

Delayed skin reactions to inhalant antigens

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A prospective study of the incidence and characteristics of spontaneously occurring delayed skin test reactions has been carried out. A total of 292 subjects were studied, 200 atopic by history; they received 2,700 intradermal skin tests to 12 common inhalant antigens. Thirteen per cent of the total group of skin tests manifested a positive immediate reaction; 6 per cent of the tests exhibited a delayed reaction with induration of 5 mm. or greater at 48 hours. There was a significantly higher incidence of immediate reactions in the atopic group for the majority of antigens studied ($p < 0.01$ for 7 of 12 antigens). However, the atopic individuals exhibited no greater incidence of delayed reactions than the nonatopic individuals ($p > 0.05$ for 10 of 12 antigens). Skin biopsies performed 48 hours after the application of the skin tests demonstrated four distinct classes of response: (1) Negative immediate-negative delayed responses were characterized by minor perivascular round cell infiltration which was indistinguishable from that seen in control injection sites. (2) Positive immediate-negative delayed sites demonstrated a similarly mild perivascular reaction, but many eosinophils were seen. (3) Negative immediate-positive delayed responses to inhalant antigens were histologically indistinguishable from tuberculin reactions, with dense perivascular and interstitial round cell infiltrations. (4) Positive immediate-positive delayed responses contained a similarly dense perivascular and interstitial infiltrate, but eosinophils were prominent.

Many observers have noted that delayed reactions are sometimes seen at the sites of intradermal skin tests to inhalant antigens. However, little is known about the incidence, characteristics, and significance of these reactions. Passing references are made to delayed reactions in some clinical allergy texts, but their significance has not been obvious.^{1, 2} A report cites the frequency of such reactions in a clinical practice.³ However, there are no reported studies which characterize these reactions or determine the incidence of delayed reactions in a nonatopic population. There is no information concerning the relationship of such delayed reactions to immediate reactions or to the atopic state.

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A prospective study has been carried out to define the immunologic and clinical characteristics of delayed reactions to the inhalant antigens which commonly induce respiratory allergic symptoms. Specifically, such delayed reactions were analyzed from the standpoint of: (a) incidence in atopic and nonatopic populations; (b) the relationship of immediate to delayed reactions; and (c) the gross and histologic characteristics of the reactions.

MATERIALS AND METHODS

The statistical studies included 292 subjects receiving a total of 2,700 skin tests to 12 antigens. The subjects were tested intradermally to a group of 12 common inhalant antigens. Intradermal tests were carried out with: (a) dust extract (Hollister-Stier, 1,000 p.n.u. per cubic centimeter); (b) pollens of mixed trees (oak, sycamore, poplar, and maple), mixed ragweed (short and giant), timothy and plantain extracted and diluted with Cocas Solution[†] to 1,000 p.n.u. per cubic centimeter; and (c) extracts of feathers, animal danders (cat, dog, horse), and molds (*alternaria*, *helminthosporium*, and *hormodendrum*).^{*} Antigen E,† a purified antigenic ragweed fraction, was utilized in selected histologic studies.⁴

Preliminary scratch tests were performed with standard concentrations of extracts from the same sources using the hemolet technique. Individuals with a strongly positive scratch reaction were not tested by the intradermal technique for reasons of safety. For each intradermal test 0.02 c.c. was injected intradermally through a No. 27 gauge needle on the posterior surface of the upper arm.

Intradermal tests were read at 20 minutes and considered definitely positive if the wheal was 3 times or greater the diameter of the wheal at the Cocas solution test site or twice the diameter of the control wheal if pseudopods were present. The test sites were marked and again examined after 48 hours by one or more of the authors. The reaction at 48 hours was considered positive if induration of 5 mm. or greater in horizontal diameter was present.

The population studied included patients being skin tested during the course of allergy evaluation at the Allergy and Immunology Clinic of the Hospital of the University of Pennsylvania and volunteer medical students. Clinical charts and questionnaires were reviewed and the individuals were separated into atopic and nonatopic groups based on the history alone. The definition of the atopic state used included only those individuals with a personal history of allergic rhinitis, asthma, or eczema.

