances liberated in the lungs during anaphylactic shock are not known, a direct comparison between the duration of PBZ aerosol inhalation necessary for protection against histamine poisoning and anaphylactic shock is not possible. It is interesting to note, however, that the results in anaphylaxis were somewhat less regular than those obtained in the histamine-shock series, since some animals died in shock even after being submitted to 2% PBZ aerosol inhalation of 30 minutes duration. Contrary to those animals protected against histamine, certain individuals,

TABLE II.
Duration of Protection in Guinea Pigs Against Anaphylactic Shock After PBZ (2%) Aerosol Inhalation for 15 and 30 Minutes.

Interval between end of PBZ inhalation and challenging serum inj.	Number of animals protected		
	Active anaphylaxis after PBZ inhalation of		Passive anaphylaxis after PBZ inhalation of
	15 min	30 min	30 min
15 min	1/2		3/3
30 ''	3/4	2/3	3/3
45 ''	0/3	2/3	2/3
60 ''	2/3	1/2	3/3
2 hr		1/2	2/2
3 ''		2/2	1/1
5 ''			2/2
Controls, no PBZ	0/8		0/3

while protected against immediate anaphylactic shock by adequate treatment, died during the ensuing 24 hours.

II. *Duration of the Protection with 2% Pyribenzamine Aerosol Inhalation.* The duration of the protection to anaphylactic shock conferred to guinea pigs by PBZ aerosol inhalation was tested in the same manner as described in the experiments with histamine. Two groups of 12 sensitized animals each were submitted to an inhalation of 2% PBZ aerosol for 15 and 30 minutes respectively. It was found that inhalation of 15 minutes duration afforded protection up to 1 hour; while inhalation for 30 minutes gave protection for at least 3 hours. Protection was even greater and more consistent in 17 passively sensitized animals, since aerosol inhalation for 30 minutes was still effective after an interval of 5 hours (Table II).

III. *Influence of Aerosols Containing Trasentin or Procaine upon Histamine Intoxication.* PBZ and other antihistaminic substances display, in addition to the antihistaminic activity, powerful local anesthetic and variable antispasmodic activities. The possibility has been suggested that the activity of the antihistaminic substances, especially their effectiveness in anaphylaxis and allergy, are at least partially due to these secondary activities. Although it is known that local anesthetics and antispasmodics, such as atropine, are capable of protecting animals to a limited degree against histamine poisoning and anaphylactic shock, there is no proof that either of these secondary activities associated with antihistaminics contribute to any significant degree to the antihistaminic and anti-anaphylactic activities of PBZ and similar substances. Nevertheless, it seemed interesting to substitute for aerosols of PBZ, aerosols of substances with pronounced antispasmodic and local anesthetic activity and to investigate their influence upon histamine intoxication. We found that neither 2% procaine nor 2% Trasentin aerosol inhalation had any influence upon histamine intoxication using 2 to 3 lethal doses of histamine; convulsions and death occurred at the same intervals as in control animals not submitted to these aerosols.

IV. *Local Action of Pyribenzamine Inhalation upon the Lungs.* As it is known that various antihistaminic substances are irritating when injected subcutaneously, it was therefore necessary to control the effect of PBZ aerosol inhalation upon the cells of the lung system. Guinea pigs were subjected to inhalation of 2% PBZ for up to 2 hours and then observed for several days. The treatment was well tolerated and did not produce any clinical signs of irritation either during treatment, or subsequently. Three other animals, after receiving a 1-hour aerosol treatment, were killed 24 hours later and the lungs histologically examined. They were found to be normal.

Discussion. These experiments indicate that it is possible to protect guinea pigs against 15 lethal doses of histamine, as well as against anaphylactic shock when 2% PBZ is administered in the form of an aerosol. Under the conditions of our experiments, the animals were protected after having inhaled, by calcu-

lation, 0.3 to 0.5 mg PBZ, which may have been absorbed, in whole or in part, by the lungs. This dose, as we have found from all our previous experiments, is ineffective when either injected subcutaneously or given orally. These results constitute, in our opinion, further evidence supporting the hypothesis that PBZ specifically counteracts histamine not by central action, but by a purely local influence at the very site of the peripheral histamine action.[2,3,7,8]

The evidence that the point of attack by PBZ is the peripheral receptor may explain why the protection against histamine intoxication or anaphylactic shock, persists for a surprisingly long period of time in spite of the exceedingly small amounts of PBZ which reach the lung tissue during the brief aerosol treatment. Indeed, the protective effect lasts almost as long as that protection which is conferred when PBZ is given subcutaneously or by mouth in considerably higher dosages.

It may be concluded from our results that PBZ is either selectively fixed within the receptor cells of the lungs and does not rapidly enter the blood stream as do most other substances introduced therapeutically into the lungs, or that the minute amounts of antihistaminic substances, by direct contact with the cells susceptible to histamine, render the receptive organs, by some functional mechanisms, refractive for long periods of time to subsequent attacks by histamine.

The introduction of antihistaminic substances into the lungs and the selective protection of this organ against histamine in guinea pigs have enabled us to detect signs of histamine intoxication previously not observed. Under normal conditions, the picture of histamine intoxications in guinea pigs consists almost entirely of the constriction of smooth muscles and its immediate consequences, and before any other signs can develop, the animal dies of asphyxia. On the other hand, smaller doses which do not produce this type of death are apparently too small to produce other symptoms. However, when the animals are protected against death by asphyxia, as in the present experiments, histamine in significantly high dosage can be introduced and unusual symptoms are observed, as described in these experiments; namely, a pronounced narcotic effect which lasts from 30 minutes to 1 hour. At the end of this period the animals slowly recover, as from deep narcosis.

It may be more than a fortuitous occurrence that in certain patients the various antihistaminic substances exert a definite depression and an almost narcotic action. This symptom may be due to the local anesthetic power of these substances. It also could be explained in the light of the foregoing observations; namely, that the competitive action of PBZ with histamine, in some instances, may lead to a duplication of certain histamine effects; while in others, to a suppression of other typical histamine activities.

PBZ aerosol inhalation not only confers a powerful protection against histamine intoxication in guinea pigs, but also protects against anaphylaxis. The doses necessary to prevent anaphylactic shock are approximately the same as those which are active against histamine poisoning, and in both cases the duration of protection is essentially the same. The fact that exceedingly small doses of PBZ introduced directly into the lungs are equally effective in both histamine poisoning and anaphylaxis adds another argument in favor of the theory that anaphylactic death and histamine death in guinea pigs are produced by the same poison, or at least by substances closely related.

Conclusions. 1. PBZ inhaled in the form of an aerosol and in minute amounts, protects guinea pigs against histamine intoxication and anaphylactic shock. This protection is relatively long-lasting. 2. The selective protection which PBZ aerosols confer to the lung tissue permits the observation in guinea pigs of hitherto unrecorded histamine effects. 3. The high activity of PBZ aerosol inhalation and the apparent lack of irritation suggest the possible therapeutic use of PBZ as an aerosol, since it may be predicted that this method of administration produces a lasting therapeutic effect with doses too small to produce undesirable side reactions.

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[7] Wells, J. A., Morris, H. D., Bull, H. B., and Dragstedt, C. A., *J. Pharm. Exp. Ther.*, 1945, **85**, 122.

[8] Yonkman, F. F., Chess, D., Mathieson, D., Hansen, N., *J. Pharm. Exp. Ther.*, 1946, **87**, 256.