

STUDIES IN REAGINIC AND HISTAMINIC WHEELS

I. THE EFFECTS OF REAGINIC AND HISTAMINIC WHEELS UPON THE SUBSEQUENT RESPONSIVENESS OF PASSIVELY SENSITIZED CUTANEOUS SITES

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INTRODUCTION

IN 1921, Prausnitz and Küstner¹ reported the passive local sensitization of human skin by the intracutaneous injection of blood serum from a fish-sensitive individual with positive skin reactions to this allergen. This phenomenon was confirmed by De Besche² with sera of patients sensitive to other allergens. Coea and Grove³ described the immunologic properties of the skin-sensitizing antibody which they named the "atopic reagin."

The Prausnitz-Küstner phenomenon opened a new approach to the study of the immunologic mechanisms involved in immediate positive skin reactions and in atopic hypersensitiveness. The phenomenon met its first practical application in the suggestion of Walzer and Kramer⁴ that it be used, in a modified form, as an "indirect" method of testing atopic patients in whom direct testing was impractical. This technique was developed by Walzer and Bowman,⁵ who later reported other applications for passive transfer techniques.

Early in the course of these investigations, it became apparent to us that positive reactions at passively sensitized sites produced local changes which could not be accounted for solely on the basis of specific neutralization of antibodies. This fact was brought out in a preliminary report⁶ to the Society for the Study of Asthma and Allied Conditions in April, 1932. The studies were continued but were repeatedly interrupted for long periods of time because the available techniques were inadequate for the solution of the difficulties encountered. More recent experiments have yielded information which clarifies some of the inexplicable phenomena previously encountered in passive transfer studies.

Although we referred to different phases of this problem in our previous publications, the complete series of experiments are now about to be presented in detail for the first time. The scope of each paper in this series will be restricted more or less to that of the original preliminary report on which it is based and consequently will include only that bibliography which antedated the presentation of the preliminary report. Subsequent studies on these subjects by other workers will be reviewed in later papers.

Throughout this series of studies the nature and properties of histaminic and reaginic wheals were compared. These experiments were designed, in part, to test the validity of the concept advanced by Lewis⁷ and his co-workers, Dale,⁸

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and others that histamine, or a histamine-like ("H") substance, liberated locally from the tissue cells was responsible for the immediate whealing response of the allergic skin to a test with an offending allergen.

The experiments in this communication deal with the effects of wheal formation at a passively sensitized site upon the subsequent whealing potentialities of that site.

The wheals at the passively sensitized sites were produced either by an immunologic reaction between the atopic reagin and its related atopen (allergen, antigen) or by nonimmunologic excitation with a nonspecific urticariogenic excitant, such as histamine or codeine. After an appropriate interval, the sites were restimulated with the same or with the alternate type of excitant. In this way, comparisons were made of the effects of immunologic and non-immunologic types of wheals upon the subsequent responsiveness of passively sensitized sites to the two different types of excitants.

TECHNICAL CONSIDERATIONS

1. *Recipients*.—Nonatopic adults were used as recipients for sensitization in these studies. Many of them were student nurses or patients in a psychiatric hospital. As far as could be ascertained, they had negative family and personal histories for atopic illnesses. All had been intracutaneously tested with a series of representative food and inhalant atopens and those exhibiting positive reactions were eliminated. Subjects with sunburned, rough, or otherwise abnormal skins were also excluded.

2. *Sera*.—The sera employed for passive cutaneous sensitization were obtained from clinically sensitive atopic patients with positive cutaneous reactions. In the preparation of these sera every effort was made to avoid hemolysis since, in our experience, the use of hemolyzed serum for sensitization perceptibly impaired the reactivity of the passively sensitized sites for several weeks.

Blood obtained by venipuncture with a dry sterile syringe was allowed to cool at room temperature for about one hour. The coagulum was separated from the wall of the test tube with a sterile applicator and the tube placed in the refrigerator. On the following day, the serum was removed from the clot, centrifuged, Seitz-filtered, and then stored without preservative in the refrigerator.

3. *Passive Cutaneous Sensitization*.—For the passive local sensitization of each site on the recipient, 0.05 ml. of serum was injected intracutaneously. Not more than 6 sites were sensitized at one time on any recipient, 4 being the number prepared in most instances.

4. *Testing Techniques*.—An interval of from three to seven days was allowed to elapse before the sensitized sites were tested. In only a few instances did the objectives of the experiment require a shortening of this interval. The passively sensitized sites were tested by the intracutaneous injection of approximately 0.01 ml. of an excitant.

In these passive transfer experiments, the techniques developed for comparative testing in the direct method* were employed. The following considerations were considered pertinent and important: (a) extreme care was exercised in the performance of the skin tests with respect to depth of the test injection and with respect to the amount of test material introduced; (b) comparative tests were placed at least 6 cm. apart; (c) wheals which were to be compared ranged from one-plus to two-plus in size, since it was found that the irregular outlines of larger wheals made comparisons difficult; (d) reactions were graded by palpation as well as by inspection; (e) a sufficient number of comparative tests were made in each experiment to permit reliable conclusions to be drawn.