Punch biopsies of representative reactions were obtained with a 3 mm. dermal punch. The surrounding area was initially infiltrated with 1 per cent procaine; care was taken to avoid the test area. The biopsy specimens were fixed immediately in 10 per cent formalin. Paraffin sections were prepared and stained with hemotoxylin and eosin in the Dermatology Pathology Laboratory of the Hospital of the University of Pennsylvania.

^{*}Obtained from the Hollister-Stier Laboratory, Spokane, Wash.

[†]Antigen E was kindly supplied by Phillip Norman, M.D., Johns Hopkins Medical School, Baltimore, Md.

The slides were coded and then interpreted independently in a blind fashion by 3 of the authors (G. G., B. Z., and H. B.). A semiquantitative (1 to 4+) grading system was used with regard to the percentage of eosinophils in the infiltrate, the degree of perivascular and interstitial inflammatory reaction, and the depth of tissue involved.

RESULTS

Incidence of immediate and delayed reactions

Of the 292 subjects, 200 were considered atopic by history. Seventeen per cent of the skin tests performed on atopic subjects manifested an immediate positive reaction (Table I). Positive immediate reactions were seen in only 5 per cent of the nonatopic group. The difference in incidence of immediate reactions between the atopic and nonatopic individuals was highly significant ($p < 0.01$) for 7 and significant ($p < 0.05$) for 1 of the 12 antigens used. Only the animal danders and hormodendrum failed to show a significant difference between the atopic and nonatopic groups with regard to the incidence of positive immediate reactions.

By contrast, there was no significant difference in the incidence of delayed reactions between the atopic and nonatopic groups. Positive delayed reactions were seen in 7 per cent of the tests performed on atopic individuals; 5 per cent of similar tests performed on nonatopic individuals manifested a delayed reaction (Table II). The difference in incidence of delayed reactions between the atopic and nonatopic groups was significant ($p < 0.05$) for only 2 antigens, timothy and helminthosporium; in no case was this difference highly significant.

Table I. *Immediate reactions*

<i>Antigen</i>	<i>Skin tests with positive immediate (20 minute) reactions (per cent)</i>		
	<i>All subjects</i>	<i>Atopic subjects</i>	<i>Nonatopic subjects</i>
Dust	19	27	4*
Feather	13	18	2*
Ragweed	24	33	9*
Plantain	20	24	0*
Tree	14	18	6*
Timothy	18	23	10†
Alternaria	9	13	0*
Helminthosporium	8	12	0*
Hormodendrum	5	6	0
Cat	11	12	9
Dog	7	8	3
Horse	6	7	0
Average	13	17	5

*Highly significant ($p < 0.01$) difference in incidence of positive immediate reactions between atopic and nonatopic groups.

†Significant ($p < 0.05$) difference in incidence of positive immediate reactions between atopic and nonatopic groups.

All others are not significant ($p > 0.05$).

The relationship of delayed reactions to immediate reactions is presented in Table III. In order to determine this relationship, the skin test results in atopic and nonatopic subjects are not separated. Hence the relationship between immediate and delayed reactions occurring at individual test sites was studied independently of the clinical status of the subject. A positive delayed reaction tended to occur more commonly at a test site which had initially manifested an

Table II. *Delayed reactions*

<i>Antigen</i>	<i>Skin tests with positive delayed (48 hour) reactions (per cent)</i>		
	<i>All subjects</i>	<i>Atopic subjects</i>	<i>Nonatopic subjects</i>
Dust	5	6	3
Feather	11	12	7
Ragweed	9	8	12
Plantain	4	4	0
Tree	3	3	2
Timothy	5	7	1*
Alternaria	8	8	8
Helminthosporium	13	16	6*
Hormodendrum	3	3	0
Cat	6	4	8
Dog	2	3	0
Horse	2	3	0
Average	6	7	5

*Significant ($p < 0.05$) difference in incidence of positive delayed reactions between atopic and nonatopic groups.

All others are not significant ($p > 0.05$).

Table III. *Relationship of delayed to immediate reactions*

<i>Antigen</i>	<i>Skin tests with positive delayed (48 hour) reactions (per cent)</i>	
	<i>Positive immediate reaction</i>	<i>Negative immediate reaction</i>
Dust	8	5
Feather	18	10
Ragweed	12	9
Plantain	7	3
Tree	11	1*
Timothy	10	4
Alternaria	22	7†
Helminthosporium	32	11†
Hormodendrum	22	2*
Cat	15	4†
Dog	8	2
Horse	0	3
Average	13	5

*Highly significant ($p < 0.01$) difference in incidence of positive delayed reactions between tests which were positive or negative on immediate reading.

