

STUDIES IN REAGINIC AND HISTAMINIC WHEELS.
II. THE EFFECT OF LOCALLY ADMINISTERED ANTIHISTAMINE ON
THE PASSIVE TRANSFER REACTION*

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THE effectiveness of locally applied or injected antihistamines in diminishing the immediate whealing response of the skin to atopens and histamine has been repeatedly demonstrated.^{1, 2} In passive transfer experiments, the interest of previous workers has been focused primarily upon the ability of the antihistamines to diminish or to prevent the development of a positive reaction at the passively sensitized site.^{3, 4}

We have been concerned with another phase of the Prausnitz-Küstner phenomenon. We have sought to determine the effect of antihistamine upon the wheal produced by the *sensitizing injection* of serum in the preparation of the passively sensitized site. Bowman and Walzer⁵ had reported that when a pronounced immediate wheal results from the injection of the sensitizing serum into the skin of the recipient, hyporesponsiveness to subsequent testing tends to develop at this site. The purpose of the present study was to determine whether the addition of an antihistamine to the sensitizing serum would diminish the troublesome whealing produced by the sensitizing injection and thus reduce the amount of hyporesponsiveness which, as a consequence, developed at the site.

Method and Material.—Most of the subjects employed as recipients in the passive transfer experiments were physically sound adult patients in a psychiatric institution. Patients with a positive personal history of atopic diseases and those who demonstrated positive reactions to preliminary intracutaneous tests with a number of common allergens were excluded from the study.

Preliminary comparative studies were made with several brands of injectable antihistamine solutions, including Pyribenzamine, Neo-Antergan and Thephorin. All of these antihistamines, in concentrated solution, were found to be irritating when injected intracutaneously. Because Pyribenzamine proved to be less irritating than the other brands tested, it was selected for use in this study. The stock or concentrated solution of Pyribenzamine contained 25 mg. of the drug per cubic milliliter. It was free of preservative. Fresh dilutions of the antihistamine in physiologic saline were prepared daily. The solutions of histamine phosphate diluted in terms of the base were prepared in physiologic saline prior to each experiment. For intracutaneous testing, the histamine solution was used in a dilution of 1:30,000 or of 1:15,000. Preliminary studies had

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Read at the Eighth Annual Meeting of the American Academy of Allergy, Chicago, Feb. 19, 1952.

Received for publication Dec. 22, 1952.

*Aided by a grant from the Celia G. Antonoff Research Fund.

shown that these dilutions would elicit reactions of slight (+) or moderate (++) size, the zone of reactivity most suitable for detecting the relative size of the wheals.

The antigens employed for skin testing were those in routine use at the Allergy Clinic of The Jewish Hospital of Brooklyn. They had been prepared according to the method of Coca and Milford.⁷ For testing, those dilutions of antigens were chosen which preliminary titrations of the sera had shown were required to produce reactions within the slight (+) or moderate (++) zone on the passive transfer sites.

The reaginic sera were obtained under sterile precautions from clinically sensitive patients who, at the time of bleeding, were receiving no medication. The usual precautions to prevent hemolysis and contamination of blood were observed. Sera were Seitz-filtered and stored without preservative in the refrigerator. They were used within eight months of the time they were drawn.

Serum "C1" and "C2" were taken on separate occasions from an asthmatic patient who was clinically sensitive to rabbit epithelium. Serum "S" was obtained from a boy with atopic dermatitis who was clinically sensitive to mustard. Serum "N" was drawn from a physician with bronchial asthma who was sensitive to cottonseed.

Tests were performed by the intracutaneous technique with due regard to the recommendations for comparative testing made by Bowman.⁶ The test dose of histamine and of specific antigen was 0.01 ml. of the test material. The skin reactions were read at about fifteen minutes.

To simplify the presentation of results to the reader, no attempt will be made to report the exact size of the reactions obtained at the various sites. Instead reactions at the sites treated and untreated with antihistamine will be compared and the relative size of the two will be recorded.

The technique employed for passive transfer testing will be described in the experiments which are to be presented.

Preliminary Studies With Pyribenzamine.—Since it was planned to investigate the effect of antihistamine solution upon the Prausnitz-Küstner technique, it became necessary to run preliminary studies to ascertain to what extent this drug was irritating to the skin when employed intracutaneously.