5. *Test Solutions*.—Extracts of atopens were prepared with buffered saline by the method of Coca and Milford¹⁰ and their total nitrogen contents were determined by the Kjeldahl method. Dilutions of the concentrated extracts were prepared in buffered saline. Nonspecific wheals at passively sensitized sites were produced with solutions of histamine diphosphate and codeine sulfate, diluted in physiologic saline on the basis of salt content.

6. *Size of Reactions*.—To study the effects of a reaction upon a passively sensitized site, it was found necessary to make the initial or primary test wheal large enough to exercise a pronounced effect upon the site. For this reason, primary reactions usually ranged from three-plus to four-plus in size. For the retests, however, wheals between one-plus and two-plus in size were preferred for the reason previously noted.

In order to insure the production of reaginic wheals of the proper size, it was necessary to run a series of preliminary titrations with each serum and atopen. Passively sensitized sites were prepared on several subjects with the serum which had been selected for the experiment. These sites were then tested with varying dilutions of the related allergenic extract so that the dilution producing wheals of a size most suitable to the needs of the experiment might be determined. Similarly, in experiments involving nonspecific wheals, it was necessary to perform preliminary titrations on each subject with varying dilutions of histamine or codeine.

7. *The Reading and Recording of Reactions*.—We were handicapped by the lack of a standard system for the reading and recording of skin reactions resulting from the tests. We were well aware that a system, based solely on dimensional measurements, failed to take into full consideration the amount of edema present in a wheal, a factor which could be estimated only by palpation of the reaction.⁹ Neither a suitable scale for the measurement of this factor nor a means for recording it existed.

Since the whealing component of the cutaneous reaction was considered much more significant than the erythematous response, the two factors, although read simultaneously, were recorded separately. Because the dimensional measurements of a wheal represented the component which could be most accurately estimated, it was used as the basis of a system for grading wheal reactions which is presented herewith. The results recorded in the protocols, however,

*These studies were described in a subsequent publication.⁹

represent, in many instances, readings after slight adjustments had been made upward or downward to take into account definite differences in edema detected by palpation of the wheals under comparison.

With respect to size of wheals, the following reading scale was employed:

A wheal 8 mm. in diameter was graded one-plus (+).

A wheal 10 mm. in diameter was graded two-plus (++)

A wheal 12 mm. in diameter was graded three-plus (+++).

A wheal 14 mm. in diameter with irregular outlines or with true pseudopods was graded four-plus (++++).

With respect to erythema the following reading scale was adopted:

A one-plus (+) represented 1 cm. of solid erythema surrounded by an areola of punctate erythema.

A two-plus (++) represented 2 cm. of solid erythema surrounded by an areola of punctate erythema.

A three-plus (+++) represented 3 cm. of solid erythema surrounded by an areola of punctate erythema.

A four-plus (++++) represented 4 cm. of solid erythema surrounded by an areola of punctate erythema.

Intermediate degrees of reactivity in both scales were designated as $0 \pm$, $\pm$, $+\pm$, $++\pm$, etc.

The obvious inadequacies of these systems of grading were, to some extent, minimized by the fact that the results in all experiments depended upon the comparison of two or more cutaneous reactions which had been simultaneously produced. The readings could therefore be adjusted to indicate *relative* differences in the wheals under comparison. Such liberties were considered permissible since, in no instance, were readings obtained in different experiments to be compared.

The effect of the deficiencies in this system of grading was further diminished by having all tests performed and reactions read by the same investigator (K. B.).

EXPERIMENT 1

The Effect of Wheal Formation at a Passively Sensitized Site Upon Its Capacity to Respond to Restimulation.—The first experiment was designed to show that wheal formation at a passively sensitized site affects the subsequent responsiveness of that site.

Five nonatopic subjects were sensitized with a serum containing skin-sensitizing antibodies for ragweed pollen and for horse epithelium. On the lateral aspect of one arm of each subject, six sites were prepared in two vertical rows of three, neighboring sites being about 6 cm. apart. After an interval of from three to five days, the 3 sites in one of the vertical rows on each subject were tested with a ragweed pollen extract which produced wheals approximately three-plus in size on all sites.

At intervals of from two to thirty-one days thereafter, a site in the previously tested row and its counterpart in the untested row were injected with an extract of horse epithelium. Only one pair of sites were tested on a single day.

The results of the 13 comparative tests are recorded in Table I. In every instance, the wheal on the previously tested site was smaller than that on the previously untested site. The erythema on the previously tested site was smaller than that on the control (i.e., previously untested) site in 11 instances, or 85 per cent of trials.

