

THE EFFECTS OF DRUGS UPON A GRADED COUGH RESPONSE
OBTAINED IN SENSITIZED GUINEA PIGS EXPOSED TO
AEROSOL OF SPECIFIC ANTIGEN

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In passively sensitized guinea pigs anaphylaxis has been elicited with known amounts of antibody and antigen (1). Thus, some information has been obtained regarding a quantitative relationship between antigen and antibody in the production of fatal anaphylactic shock. Since sublethal degrees of shock do not lend themselves to accurate and objective grading, little or no attempt has been made to construct dose-response curves based on graded responses of the animals to graded doses of either antigen or antibody. The experiments to be presented in this paper describe the effects of drugs upon a non-fatal anaphylactic reaction for which dose-response curves can be constructed when graded amounts of antibody are administered.

Feinberg and Malkiel (2) observed that when passively sensitized guinea pigs were exposed to an aerosol of the specific antigen, the time required for the production of dyspnea and cough was relatively constant. They did not, however, determine the amount of injected antibody, nor did they administer graded doses. Herxheimer (3) and Armitage *et al.* (4) used a similar procedure with actively sensitized guinea pigs. In none of these experiments was an objective measure of the end-point possible. Herxheimer pointed out that determination of the end-point was difficult and subjective; he relied upon the combined judgment of two experienced observers.

The technique developed in this laboratory permits one to determine objectively and quantitatively the reaction of guinea pigs sensitized with known amounts of antibody and exposed to an aerosol of the specific antigen. The principle of the method consists in obtaining by mechanical registration a count of the number of coughs produced by the animal within a fixed exposure time.

Methods

Guinea pigs of both sexes, weighing 250 to 350 gm., were injected intravenously with known amounts of rabbit antiserum in which a determination of antibody nitrogen had previously been made.¹ The antigen used, both for sensitizing the rabbits and for exposing the

¹ The authors are indebted to Dr. Morris Solotorovsky and Dr. Curt Porter, of Merck Institute for Therapeutic Research, Rahway, New Jersey, for assistance in this determination.

guinea pigs, was Armour's crystalline bovine plasma albumin. Although intracardiac injections are frequently employed in guinea pigs, it is our feeling that such a procedure entails unnecessary risk of injury to the animal. Contrary to the impression of many workers, intravenous injection of guinea pigs is quick and easy after some practice (see Appendix to Kabat and Mayer (1)). We generally use the vein on the outer surface of the left foreleg.

About 48 hours after administration of the antiserum, the animals were exposed in individual chambers to an aerosol of specific antigen. Exposure to the aerosol, which consisted of particles of $1\ \mu$ or less in diameter, induced coughing. By means of a rubber diaphragm and an electric circuit described in a previous paper dealing with antitussive drugs (5), a count of the number of coughs was semi-automatically obtained.

In the present experiments, the animals were left in the chamber for 10 minutes. Severe anaphylactic shock was seldom observed with this procedure; the reactions of the animals seldom went beyond the stage of coughing and moderate dyspnea.

TABLE I

Effect of Varying the Concentration of Antigen in the Aerosolized Solution

Guinea pigs sensitized with 80γ of antibody nitrogen, and exposed 48 hours later for 10 minutes in the aerosol chamber.

Concentration of antigen, per cent.	0.1	0.2	1.0	5.0	8.1
No. of animals	5	10	5	5	5
Mean no. of coughs	14.2	25.8	31.2	28.6	29.0

RESULTS

Effect of Varying the Concentration of Antigen.—Table I shows the results of employing varying concentrations of antigen in the aerosolized solution when the amount of antibody was kept constant at 80γ of antibody nitrogen for each animal. It was impossible to estimate how much antigen was actually absorbed by the animal, so no attempt was made to establish a dose-response relationship. Rather, the results of this experiment were used to establish the concentration of antigen to be used in later experiments. It is clear that 0.1 per cent gives a submaximal response, while a maximal effect is obtained by concentrations of 0.2 to 1 per cent or above. For routine use in further experiments, the 1 per cent solution was adopted.