It was found that 0.01 ml. of the concentrated (2.5 per cent) solution of Pyribenzamine, when injected intracutaneously, was more irritating to the skin than a similar amount of physiologic saline. Tests with dilutions of Pyribenzamine up to 1:10 dilution of the stock (2.5 per cent) solution produced larger reactions than similar tests with saline. When the antihistamine was diluted from 1:20 to 1:60, reactions resulted which were either larger or equal to similar tests with saline. Pyribenzamine, in dilutions greater than 1:60, produced no reactions larger than those with saline. Pyribenzamine in this dilution, therefore, was considered nonirritating to the skin.

Since it was intended to use the Pyribenzamine in the sensitizing injection in the passive transfer experiments, the above studies were repeated using 0.025 ml. for each intracutaneous injection. This amount represents the volume of

serum ordinarily injected for sensitization of the cutaneous sites. In these trials, Pyribenzamine in dilutions up to 1:100 of the stock solution consistently produced reactions which were larger than the saline controls. In dilutions between 1:100 and 1:1000, the results were inconstant. With antihistamine dilutions of 1:1,000 or greater, the injection of 0.025 ml. produced reactions no larger than those produced by similar amounts of saline. The irritating property of Pyribenzamine solution was therefore considered absent at this dilution.

In another preliminary investigation, we studied the ability of Pyribenzamine, when admixed with histamine, to counteract the urticariogenic effect of the latter. Intracutaneous tests were performed with 0.01 ml. of fresh mixtures of equal parts of saline dilutions of Pyribenzamine stock solution (2.5 per cent) and of histamine (1:15,000). The wheal which resulted was compared with that produced by a similar test with a mixture of equal parts of the histamine solutions and saline. The tests were introduced simultaneously on the same subject. The reactions were read after about fifteen minutes. In dilutions of Pyribenzamine up to and including 1:40, an antihistaminic effect against the histamine solution (1:15,000) was regularly noted. (See Table I.) With greater dilutions of Pyribenzamine, its antihistaminic effect diminished until at 1:2,560, only 68.2 per cent of the comparative tests showed the inhibiting effect of the drug. At a dilution of 1:10,240, the antihistaminic effect of the Pyribenzamine was no longer apparent, since only 52.9 per cent of the wheals produced by the Pyribenzamine-histamine mixture were smaller than the controls. The remainder of the reactions in this series were equal to or larger than the controls.

Summarizing the results of these preliminary studies, it may be stated that the irritating property of the stock Pyribenzamine solution (2.5 per cent) was lost at a dilution of 1:60, when the injection volume was 0.01 ml., and at a dilution of 1:1,000 when the volume was 0.025 ml. The antihistaminic effect of the stock solution was not lost until a dilution of 1:10,240 was reached.

TABLE I. EFFECT OF PBZ* DILUTION ON ITS ANTIWHEALING POWER

DILUTION OF PBZ STOCK SOLUTION ADDED TO HISTAMINE	NUMBER OF COMPARISONS WITH HISTAMINE-SALINE CONTROLS	NUMBER OF HISTAMINE REACTIONS DIMINISHED BY PBZ	PERCENTAGE OF REACTIONS AFFECTED BY PBZ
1:2	8	8	100
1:10	9	9	100
1:40	8	8	100
1:160	8	7	87.5
1:640	14	12	85.7
1:2,560	22	15	68.2
1:10,240	17	9	52.9

Histamine dilution 1:15,000.

Stock PBZ 2.5 per cent solution of the drug.

Test mixture made of equal parts of PBZ dilution and histamine 1:15,000.

Control mixtures made of equal parts of saline and histamine 1:15,000.

*Pyribenzamine.

PASSIVE TRANSFER STUDIES

Sites were prepared on nonatopic subjects by the intracutaneous injection of 0.025 ml. of two freshly prepared mixtures. One contained equal parts of a sensitizing serum and stock (2.5 per cent) Pyribenzamine solution. The second,

or control, mixture contained equal parts of the sensitizing serum and saline. Two pairs of sites were sensitized on each subject. The sensitized areas were placed at least 6 cm. apart. The positions of the test and control mixture sites were rotated on the arm in order to eliminate the possible importance of this factor in the results obtained.⁶

The immediate wheals produced by the sensitizing injections of the two mixtures were compared after about thirty minutes in order to determine the effect of the presence of the Pyribenzamine in the one mixture. The sites were tested seven days later. One pair of sensitized sites was injected with 0.01 ml. of the related antigen and the resulting reactions were compared. The second pair of sites was tested with a solution of histamine phosphate (1:15,000 or 1:30,000) and their reactions were also compared.