TABLE I. THE SITES WERE PASSIVELY SENSITIZED WITH A SERUM CONTAINING REAGINS FOR RAGWEED POLLEN AND HORSE EPITHELIUM. THE INITIAL TESTS WERE MADE WITH RAGWEED EXTRACT* AND THE RETESTS WITH HORSE EPITHELIUM EXTRACT.*

SUBJECT	INTERVAL BETWEEN TESTS IN DAYS	RESPONSE TO HORSE EPITHELIUM				SITE YIELDING LARGER REACTION	
		PREVIOUSLY TESTED SITE R		PREVIOUSLY UNTESTED SITE C			
		WHEAL	ERYTHEMA	WHEAL	ERYTHEMA	WHEAL	ERYTHEMA
C. O.	2	+	0 ±	++ ±	++	C	C
	7	+	0 ±	++	+	C	C
	14	+	0 ±	++	+	C	C
K. O.	4	+ ±	±	+++	±	C	C
	7	+ +	±	+++	+	C	C
	11	+	±	++ ±	+ ±	C	C
B. F.	4	+ ±	±	++	+ ±	C	C
	7	+ ±	+	++	±	C	R
	11	+	0	++	0	C	=
B. W.	5	+	±	++ ±	++	C	C
	16	+ ±	±	++	+	C	C
W. A.	21	+	0 ±	++	+ ±	C	C
	31	+	0	++	±	C	C
						Wheal	Eryth.
Number of comparative tests						13	13
Number of responses larger on control site (C)						13 (100 %)	11 (85 %)
Number of responses larger on retested site (R)						0	1
Number of responses equal on both sites (=)						0	1

*The dilutions used in these and subsequent experiments were determined by preliminary titrations with each serum and atopen as described under "Technical Considerations."

The significance of the finding that the alteration in the response of the passively sensitized site was still demonstrable after a period of thirty-one days will be discussed in subsequent experiments.

This experiment yielded evidence to indicate that an immunologic (reagin-atopen) wheal at a passively sensitized site definitely reduced the subsequent responsiveness of that site to excitation with another atopen. This finding raised the following important questions: (1) Would a passively sensitized site which had been the seat of an immunologic (reaginic) wheal also show subsequent diminished responsiveness to a nonspecific urticariogenic excitant, such as histamine or codeine, which produce wheals by a direct effect upon the capillaries without the mediation of an immunologic mechanism? (2) Would a primary histaminic wheal at a passively sensitized site also affect the subsequent responsiveness of that site to an atopen or to histamine?

The experiments which follow were designed to provide the answers to these basic questions.

EXPERIMENT 2-A

The Comparative Effects of Reaginic and Histaminic Wheals at Passively Sensitized Sites Upon Their Subsequent Responsiveness to Atopens.—In this

experiment, each of 7 subjects was passively sensitized at 3 different sites (T, H, and C) with a serum containing skin-sensitizing antibodies for timothy and ragweed pollens. Seven days later, site T on each subject was tested with timothy pollen extract in a dilution which preliminary studies had indicated would produce a four-plus (++++) reaction. At the same time, site H was tested with a previously determined dilution of histamine which produced a wheal comparable in size to the specific wheal at site T. Site C was left untested as a control. At intervals varying from seven to twenty-one days thereafter, sites T, H, and C were tested with ragweed pollen extract. The results obtained in this experiment are listed in Table II.

In 7 comparative tests, the wheal obtained with the ragweed test at site T, the seat of a previous reaginic wheal, was smaller in every instance than that elicited with a similar test at site C, the previously untested control site. Similarly, the wheal obtained with a ragweed test at site H, the site of a previous histaminic wheal, was smaller than that at the control site in 5 out of 7 trials, or 71 per cent of the cases. The ragweed wheal at site T was smaller than that obtained at site H in 71 per cent of cases. The erythemas associated with these reactions generally conformed in extent to the whealing reactions which they accompanied.

This experiment was repeated using codeine instead of histamine as the nonspecific excitant. Similar results were obtained but the impairment in whealing capacity of a sensitized site following the use of codeine was not quite so great as that produced by a histaminic wheal of comparable size. The protocols of the codeine experiments will not be presented here, since the results did not materially vary from those obtained with histamine.

It was demonstrated in these experiments that reaginic wheal formation at a passively sensitized site reduced its subsequent responsiveness to immunologic stimulation with another atopen. Similarly, hyporesponsiveness* at a passively sensitized site followed wheals induced by nonimmunologic (histamine or codeine) excitants. However, a reaginic wheal seemed to produce a more deleterious effect upon the subsequent whealing capacity of the site than did a histaminic wheal of the same size.

The fact that timothy and ragweed pollens, which are antigens similar in nature, were used for the testing and retesting of some of the passively sensitized sites had no bearing on the results obtained. It will be demonstrated in a sub-

*The diminished responsiveness which followed wheal formation at a passively sensitized site was designated in our preliminary report as a type of "refractoriness." This term had been borrowed from Lewis¹ who had described a "partial refractoriness to whealing" which he detected at the site of an experimentally produced histamine wheal. The phenomenon was attributed to a transient diminution in capillary permeability which disappeared in a matter of minutes or hours. The inadequacy of the term for the changes noted in our experiments became apparent at an early date when it was recognized that immunologic as well as tissue factors might be involved and that these changes might persist for days or weeks, or might even be permanent. The term "refractoriness," nevertheless, was temporarily retained and the choice of a more adequate term postponed until the nature of the changes involved could be more carefully analyzed.