Dose-response Curve for Antibody Nitrogen.—With doses of antibody nitrogen between 20 and 80γ , the number of coughs produced by the animal in a 10 minute exposure was found to be linearly related to log dose. An analysis of the data is presented in Table II. These are pooled results from five experiments, with 5 to 6 animals per group in each experiment; the lowest dose, 10γ of antibody N, was tested in only two of the experiments. When the responses of 10γ of antibody nitrogen were included in the statistical analysis, they were found to be non-linear with respect to log dose. However, in the range of 20γ to 80γ , there was no deviation from linearity; this range has therefore been used for the statistical analysis shown in Table II. The analysis demon-

strates a very highly significant correlation between individual responses and log dose (coefficient of correlation, 0.56, $P < < 0.01$). There was considerable variation in the regression coefficients of the individual experiments (range, 25.0 to 42.5) but the differences were not statistically significant.²

Fig. 1 shows the mean regression for these data, and the points are the group averages for the individual experiments.

TABLE II

Dose-Response Relationship between Antibody Nitrogen and Number of Coughs

Guinea pigs sensitized with varying amounts of antibody 48 hours prior to exposure for 10 minutes in the aerosol chamber. Concentration of aerosolized solution: 0.2 per cent to 1 per cent antigen.

Antibody nitrogen, γ	10	20	40	80
No. of animals.....	12	28	27	28
Mean No. of coughs.....	2.17	7.29	17.30	27.36
\pm standard error.....	± 1.24	± 1.20	± 2.28	± 3.13

Analysis of variance, 20, 40, and 80 γ

Source of variation	Degrees of freedom	Sum of squares	Mean square
Total.....	82	17,812	
Within groups (error).....	80	12,172	152
Doses antibody N.....	2	5,640	2,820
Linear regression.....	1	5,640	
Deviations from linear regression.....	1	0	0

$$F = \frac{2820}{152} = 18.55 \text{ very highly significant.}$$

Standard deviation of single determination = 12.33.

Regression equation: $Y = 33.45 \log X - 36.21$, in which $Y =$ No. of coughs, $X = \gamma$ antibody N.

Index of precision (λ) = $12.33/33.45 = 0.37$.

Specificity of the Reaction.—Two groups of non-sensitized control animals, 5 animals per group, were exposed to the aerosolized antigen. In one group, one animal coughed once, and in the other group one animal coughed three times during a 10 minute exposure. In each group, 4 of the animals had no coughs. Therefore, when a significant number of coughs was produced in sensitized animals, it was considered to be a specific response.

² Two of these experiments were performed with antiserum kindly supplied by Dr. Joaquin Munoz, of Sharp & Dohme, Division of Merck & Co., Inc., West Point, Pennsylvania. The other three were performed with antiserum prepared in our own laboratories. The results with the two antisera were identical.

Effect of Antitussive Drugs.—The cough produced by this technique does not respond to the antitussive drug, codeine, but can be inhibited by anti-allergic agents, such as antihistaminic drugs and cortisone (see below). We have shown elsewhere (5) that when guinea pigs are exposed in the glass chambers to a simple irritant such as ammonia or aerosolized dilute acid, they

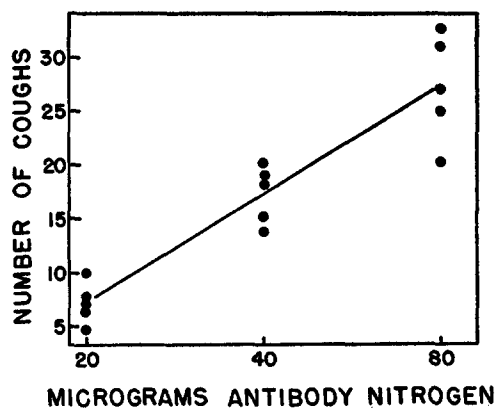


FIG. 1 Dose-response curve showing the relationship between log dose of antibody and the number of coughs. Guinea pigs were passively sensitized 48 hours before exposure to aerosolized 1 per cent antigen solution for 10 minutes. Solid line, untreated controls, pooled data from 5 experiments. The points are group averages, 5 to 6 animals per group.

TABLE III

Effect of Antitussive Drugs upon the Cough

Guinea pigs sensitized with 80 γ of antibody nitrogen and exposed for 10 minutes to aerosolized antigen. 6 animals per group.

Experiment	Controls	No. of coughs			
		Narcotine 1 mg./kg.	Narcotine 2 mg./kg.	Codeine PO ₄ 4 mg./kg.	Propadrine HCl 8 mg./kg.
1	30.5	—	—	36.5	—
2	16.8	1.7	—	—	—
3	23.2	13.8	5.8	—	22.0

respond by a cough which is readily inhibited by such drugs as codeine, morphine, propadrine, and narcotine. These drugs are effective in doses on the order of 0.5 to 1 mg. per kg. against cough produced by simple irritants. Table III shows the results of three experiments with antitussive drugs in the cough produced by the present technique in sensitized guinea pigs. It is clear that narcotine was the only antitussive drug effective in this test, even though both codeine and propadrine were used at higher dose levels.