A. *The Effect of the Presence of Undiluted Pyribenzamine Solution in the Sensitizing Injection on the Sensitization Wheals.*—At the sites injected with 0.025 ml. of the two mixtures, wheals started to develop as the skin reacted to the injection of a foreign substance. At the end of thirty minutes, the wheal resulting from the introduction of the mixture made of equal parts of stock Pyribenzamine solution and reaginic serum was compared with that produced by the injection of the saline-serum mixture. The Pyribenzamine-serum mixture produced larger immediate wheals than the saline-serum controls in 45 of the 46 comparisons or 97.8 per cent of trials. (See Table II.) The Pyribenzamine-serum wheals were also more indurated than the control wheals. Another difference between the reactions was the narrow halo of intense erythema which surrounded the Pyribenzamine-serum wheals in contrast to the more diffuse pale erythema which circumscribed the serum-saline wheals.

TABLE II. EFFECT OF CONCENTRATED PBZ SOLUTION IN THE SENSITIZING SERUM ON THE PASSIVE TRANSFER REACTION

SERUM	ANTIGEN	EFFECT OF DILUTED PBZ SOLUTION IN SENSITIZING INJECTION								
		ON IMMEDIATE SENSITIZING REACTION			ON SUBSEQUENT TEST WITH ANTIGEN			ON SUBSEQUENT TEST WITH HISTAMINE		
		NO. OF COMPARISONS	WHEEL LARGER ON		NO. OF COMPARISONS	WHEEL LARGER ON		NO. OF COMPARISONS	WHEEL LARGER ON	
			PBZ-SERUM SITE	SALINE-SERUM SITE		PBZ-SERUM SITE	SALINE-SERUM SITE		PBZ-SERUM SITE	SALINE-SERUM SITE
S	Mustard	8	7	1	6	1	5	2	1	1
C1	Rabbit epithelium	18	18	0	9	0	9	8	3	5
C2	Rabbit epithelium	20	20	0	10	1	9	10	2	8
Totals		46	45	1	25	2	23	20	6	14

Sensitizing mixture made of equal parts of PBZ (2.5 per cent solution) and serum.

Control mixture made of equal parts of saline and serum.

Passively sensitized sites tested after seven days.

Sensitizing intracutaneous injection made with 0.025 or 0.05 ml.

Intracutaneous test made with 0.01 ml. of related antigen or of histamine (1:15,000 or 1:30,000.)

B. *The Effect of the Presence of Undiluted Pyribenzamine Solution in the Sensitizing Injection on the Subsequent Reaction to Tests With Allergens.*—Seven days after the sensitization of the skin sites with the two mixtures, the sites

were tested for sensitivity by the intracutaneous injection of 0.01 ml. of the related allergens. For sites prepared with Serum "S," mustard extract was used; for Serum "C," rabbit epithelium was employed. After fifteen minutes, the reactions which developed at each pair of sites were compared. In 23 out of 25 comparisons (92 per cent), the specific reactions on the control (saline-serum) sites were larger than those on the Pyribenzamine-serum sites. Hence, it appeared that the presence of undiluted Pyribenzamine in the sensitizing mixture had reduced the subsequent responsiveness of the sensitized sites to antigenic stimulation.

On 8 of the 25 Pyribenzamine-serum sites, atypical wheals with hollow centers ("crater" wheals) or "dispersed" wheals developed. These unusual whealing phenomena will be described in a future communication.

C. The Effect of the Presence of Undiluted Pyribenzamine Solution in the Sensitizing Injection on the Subsequent Reactions to Tests With Histamine.—In a previous communication, Bowman and Walzer⁵ demonstrated that the hyporesponsiveness which developed at a passively sensitized site could be attributed mainly to two factors. The first was a tissue factor, nonspecific in nature, which was based on injury to cells. The second was an immunologic factor and was the result of injury to the injected reaginic antibodies. The former effect was found to be transient, the latter was found to be irreversible.