At present, we prefer, and employ in this communication, the term "hyporesponsiveness" to designate the diminished whealing capacity found at a cutaneous site which has been altered in some manner. The term is intended to be all-inclusive and to indicate the presence of a diminished responsiveness at a skin site without regard to the mechanisms which produced it. It covers diminutions in response, either transient or permanent, which are the result of either immunologic or nonimmunologic mechanisms and which occur at unsensitized as well as at passively sensitized cutaneous sites. In the studies which follow, the term "hyporesponsiveness" will be broken down into the various factors which seem to participate in its production.

sequent experiment (4-B) that the primary factors under consideration in these experiments are independent of any antigenic relationships which may exist between the antigens employed.

In this experiment, some of the wheals produced on retesting the passively sensitized sites were larger than had been considered suitable for purposes of comparison. However, the results obtained were repeatedly confirmed in subsequent studies in which this technical error was avoided, thus rendering a repetition of this experiment unnecessary.

EXPERIMENT 2-B

The Comparative Effects of Reaginic and Histaminic Wheals at Passively Sensitized Sites Upon Their Subsequent Responsiveness to Histamine.—The previous experiment, with slight modifications, was repeated to determine whether the same results would be obtained when a nonspecific excitant (histamine) instead of a specific excitant was used for retesting the sites.

Accordingly, 7 subjects were each sensitized at 3 sites (R, H, and C) with a serum containing skin-sensitizing antibodies for ragweed. Seven days later, site R was tested with a ragweed pollen extract, and site H with histamine. Dilutions of both excitants were chosen which preliminary titrations had shown would produce marked (++++) wheals of equal size. The control site was left untested. After another seven-day interval, sites R, H, and C were tested with histamine in a dilution of 1:5000, which, it was known, would produce wheals of two-plus degree. The results of this experiment are recorded in Table III.

In all 7 comparisons, the histaminic wheal at previously tested site R was smaller than that at control site C. Likewise, in every retest, the histaminic wheal at site R was smaller than that at site H. The wheal at site H was smaller than that at its control site C in 4 (57 per cent) instances, equal to it in 2 (28 per cent), and larger in only 1 (14 per cent). The erythema responses generally conformed to those of the wheals.

Thus, it was found that *after an interval of seven days*, a passively sensitized site, which had been the seat of either a reaginic or histaminic wheal, tended to show a hyporesponsiveness on restimulation with histamine. The results in this experiment closely corresponded to those which had been obtained in the previous experiment when the sites had been restimulated with a specific excitant. In this, as in the previous study, the primary reaginic wheal produced a more deleterious effect, as expressed in subsequent hyporesponsiveness, on the passively sensitized site than did a histaminic wheal of comparable size.

EXPERIMENT 3-A

The Duration of Hyporesponsiveness to Histamine Produced by a Histaminic Wheal at a Passively Sensitized Site.—It seemed important at this point to determine the duration of the hyporesponsiveness which followed a reaction at a passively sensitized site. Since reaginic and histaminic wheals seemed to affect their sites unequally, they were studied separately. The following experiment was designed to determine the duration of the hyporesponsiveness produced at a passively sensitized site when histamine was used both for the stimulation and restimulation of the site.

On each of 35 subjects, 4 sites were sensitized with either of two reagin-bearing sera. Although reagins played no active role in this experiment, it was necessary to use such sera since this study was concerned with the responsiveness of passively sensitized sites.

Four days after sensitization, wheals of three- or four-plus magnitude were produced at these sites with histamine in a dilution of 1:1,000. At intervals varying from ten to twenty-eight days thereafter, one of these sites and a neighboring normal nonsensitized cutaneous area were tested with histamine in a 1:2,500 or 1:5,000 dilution and the resulting reactions compared. The results of 40 such comparative tests are summarized in Table IV.

TABLE IV. ALL TESTS AND RETESTS AT PASSIVELY SENSITIZED AND NORMAL CUTANEOUS SITES WERE MADE WITH HISTAMINE.

INTERVAL IN DAYS BETWEEN TEST AND RETEST WITH HISTAMINE	NUMBER OF COMPARATIVE TESTS	RESPONSE TO HISTAMINE AT PREVIOUSLY TESTED SITE					
		SMALLER THAN NORMAL		EQUAL TO NORMAL		LARGER THAN NORMAL	
		WHEAL	ERYTH.	WHEAL	ERYTH.	WHEAL	ERYTH.
10	6	5 (83%)	5 (83%)	1 (17%)	1 (17%)	0	0
19 to 21	9	4 (44%)	4 (44%)	5 (56%)	3 (33%)	0	2 (22%)
25 to 28	25	4 (16%)	6 (24%)	16 (64%)	16 (64%)	5 (20%)	3 (12%)

Comparative tests with histamine performed ten days after the original tests produced smaller wheals on the retested sites in 5 (83 per cent) of 6 trials. At from nineteen to twenty-one days, smaller wheals were obtained on the retested sites in 4 (44 per cent) of 9 comparisons, the remaining 5 (56 per cent) being equal in size to the controls. (At this time interval, 4 additional unlisted comparisons were made, using codeine in a dilution of 1:120 as the nonspecific excitant for restimulation of the sites. Hyporesponsiveness was found at the retested sites in every instance.) In 25 comparative tests performed at from twenty-five to twenty-eight days after the initial tests, smaller reactions were obtained on the retest site in only 4 (16 per cent) instances. Sixteen (64 per cent) reactions were equal on retest and control sites at this time and, for the first time in this experiment, some of the wheals on the retested sites were larger than those on the control sites. This occurred in 5 instances, or 20 per cent of the comparative tests. The erythema responses in this experiment tended to run parallel to the whealing reactions.