†Significant ($p < 0.05$) difference in incidence of positive delayed reactions between tests which were positive or negative on immediate reading.

All others are not significant ($p > 0.05$).

immediate reaction as opposed to a test site which had been negative on immediate reading. However, the increased incidence of delayed reactions in test sites that had shown an immediate positive reaction was statistically significant ($p < 0.05$) for only 5 of the 12 antigens studied.

Histologic studies

Skin test reactions were classified into four categories based on the gross appearance of the reactions when examined at both 20 minutes (immediate reading) and 48 hours (delayed reading):

1. Negative immediate-negative delayed (neg.-neg.): test sites manifesting no significant wheal and erythema reaction at the immediate reading or significant induration at the delayed reading.

2. Negative immediate-positive delayed (neg.-pos.): test sites manifesting no reaction at immediate reading but exhibiting 5 mm. or greater of induration at delayed reading.

3. Positive immediate-negative delayed (pos.-neg.): test sites manifesting a positive wheal and erythema response at the immediate reading but manifesting no significant induration at the delayed reading.

4. Positive immediate-positive delayed (pos.-pos.): test sites manifesting a positive wheal and erythema response at the immediate reading and also manifesting induration of 5 mm. or greater at the delayed reading.

Biopsies of skin test sites in each category were carried out 48 hours after the intradermal injection of antigen (Table IV). Each category of reaction exhibited characteristic histologic findings as noted below. The semiquantitative results of the interpretation of the biopsies will be presented with particular reference to: (1) the type of infiltrating cell, especially the degree of eosinophilic infiltrate; (2) the degree of perivascular infiltration; (3) the degree of interstitial inflammatory reaction; and (4) the depth of tissue involved.

Negative immediate and negative delayed (Fig. 1). Fifteen biopsies were performed 48 hours after an injection of an inhalant antigen which manifested neither an immediate nor a delayed reaction:

1. The cellular infiltrate in the neg.-neg. reactions was composed of mononuclear cells, mainly lymphocytes with some histiocytes. One of the 15 neg.-neg. sites contained a few scattered eosinophils which were graded as 1+. No eosinophils were noted in the other 14 neg.-neg. biopsies. Only a rare polymorphonuclear leukocyte was seen in any of these 15 biopsies.

2. The degree of perivascular cellular infiltration was very slight (0 to 1+) in

Table IV. *Histologic studies*

<i>Categories</i>	<i>Number of biopsies</i>	<i>Antigens used</i>
Neg.-neg.	15	Timothy, tree, ragweed, feather, alternaria, Antigen E
Neg.-pos.	7	Ragweed, tree, aspergillus
Pos.-neg.	13	Timothy, ragweed, Antigen E
Pos.-pos.	12	Timothy, ragweed, tree, plantain