In order to determine whether these findings would apply to sites prepared with the Pyribenzamine-serum mixtures, 20 pairs of sites, prepared with the two previously described mixtures, were tested, after an interval of seven days, with histamine solution in dilutions of 1:15,000 or 1:30,000. After fifteen minutes, the wheals which developed were compared. Here again the wheals on the Pyribenzamine-serum sites tended to be smaller than those on the saline-serum control sites. This occurred in 14 of the 20 comparisons, or in 70 per cent of trials. (See Table II.) Hence, the hyporesponsiveness of the sites to histamine stimulation was not so regularly demonstrable as that to tests with antigens, which, in the previous experiment, had been found in 92 per cent of trials. Nevertheless, the findings pointed to the participation of both the tissue and antibody factors in the causation of the hyporesponsiveness demonstrated at the Pyribenzamine-serum sites.

Since the serum factors in the Pyribenzamine-serum and saline-serum mixtures were identical, it was reasonable to attribute the differences in the results obtained to the presence of the antihistamine solution in one mixture. It seemed likely that, because of its concentration, the stock Pyribenzamine solution was irritating to the skin and was responsible for the increase in the size of the immediate sensitization reactions which these sites exhibited over those produced by the control mixture. This resulted in a greater degree of hyporesponsiveness at the Pyribenzamine sites and a smaller specific reaction at these sites when they were subsequently tested with antigens.

D. The Effect of Diluting the Pyribenzamine Solution in the Sensitizing Injection Upon the Subsequent Tests With Allergens.—The attempt was then made to eliminate the irritating property of the Pyribenzamine by dilution. Pyribenzamine dilutions of 1:40 to 1:640 were prepared with physiologic saline and mixed with equal parts of sensitizing Serum "N." The immediate reactions

to sensitizing injections of these mixtures were compared with those produced by the saline-serum controls. In these experiments, the sensitizing reactions were *larger* on the *control* sites in 12 of the 15 comparisons, or in 80 per cent of the trials. (See Table III.) These results were in marked contrast to those previously obtained with undiluted Pyribenzamine solution (Table II) which almost invariably produced larger immediate reactions on intracutaneous injection than the controls.

TABLE III. EFFECT OF DILUTED PBZ SOLUTION IN THE SENSITIZING SERUM ON THE PASSIVE TRANSFER REACTION

PBZ DILUTION	EFFECT OF DILUTED PBZ SOLUTION IN SENSITIZING INJECTION					
	ON IMMEDIATE SENSITIZING REACTION			ON SUBSEQUENT TEST WITH ANTIGEN		
	NO. OF COMPARISONS	WHEEL LARGER ON		NO. OF COMPARISONS	WHEEL LARGER ON	
		PBZ-SERUM SITE	SALINE-SERUM SITE		PBZ-SERUM SITE	SALINE-SERUM SITE
1:40	5	1	4	5	4	1
1:160	5	1	4	5	3	2
1:640	5	1	4	5	4	1

Sensitizing mixture made of equal parts of serum and PBZ dilutions.

Passively sensitized sites tested after seven days with diluted antigen.

When the sensitized sites were tested with the related allergen, cottonseed, after an interval of seven days, larger wheals were obtained on the dilute Pyribenzamine-serum sites than on the control sites in 11 of 15 comparisons, or in about 75 per cent of the trials. The results obtained with the various dilutions of the antihistamine which were employed were quite similar.

Hence, it had been demonstrated in these experiments that it was possible to dilute the Pyribenzamine solution in the sensitizing injections down to the point where its irritating property was eliminated but its antiwhealing power was still retained. By diminishing the size of the immediate sensitizing reaction, tissue trauma and injury to the skin-sensitizing antibodies, the two most important factors responsible for the hyporesponsiveness of passively sensitized sites, were reduced. For this reason, the passively sensitized sites prepared with the dilute antihistamine responded more favorably to subsequent tests with antigen than did the saline-serum sites.