From this experiment, it was ascertained that a passively sensitized site which had been the seat of a histaminic wheal usually regained its normal responsiveness to histamine (or codeine) within a period of four weeks.

EXPERIMENT 3-B

The Duration of Hyporesponsiveness to Atopen Produced by a Histaminic Wheal at a Passively Sensitized Site.—The preceding experiment was then repeated to ascertain whether normal responsiveness to histamine would return to a passively sensitized site within four weeks after a reaginic wheal, as it usually did following a histaminic wheal.

Eleven subjects were passively sensitized at cutaneous sites with the sera used in the previous experiment (3-A). These contained skin-sensitizing antibodies for horse and rabbit danders and for ragweed pollen. After four days, these sites were tested with horse or rabbit epithelium or with ragweed pollen extract. Three- or four-plus reactions were obtained at all sites.

After an interval of from twenty-one to twenty-eight days, a site (A) previously tested with atopen and a normal (unsensitized) cutaneous area (N) on each subject were tested with histamine (1:2,500). In 3 instances, a solution of codeine sulfate (1:120) was substituted for the histamine. The results of all comparative tests are shown in Table V.

TABLE V. THE INITIAL REACTION AT THE PASSIVELY SENSITIZED SITE A WAS INDUCED WITH AN ATOPEN (HORSE EPITHELIUM, RABBIT EPITHELIUM, OR RAGWEED). THE RETEST WAS MADE WITH HISTAMINE OR CODEINE. A PREVIOUSLY UNTREATED CUTANEOUS AREA (N) WAS USED FOR THE CONTROL TEST.

SUBJECT	INTERVAL IN DAYS BE- TWEEN TEST AND RETEST	NONSPECIFIC EXCITANT USED FOR RETEST	RESPONSE TO HISTAMINE (OR CODEINE) ON					
			SITE A PREVIOUSLY TESTED WITH ATOPEN		NORMAL SITE N		SITE YIELDING LARGER RESPONSE	
			WHEAL	ERYTH.	WHEAL	ERYTH.	WHEAL	ERYTH.
1	21	Histamine	+++	+±	+++	+±	=	=
2	21	Histamine	++	+	+++	++	N	N
3	21	Histamine	+++	+++	+++±	+±	A	A
4	25	Histamine	++	++	++	++	=	=
5	25	Histamine	+++	++	++	+±	A	A
6	25	Histamine	++	+	++	+	=	=
7	28	Histamine	+±	+	+±	+	=	=
8	28	Histamine	+±	+±	+±	+	=	A
9	28	Codeine	+++±	++	+++±	+++	=	N
10	28	Codeine	++	+±	++	+	=	A
11	28	Codeine	+++	+++±	+++	+++	=	N

Of the 3 cases retested on the twenty-first day, 1 showed a larger wheal at the normal site (N), 1 at the retested site (A), while the third case showed equal reactions at the two sites. At twenty-five days, 2 of 3 retested sites (A) responded with wheals equal in size to those obtained at their control sites (N). On the twenty-eighth day, 5 cases were retested, 2 with histamine and 3 with codeine. Equal responses were obtained at the retested and normal sites in all 5 comparative tests. In the majority of instances, the erythema and wheal responses corresponded.

This experiment demonstrated that a passively sensitized site, which had been the seat of a reaginic wheal, recovered its normal whealing responsiveness to nonspecific excitants, such as histamine or codeine, within four weeks. A recovery period of similar duration had been found in the preceding experiment when the primary wheal had been produced with histamine.

EXPERIMENT 4-A

The Duration of Hyporesponsiveness to Atopen Produced by a Histaminic Wheal at a Passively Sensitized Site.—It had been demonstrated in Experiments 2-A and 2-B that both reaginic and histaminic wheals impaired the capacity of passively sensitized sites to form reaginic and histaminic wheals on restimulation.

This alteration in the responsiveness of the previously tested sites might be accounted for in several ways: (a) It might represent an alteration in the tissue cell, nonimmunologic in nature, produced by the primary wheal. The results of Experiments 3-A and 3-B demonstrated the existence of such a tissue factor. (b) It might be the expression of an immunologic change, namely, an alteration in the state or quantity of the skin-sensitizing antibodies at the passively sensitized site produced by the primary wheal. This possibility was suggested by the results of Experiments 1 and 2-A, but its actuality had not been established. (c) It might be an expression of a combination of these tissue and immunologic factors.

In Experiments 1, 2-A, and 2-B, all, except two of the retests, were made at periods varying from a few days to three weeks after the primary wheal. During this period, both the tissue and immunologic factors under consideration might have contributed to the hyporesponsiveness of the passively sensitized site.

The fact that the nonspecific tissue factor was of limited duration afforded the means of ascertaining whether an immunologic component was also involved in the hyporesponsiveness which followed wheal formation at a passively sensitized site. A loss in reactivity, persisting beyond four weeks and exceeding the natural decrease in response caused by aging of the site, could only be attributed to an immunologic change produced by the initial wheal at the passively sensitized site.