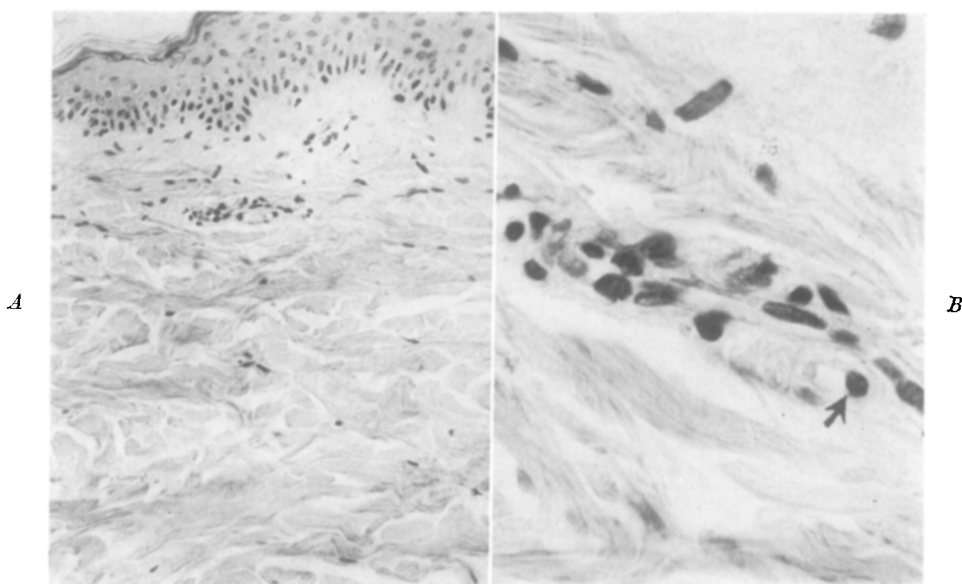


Fig. 1

Biopsy of a neg.-neg. test site 48 hours after injection of 20 p.n.u. timothy antigen which was negative on immediate and delayed reading. *A*, Low power ($\times 230$) shows only scattered (0+) perivascular and interstitial infiltrate in the superficial part of the dermis. *B*, High power ($\times 1100$) shows that the scattered infiltrating cells are mononuclear in type (see arrow).

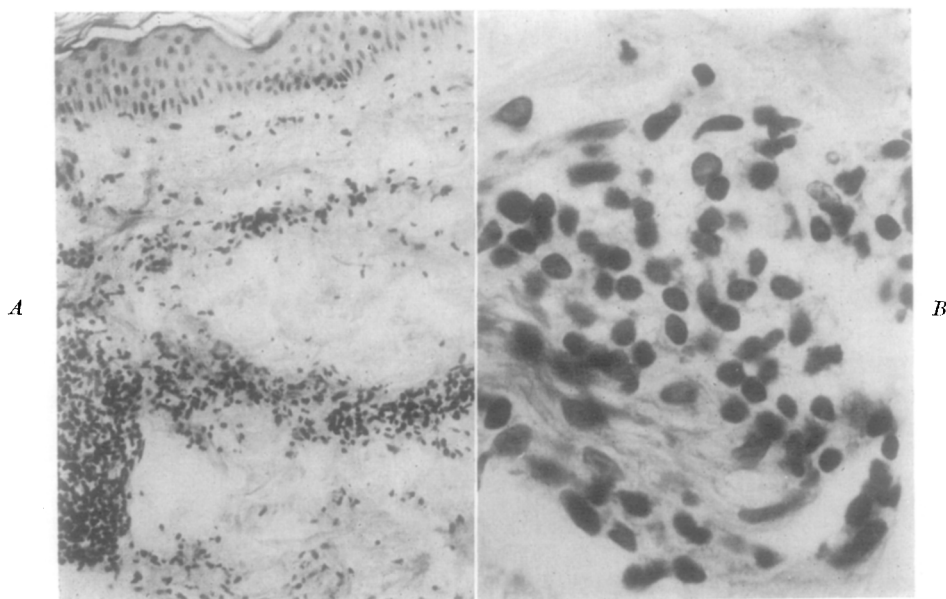
13 of the 15 neg.-neg. sites. The remaining 2 biopsies showed somewhat more marked perivascular mononuclear cell infiltrates which were graded as 2 and 3+, respectively.

3. The intensity of the interstitial cellular infiltration was very slight (0 to 1+) in each case.

4. The location of the perivascular and interstitial infiltration was superficial and limited to the upper one third of the corium in 11 of the neg.-neg. sites. In 2 cases, the infiltrating cells reached the middermis, but the fat layer of the dermis was not involved in any of the 13 biopsies which were of sufficient depth to make this determination. Two biopsies of neg.-neg. sites did not include the deeper areas of the dermis in the specimen.

Negative immediate and positive delayed (Fig. 2). Seven biopsies were performed 48 hours after an injection of an inhalant antigen; they manifested no reaction when read at 20 minutes but demonstrated an indurated area of 5 mm. or greater at 48 hours.

1. All 7 manifested a marked cellular infiltrate. This infiltrate was composed of lymphocytes, histiocytes, and an occasional neutrophil in 5 of the 7 specimens. Two biopsies of neg.-pos. reactions to alternaria and cat antigens on the same subject contained a moderate number (2 and 3+, respectively) of eosinophils together with mononuclear cells in the cellular infiltrate. No eosinophils were seen in the other 5 biopsies of neg.-pos. reactions.