Since the antihistaminic drug, pyrilamine maleate, is very effective in inhibiting the cough of the sensitized animals (see below), it was of interest to determine whether narcotine possessed antihistaminic activity. Six animals were injected with 10 mg. per kg. of narcotine, and an equal number with 0.1 mg. per kg. of pyrilamine maleate. They were then exposed to histamine aerosol in the same chambers. All of the narcotine-treated animals suffered severe histamine shock within an average of 1.5 minutes of exposure, comparable to the average of 2.1 minutes for uninjected controls. All of the pyrilamine-treated animals survived 10 minutes of exposure without severe shock. Narcotine showed no trace of antihistaminic activity, although it was

TABLE IV

Synergistic Effect of Cortisone and Pyrilamine in Inhibiting Cough

Guinea pigs sensitized with 80 γ of antibody N and exposed for 10 minutes to aerosolized antigen, 1 per cent solution. 6 animals per group.

Treatment*	Mean No. of coughs		Mean latent period	
	Experiment 1	Experiment 2	Experiment 1	Experiment 2
Pyrilamine.....	9.5	7.8	<i>min.</i> 6.12	<i>min.</i> 6.21
Cortisone.....	14.7	3.3	7.67	7.97
Pyrilamine + cortisone.....	<0.2‡	0	>10	>10
Controls.....	22.6	14.8	2.55	3.33

* Subcutaneous injections: pyrilamine maleate (neo-antergan, Merck) 10 γ /kg. 1 hour before exposure; cortisone acetate (Cortone, Merck) 5 mg. daily for 3 days, last dose 24 hours before exposure.

‡ One animal gave 1 cough.

administered in a dose ten times as great as that which was found to be effective in the sensitized animals.

In additional experiments, neither cortisone acetate nor the antihistaminic drugs, phenergan hydrochloride and pyrilamine maleate, inhibited the cough owing to a simple irritant. For example, one group of 7 animals, after receiving 5 mg. daily of cortisone acetate for 3 days, gave 73.4 ± 9.7 coughs when exposed to ammonia vapor in the chamber for 3 minutes. This did not differ significantly from the figure of 54.1 ± 6.6 obtained in a control group of 7 animals. Another group of 6 animals exposed to ammonia vapor for 3 minutes gave an average of 49.8 coughs before injection of 8 mg. per kg. of pyrilamine maleate, and 49.2 coughs 1 hour after injection; corresponding figures for a group observed on the same day and treated with 1 mg. per kg. of narcotine were 54.8 and 14.6. Narcotine is therefore unique among the drugs tested, in that it inhibits both types of cough.

Synergism between Cortisone and Antihistaminic Drug.—Subcutaneous

injection of 10γ per kg. of pyrilamine maleate (neo-antergan) 1 hour prior to exposure in the chamber markedly inhibited the coughing response to aerosolized antigen in the sensitized animals (Table IV). Increasing the dose of antihistaminic drug did not increase its effectiveness. For example, in one experiment, 3 groups of animals sensitized with 80γ of antibody nitrogen were given 5, 10, and 1000γ per kg., respectively, of pyrilamine maleate. The mean number of coughs obtained was 22.6, 11.8, and 8.5, respectively. The first group did not differ significantly from the controls (32.7) and the last two groups did not differ significantly from each other.

On the other hand, Table IV demonstrates that the inhibiting effect of 10γ per kg. of antihistaminic drug can be augmented by prior treatment with cortisone. This table shows two identical experiments on groups of 6 animals, sensitized with 80γ of antibody nitrogen. Cortisone was administered subcutaneously, 5 mg. daily for 3 days, with exposure in the chamber on the 4th day. Not only were the coughs counted, but the times required for the first bout of coughing to occur was also measured. Some of the treated animals did not show a clear-cut bout of coughing. For purposes of calculating the mean latent period, such animals were arbitrarily assigned a time of 10 minutes.

Either cortisone or pyrilamine reduced the number of coughs and prolonged the latent period, but neither treatment completely suppressed the reaction. When pyrilamine was administered to animals pretreated with cortisone, inhibition was complete. Not only was there no cough in these animals, but other signs of reaction, such as hyperpnea, dyspnea, and restlessness were also notably absent.