The two experiments which follow were designed to ascertain whether the hyporesponsiveness in specific response produced by a wheal at a passively sensi-

TABLE VI. CUTANEOUS SITES WERE PASSIVELY SENSITIZED TO COTTONSEED, FLAXSEED, HORSE EPITHELIUM, MILK, RABBIT EPITHELIUM, AND RAGWEED. INITIAL WHEELS WERE PRODUCED WITH HISTAMINE. RETESTS WERE MADE WITH AN ATOPEN.

SUBJECT	INTERVAL IN DAYS BE- TWEEN TESTS	ATOPEN USED FOR RETEST	RESPONSE TO TEST WITH ATOPEN				SITE OF LARGER RESPONSE	
			ON SENSITIZED SITE H 2 PRE- VIOUSLY TESTED WITH HISTAMINE		ON SENSITIZED SITE C PREVIOUSLY UNTESTED			
			WHEAL	ERYTH.	WHEAL	ERYTH.	WHEAL	ERYTH.
1	28	Milk	++	+±	++±	++	C	C
2	28	Milk	++	++	+±	+±	H 2	H 2
3	28	Milk	+±	+±	++	++	C	C
4	28	Cottonseed	++	+	+++	++	C	C
5	35	Cottonseed	+	±	+±	++	C	C
6	28	Cottonseed	++	±	+++	++	C	C
7	35	Cottonseed	++	+	++±	+	C	=
8	35	Cottonseed	±	0	+±	0±	C	C
9	35	Cottonseed	+±	0±	++	±	C	C
10	35	Cottonseed	+	0±	+±	+	C	C
11	35	Cottonseed	+	0	+±	0±	C	C
12	35	Cottonseed	+±	±	++	+	C	C
13	35	Ragweed	+	±	+±	+	C	C
14	35	Ragweed	+	+	++	+++	C	C
15	35	Ragweed	+±	±	++	+	C	C
						Wheal	Eryth.	
Number of comparative tests						15	15	
Number of responses larger on previously tested site (H 2)						1	1	
Number of responses larger on untested site (C)						14 (93%)	13 (87%)	
Number of responses equal on both sites (=)						0	1	

tized site outlasted the four-week period needed for the re-establishment of normal tissue responsiveness at that site. In the first experiment, 4-A, the initial wheal was produced by histamine, in the second, 4-B, by an atopen.

Three cutaneous sites (H 1, H 2, and C) on each of 15 subjects were passively sensitized with reagin-bearing sera. For sensitization of 6 of these subjects, a serum containing reagins for rabbit epithelium, horse epithelium, flaxseed, and milk was employed. For the other 9 subjects, a serum with reagins for ragweed pollen and cottonseed was used.

Four days following passive cutaneous sensitization, sites H 1 and H 2 were tested with histamine in a dilution of 1:1,000. Three-plus reactions resulted at all sites. Site C was not tested. On the twenty-seventh day thereafter, site H 1 and a neighboring normal unsensitized skin area were tested with histamine in a dilution of 1:5,000. In all instances, H 1 responded as well to histamine as did the normal skin.

From one to eight days later, sites H 2 and C were tested with one of the atopens related to the reagins in the sensitizing serum. The results of these tests are shown in Table VI.

In 14 (93 per cent) of the 15 comparative tests, the wheal on the previously tested site H 2 was smaller than that on the passively sensitized but previously untested control site C. In 13 (87 per cent) of these tests, the erythema on the retested site H 2 was also smaller than that on the control site C.

It was, therefore, conclusively demonstrated that a sensitized site previously tested with a nonspecific excitant, histamine, showed a persistent loss in its subsequent capacity to produce reaginic wheals. That this permanent loss in sensitiveness exceeded that due to aging of the site was shown by the fact that site H 2, which had been previously tested, responded with a smaller reaction than control site C, which had been sensitized at the same time but was tested for the first time from thirty-two to forty days after sensitization.

EXPERIMENT 4-B

The Duration of Hyporesponsiveness to Atopen Produced by a Reaginic Wheal at a Passively Sensitized Site.—The previous experiment was repeated except that an atopen rather than histamine was used to induce the initial wheal at the passively sensitized site. Since this atopen would tend to neutralize all reagins antigenically related to it, an immunologically unrelated atopen was chosen for restimulation of the site.

Three cutaneous sites (H, A, and C) were passively sensitized on each of 14 nonatopic subjects with one of the two sera employed in Experiment 4-A. Four days later, site H was tested with histamine in a dilution of 1:1,000 and site A was tested with an atopen. In all subjects, reactions (+++) of approximately equal size were obtained at sites H and A. Site C, the control, was not tested at this time.

As in the preceding experiment, site H and a neighboring normal (unsensitized) cutaneous area were tested, after an interval of approximately four weeks, with histamine in a dilution of 1:5,000, in order to verify the return of normal tissue responsiveness at the previously tested site. Several days there-

after, site A and site C were tested with a second atopen antigenically unrelated to that previously employed at site A. The results of these comparative tests are shown in Table VII.