**Fig. 2**

Biopsy of a neg.-pos. test site 48 hours after the injection of 20 p.n.u. of ragweed antigen which was negative on the immediate reading but which manifested an indurated area of 5 mm. in diameter when biopsied at 48 hours. *A*, Low power ($\times 230$) shows a moderately intense (3+) perivascular and a moderate (2+) interstitial inflammatory response which extended through the full depth of the biopsy specimen. *B*, Under high power ($\times 1100$), the cells are identified as being of mononuclear type.

2. The perivascular infiltration was graded as 3+ or greater in 6 of the neg.-pos. biopsies and 2+ in the seventh.

3. The degree of interstitial cellular infiltrate was graded 3+ in 1, 2+ in 5, and 1+ in the seventh biopsy of a neg.-pos. site.

4. The cellular infiltration extended to the fat layer in 5 of the 7 biopsies, but to only the middermis in 1. The seventh biopsy did not contain the deep layers of the dermis or subcutaneous fat but had a marked infiltration throughout the areas of the dermis seen.

Positive immediate and negative delayed (Fig. 3). Thirteen biopsies were performed 48 hours after the injection of an inhalant antigen which manifested a positive wheal and erythema response at 20 minutes without any visible delayed component when read at 48 hours.

1. These specimens were characterized by the high percentage of eosinophils in the mild inflammatory response. The degree of tissue eosinophilic infiltrate was graded as 3 or 4+ in 11 and 2+ in 1 biopsy in the pos.-neg. group. One pos.-neg. site was devoid of eosinophils. The remainder of the cells were lymphocytes, histiocytes, and an occasional polymorphonuclear cell, but eosinophils were often the predominate cell in those pos.-neg. sites.

2. The degree of perivascular infiltration was mild (1 to 2+) in 10 of the 13 biopsies and moderate (3+) in the remaining 3 biopsies.

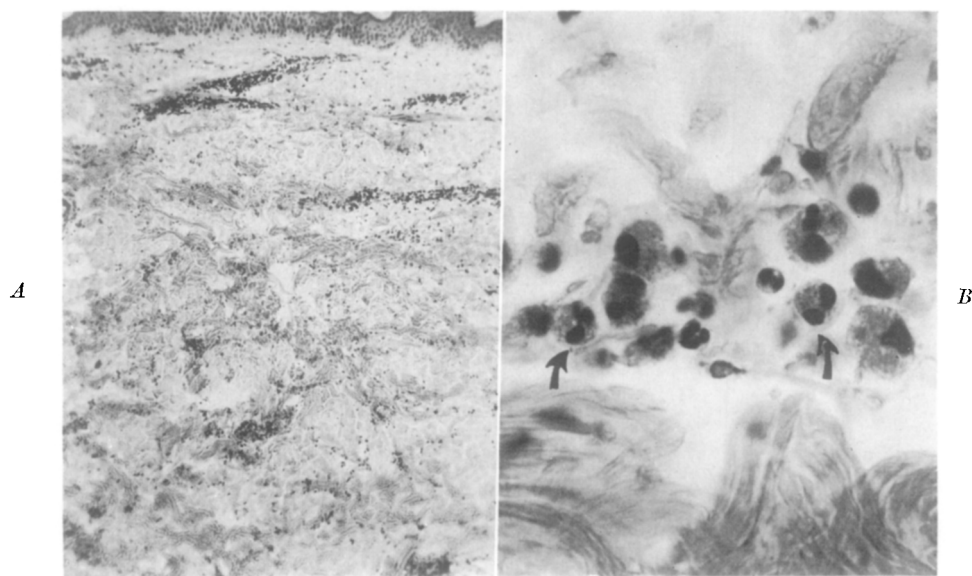


Fig. 3

Biopsy of a pos.-neg. test site performed 48 hours after the injection of 20 p.n.u. of ragweed antigen which produced a strongly positive reaction in 20 minutes but which had no visible reaction when biopsied at 48 hours. *A*, Low power ($\times 75$) shows a moderate (2+) perivascular and mild (1+) interstitial reaction which extends somewhat into the deeper dermis. *B*, High power ($\times 1100$) shows that the majority of the infiltrating cells are eosinophils (see arrows).

3. The interstitial infiltration was graded as 1+ in 9 cases and 2+ in the other 4.

4. The cellular infiltration extended to the middermis in 8 specimens and through the deep dermis and fat layer in 1. Only the superficial one third of the dermis was involved in 3 biopsies. The thirteenth biopsy did not contain the deeper dermis in the specimen.