This effect of cortisone is in contrast to the failure of this steroid to inhibit fatal anaphylactic shock due to intravenous antigen (references in Solotovsky and Winsten (6)). We can confirm the latter observation, using the same antigen-antibody system as was used in the coughing experiments. Eight guinea pigs were treated for 5 days with 5 mg. of cortisone daily. On the 4th day, 80γ of antibody nitrogen was injected, and on the 6th day, 1 mg. of antigen was administered intravenously. Six of the 8 animals died in anaphylactic shock, one had severe shock but recovered, while one had mild shock. By contrast, 6 animals were similarly sensitized and given the same shocking injection on the same day, but were pretreated with 1 mg. per kg. of pyrilamine maleate 1 hour before shocking. None of these animals suffered more than very mild signs of shock.

DISCUSSION

Perhaps the principal advantage of this technique is the fact that for the first time a graded response has been found in sensitized animals which can be objectively measured with sufficient precision to show that a quantitative relationship exists between the degree of response and the amount of antibody injected. The method would be even more useful if a similarly precise

measurement could be made of the amount of antigen which entered into the reaction, and also if the linear relationship extended over a wider range of doses of antibody.

It is of interest that the cough produced by this procedure responds to the same sort of drugs which are of therapeutic value in human allergy (antihistaminic agent and cortisone) but is unaffected by the antitussive drug, codeine. Codeine is generally thought to act by central inhibition of the cough reflex, and it is quite effective in suppressing the cough of guinea pigs produced by simple irritants administered in the same way as was the antigen in the present experiments. It is not known whether the "allergic" cough produced in this procedure is due to an irritating effect of the products of the antigen-antibody reaction, or whether the stimulus for the cough is related to the degree of bronchoconstriction which probably occurs when the sensitized guinea pig is exposed to the inhaled antigen. It is evident, however, that the reaction produces such a strong stimulus to the cough reflex that the central inhibiting effect of codeine in doses eight times as great as the amount necessary to have a definite inhibiting effect on cough due to simple irritants had no trace of effect upon it. The activity of narcotine is also surprising, since it has no antihistaminic activity, and since the other antitussive drugs tested were ineffective. The mechanism of action of narcotine in this procedure is not known, but it might be interesting to test this drug for anti-allergic activity in man. In this connection, it may be noted that Bickerman and Barach (7) found narcotine to be particularly effective against experimentally induced cough in asthmatic subjects.

It is probable that cortisone is effective in this system by virtue of its general anti-inflammatory action, and that pyrilamine is active because of its antagonism to the peripheral effects of histamine and/or other noxious substances released by the antigen-antibody reaction in the respiratory tract. Both cortisone and pyrilamine would therefore hinder the development of the local tissue reaction responsible for the initiation of the cough reflex. There is a marked contrast between the ability of cortisone to inhibit the reaction in the present experimental system and its failure to affect anaphylactic shock produced by injected antigen. Narcotine in our hands (unpublished data) also is relatively ineffective in the treatment of fatal anaphylactic shock in guinea pigs. It is likely that intravenous antigen releases so much histamine that a potent antihistaminic drug is required to control shock. Factors other than histamine are of sufficient importance in the genesis of the cough that control may be obtained by anti-allergic agents not possessing antihistaminic activity. The activity of cortisone in these experiments parallels its action in human asthma rather than in fatal anaphylaxis. It does not necessarily follow that either the antigen-antibody system used in these investigations, or the type of local reaction obtained, is the same as that seen in asthma in humans.

It is also of interest that cortisone and pyrilamine proved to be synergistic

in these experiments. A degree of inhibition of the reaction was obtained with the antihistaminic drug in animals pretreated with cortisone which could not be achieved by greatly increasing the dose of antihistaminic agent alone. Whether this observation has any clinical significance remains to be seen.

SUMMARY

A technique is described for measuring objectively and quantitatively the reaction of sensitized guinea pigs when exposed to an aerosol of specific antigen. The principle involves registration by semi-automatic means of the number of coughs produced in animals passively sensitized with known amounts of antibody. The number of coughs is shown to be linearly related to log dose of antibody within a limited range and a dose-response curve is presented.

The cough produced by this procedure is not inhibited by the antitussive drugs, codeine and propadrine, but can be inhibited by anti-allergic agents, such as cortisone and an antihistaminic drug. It is also inhibited by narcotine. The last is the only compound so far tested which suppresses both the cough produced by this procedure and that produced by a simple irritant.

The action of cortisone and the antihistaminic drug, pyrilamine, is shown to be synergistic. A small dose of pyrilamine in animals pretreated with cortisone gives a degree of inhibition which cannot be obtained by increasing the dose of pyrilamine in animals not treated with cortisone.

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