TABLE VII. CUTANEOUS SITES WERE PASSIVELY SENSITIZED TO COTTONSEED, FLAXSEED, HORSE EPITHELIUM, MILK, RABBIT EPITHELIUM, AND RAGWEED. INITIAL REACTIONS WERE INDUCED WITH AN ATOPEN. RETESTS WERE MADE WITH A SECOND ATOPEN, ANTIGENICALLY UNRELATED TO THE FIRST.

SUB- JECT	INTERVAL IN DAYS BETWEEN TESTS	ATOPEN USED FOR		RESPONSE TO ATOPEN ON				SITE OF LARGER RESPONSE	
				PREVIOUSLY TESTED SITE A		PREVIOUSLY UNTESTED SITE C			
		INITIAL TEST	RETEST	WHEAL	ERYTH.	WHEAL	ERYTH.		
1	25	Rabbit	Flaxseed	++	++	++±	+++	C	C
2	28	Rabbit	Milk	+±	+	++±	++	C	C
3	25	Rabbit	Flaxseed	+++	++	++	++	C	=
4	28	Rabbit	Milk	+	+	+±	+±	C	C
5	28	Rabbit	Milk	+	+	+±	++	C	C
6	28	Ragweed	Cottonseed	+	0±	+++	++	C	C
7	35	Ragweed	Cottonseed	±	0±	+±	++	C	C
8	28	Ragweed	Cottonseed	±	0±	+++	++	C	C
9	35	Ragweed	Cottonseed	+±	±	++±	+	C	C
10	35	Ragweed	Cottonseed	±	0	+±	0±	C	C
11	35	Ragweed	Cottonseed	+	0	++	±	C	C
12	35	Ragweed	Cottonseed	+	±	+±	+	C	C
13	35	Ragweed	Cottonseed	+	0	+±	0±	C	C
14	35	Ragweed	Cottonseed	+	0	++	+	C	C
						Wheal		Eryth.	
Total number of comparative tests						14		14	
Number of responses larger on previously tested site (A)						0		0	
Number of responses larger on previously untested site (C)						14 (100%)		13 (93%)	
Number of responses equal on both sites (=)						0		1	

In all of the 14 comparative tests, the wheal on previously tested site A was smaller than that at control site C, the previously untested sensitized site. The erythema at site A was also diminished in all except one instance.

These results demonstrated that the original reaginic reaction at the passively sensitized site had *permanently* affected the immunologic responsiveness of that site. It was to be expected that the atopen-reagin reaction would completely or partially neutralize those reagins which were related to the atopen initially employed. However, the fact that, in this experiment, the loss of sensitivity at the previously tested site was a general one, extending beyond the boundaries of antigenic relationship, indicated that the change was a non-specific effect of the initial immunologic wheal and not the result of a specific neutralization of antibodies. This conclusion found support in the results of the previous experiments 2-A and 4-A in which it was demonstrated that histaminic wheals produced similar, though less pronounced, deleterious effects upon the subsequent immunologic responsiveness of a passively sensitized site.

EXPERIMENT 5

The Comparative Effects of Reaginic and Histaminic Wheels Upon the Sensitivity of Passively Sensitized Sites.—It had been noted in Experiments 2-A and 2-B that the reaginic wheal seemed to produce a more pronounced impairment of the responsiveness of a passively sensitized site than did a

histaminic wheal. In that study, however, sites had been retested at intervals of from seven to twenty-one days, when diminished tissue responsiveness was still present at some of the sites. In the light of subsequent findings, it seemed advisable to re-examine this question, in order to rule out the tissue factor as the one primarily responsible for the results obtained. To accomplish this, the following experiment was performed.

Four sites were sensitized on each of a series of subjects with the same sera employed in Experiments 4-A and 4-B.

On 2 sensitized sites, H 1 and H 2, on each subject, four-plus wheals were induced by tests with histamine in a dilution of 1:1,000. On the other two sites, A 1 and A 2, reaginic wheals of similar size were induced by tests with an extract of rabbit epithelium or of ragweed pollen.

After an interval of from twenty-five to thirty-five days, site H 1 and site A 1, as well as a normal unsensitized area on each subject, were tested with histamine in a dilution of 1:5,000. With few exceptions, the reactions obtained on the previously tested sites were equal to those on the normal skin.

On 16 of the subjects in whom normal responsiveness to histamine had returned at the previously tested sites, tests with atopens were performed on the following day. Sites H 2 and A 2 on each subject were tested with an atopen which was antigenically unrelated to that previously employed at A 1 and A 2. The results obtained are listed in Table VIII.

TABLE VIII. CUTANEOUS SITES WERE PASSIVELY SENSITIZED TO COTTONSEED, FLAXSEED, HORSE EPITHELIUM, MILK, RABBIT EPITHELIUM, AND RAGWEED. INITIAL REACTIONS WERE INDUCED WITH HISTAMINE OR WITH AN ATOPEN. RETESTS WERE MADE WITH A SECOND ATOPEN, ANTIGENICALLY UNRELATED TO THE FIRST.