PRACTICAL APPLICATION

As a practical application of the above findings, dilute Pyribenzamine was added to the serum used for the sensitization of sites for routine passive transfer studies, such as the indirect method of testing.⁸ Most satisfactory results were obtained by the use of sensitizing serum containing the antihistamine in a final dilution ranging from 1:50 to 1:150 of the 2.5 per cent stock solution. In order to avoid dilution of the serum, minute amounts of a 1:10 dilution of the stock Pyribenzamine solution were added to undiluted serum in order to obtain the desired concentration of antihistamine in the sensitizing material. For routine work, we most often employed a serum containing a 1:100 dilution of the stock 2.5 per cent solution of Pyribenzamine. As an example, 0.1 ml. of 1:10 Pyribenzamine stock solution was added to 0.9 ml. of undiluted serum immediately before sensitization. As an experimental control, a similar amount of physiologic

TABLE IV. ADVANTAGEOUS EFFECT OF PBZ TECHNIQUE IN INDIRECT METHOD OF TESTING

SÉRUM	FINAL CONCEN- TRATION OF PBZ IN SENSI- TIZING SÉRUM	REACTION TO SENSITIZING INJECTION						INTERVAL IN DAYS BETWEEN SENSITIZING AND TESTING	ANTIGEN TESTED	REACTION TO TEST WITH ANTIGEN						SITE SHOW- ING LARGER REACTION
		PBZ-SÉRUM SITE (P)		SALINE- SÉRUM SITE (S)		SITE SHOW- ING LARGER REACTION				PBZ-SÉRUM SITE (P)		SALINE- SÉRUM SITE (S)		SITE SHOW- ING LARGER REACTION		
		WHEAL	ERYTH.	WHEAL	ERYTH.	WHEAL	ERYTH.			WHEAL	ERYTH.	WHEAL	ERYTH.	WHEAL	ERYTH.	
1	1:100	±	00±	+±	+	5	Ragweed	+±	+	+	+	P	Equal			
2	1:150	+±	+	+++	+++	6	Wheat	+±	+±	+±	++	P	Equal			
3	1:150	+	0±	++	±	4	Ragweed	+±	+±	+	±	P	P			
3	1:100	+	0	++	±	7	Ragweed	++	+	+	0±	P	P			
4	1:100	±	0	++	+	4	Horse epithelium	+++	+	+	0±	P	P			
5	1:50	±	0	++	±	5	Birch	++	+±	+	±	P	P			

For scale for recording reactions, refer to Bowman and Walzer.⁵

saline was added to 0.9 ml. of undiluted serum and this mixture was used for the sensitization of additional sites on the same recipient. Several examples of the superiority of the Pyribenzamine technique over the previous routine method of indirect testing are presented in Table IV.

The addition of Pyribenzamine to the sensitizing serum was found to be particularly advantageous when dealing with an irritating serum, such as that which is frequently obtained from a child suffering from atopic dermatitis. When sites sensitized with such a serum and a normal untreated skin area on the recipient were tested from seven to ten days after sensitization with histamine 1:15,000, smaller reactions were invariably obtained on the sensitized site, showing the presence of hyporesponsiveness. When Pyribenzamine, in the dilutions mentioned previously herein, was added to such a serum before sensitizing the same recipient, it was found that equal responses to histamine were obtained on the sensitized site and on a normal untreated area within five days after sensitization. To workers faced with the many difficulties encountered in passive transfer techniques, the advantage of shortening the interval between sensitization and testing is obvious.

We do not wish to imply that one should expect to obtain a positive reaction on a site sensitized with a Pyribenzamine-serum mixture when a site sensitized with the serum alone yielded a negative reaction. It has been possible, however, by the use of this innovation in technique, to eliminate some of the tissue hyporesponsiveness resulting from the sensitizing injection and to obtain a definite positive reaction with this technique where only a doubtful reaction was elicited with the standard procedure.

The use of dilute Pyribenzamine in the sensitizing material is now being applied in some of the procedures which involve modifications of the Prausnitz-Küstner technique, such as the injection of antigen-antibody mixtures, the testing of sites by systemic administration of antigen, etc. These studies will be the subject of future communications.

CONCLUSIONS

1. In the passive transfer technique, the addition of concentrated (2.5 per cent) Pyribenzamine solutions to the reaginic serum produced large immediate whealing reactions following the sensitizing injections. This resulted in a pronounced hyporesponsiveness of the passively sensitized sites to subsequent tests with antigen or histamine.

2. Pyribenzamine, in proper dilution, in the sensitizing serum reduced the size of the sensitizing reactions and diminished the amount of hyporesponsiveness of the passively sensitized sites. This resulted in improved responses of the sensitized sites to tests with antigens.

3. The practical application of this technique in the indirect method of testing for hypersensitiveness has been demonstrated.

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