SUB- JECT	INTERVAL IN DAYS BETWEEN TESTS	ATOPEN USED ON SITE A 2 FOR		RESPONSE TO RETEST WITH ATOPEN ON				SITE OF LARGER RESPONSE
				SITE H 2 PREVI- OUSLY TESTED WITH HISTAMINE		SITE A 2 PREVI- OUSLY TESTED WITH ATOPEN		
		INITIAL TEST	RETEST	WHEAL	ERYTH.	WHEAL	ERYTH.	
1	28	Rabbit ep.	Milk	++	±	±	+	H 2
2	25	Rabbit ep.	Flaxseed	+++	++	++	++	H 2
3	28	Rabbit ep.	Milk	++	++	+	+	H 2
4	28	Rabbit ep.	Milk	±	±	+	+	H 2
5	28	Ragweed	Cottonseed	++	+	+	0 ±	H 2
6	35	Ragweed	Cottonseed	+	±	±	0 ±	H 2
7	28	Ragweed	Cottonseed	++	±	±	0 ±	H 2
8	35	Ragweed	Cottonseed	++	±	±	±	H 2
9	35	Ragweed	Cottonseed	±	0 ±	+	0	H 2
10	35	Ragweed	Cottonseed	+	0	+	0	=
11	35	Ragweed	Cottonseed	±	±	+	0	H 2
12	28	Rabbit ep.	Milk	++	±	+	+	H 2
13	28	Rabbit ep.	Milk	++	±	+	+	H 2
14	28	Rabbit ep.	Milk	+	+	++	±	A 2
15	25	Rabbit ep.	Milk	++	±	+	±	H 2
16	25	Rabbit ep.	Milk	++ ±	+	+	±	H 2
Number of comparative tests						Wheal	Eryth.	
Number of responses larger on site previously tested with histamine (H 2)						16	16	
Number of responses larger on site previously tested with atopen (A 2)						14 (88%)	14 (88%)	
Number of responses equal on both sites (=)						1	1	

In 14 (88 per cent) of the 16 comparative tests, the wheals and erythemas on the sites previously tested with atopens were smaller than those on sites previously tested with histamine. Thus, it was conclusively demonstrated that reaginic wheals were more deleterious than histaminic wheals in their effects upon the atopic reagents at a passively sensitized site.

PRACTICAL APPLICATIONS

It has been clearly demonstrated that a strong positive reaction on a passively sensitized site limits the reliability of subsequent tests on that site. Immediately following the initial test, there is an impairment in tissue responsiveness which gradually diminishes until it is completely gone. Rarely does this hyporesponsiveness last more than four weeks. There is, in addition to the expected specific neutralization of reagents by the atopen producing a positive reaction, an injury to all other skin-sensitizing antibodies at the passively sensitized site. This effect is independent of antigenic relationships and natural aging of the site and results in a general and permanent diminution of sensitivity of the site.

These tissue and antibody factors must be reckoned with in the interpretation of results obtained with techniques involving more than a single test at a passively sensitized site. They are particularly relevant in the evaluation of results obtained in studies of antigenic relationships through the *in vivo* neutralization of reagents at passively sensitized sites by repeated testing with one or several atopens. There are few exceptions to the general rule that the larger the reaginic wheal obtained at a passively sensitized site, the greater is the impairment in tissue and immunologic responsiveness which may be expected.

The fact that a wheal at a passively sensitized site produces a subsequent hyporesponsiveness of that site raises the important question as to whether the wheal produced at the time of passive local sensitization by the intracutaneous injection of reaginic serum may not also impair the responsiveness of the site to even the initial skin test with an atopen. We have indeed found this to be the case. Such hyporesponsiveness represents a handicap of variable degree which must be reckoned with in the indirect method of testing for atopic hypersensitiveness and in passive transfer experiments. Following skin sensitization, there usually develops a period of hyporesponsiveness at the passively sensitized site which may be quite pronounced for several days but undoubtedly persists in mild form for a longer period.⁵ When the immediate reaction produced by the serum injection at the time of sensitization is strong, as frequently occurs with sera obtained from cases with atopic dermatitis, hyporesponsiveness may be a troublesome factor. It has also been found particularly significant in connection with the wheals which result from the intracutaneous injection of atopen-reagent mixtures in experiments involving the *in vitro* neutralization of reagents.

All of these matters will be the subject of more detailed discussions in later papers in this series.

CONCLUSIONS

1. Wheals at passively sensitized sites produce an impairment in the subsequent responsiveness of such sites to restimulation. The term "hyporesponsiveness" has been selected to describe this diminished whealing power at a previously tested site.

2. Both reaginic (immunologic) and histaminic (nonimmunologic) wheals at passively sensitized sites reduce their responsiveness to restimulation either with histamine or with a specific excitant (atopen).

3. Such hyporesponsiveness of a passively sensitized site usually involves a tissue factor and an antibody factor in variable degree.

4. The tissue factor represents a temporary, completely reversible change which diminishes rapidly and rarely lasts beyond the fourth week.

5. The immunologic change represents injury by the wheal to all the reagins at a passively sensitized site and is not reversible.

6. Reaginic wheals produce greater reaginic injury at passively sensitized sites than do histaminic wheals of comparable size.

7. These tissue and antibody factors are of significance and must be reckoned with in *all* studies involving passive transfer techniques.

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