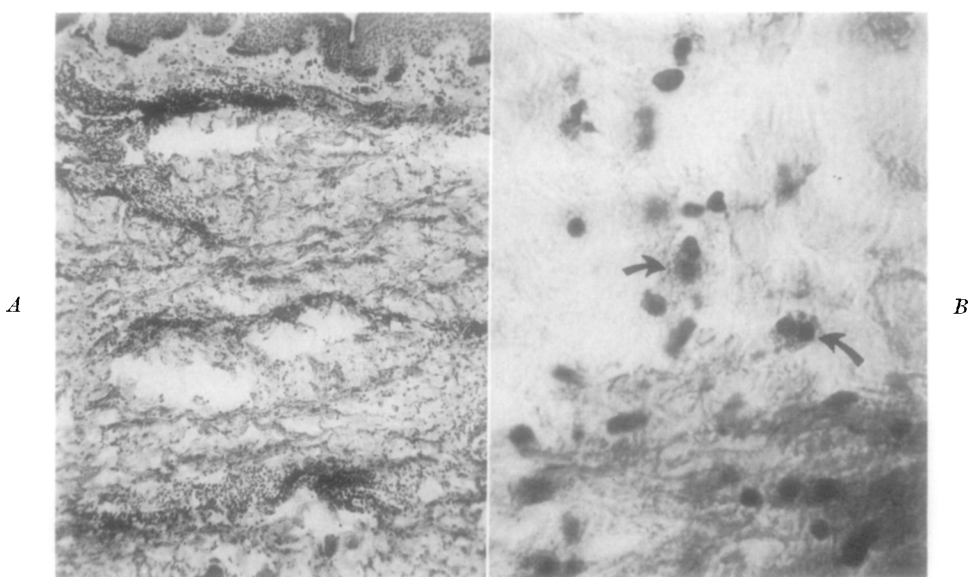
Positive immediate and positive delayed (Fig. 4). Twelve biopsies were performed 48 hours after an injection of an inhalant antigen which manifested a positive reaction at both the immediate and delayed readings. The intense cellular infiltration in these pos.-pos. specimens was characteristic in that a high percentage of the cells were eosinophils.

1. The degree of tissue eosinophilic infiltration was graded as 2+ or greater in 10 of the 12 biopsies and 1+ in the eleventh. One biopsy of a feather test site which grossly manifested a pos.-pos. reaction lacked any eosinophils. This occurred in the same subject that failed to have any eosinophils in a pos.-neg. reaction to timothy.

2. The perivascular infiltration was graded as 3 or 4+ in 9 of the 12 biopsies and 2+ in the other 3.

3. The degree of interstitial infiltration was graded as 2 or 3+ in 8 of the 12 biopsies and 1+ in the remainder.

4. Ten of these specimens manifested this mononuclear and eosinophilic

**Fig. 4**

Biopsy of a pos.-pos. test site 48 hours after the injection of 20 p.n.u. of timothy antigen which had manifested a positive response at both the immediate and delayed readings. *A*, Low power ($\times 75$) shows a moderately intense (3+) perivascular and interstitial infiltrate which extends through the full depth of the dermis. *B*, Under high power ($\times 110$), many of the cells can be identified as eosinophils (see arrows).

infiltration through the full thickness of the dermis and into the fat layer. The other 2 specimens did not contain the deeper parts of the dermis but contained a dense eosinophilic and mononuclear cell infiltration throughout the depth of the biopsy.

Control biopsies. Three biopsies were performed 48 hours after injection of 0.02 c.c. diluent alone (Cocas Solution).

1. These all manifested a mild mononuclear cell infiltration which was indistinguishable from the mild inflammatory reaction seen to an antigen injection which gave a neg.-neg. response. No eosinophils were seen in these control sites.

2. The perivascular infiltration was graded as 0 to 1+ in each case.

3. Interstitial infiltration was minimal (0+).

4. The inflammatory response was limited to the superficial one third of the dermis.

Tuberculin reactions. Five biopsies were performed on test sites which manifested 5 mm. or greater induration 48 hours after the injection of Intermediate PPD* (0.0001 mg. per 0.1 c.c.)

1. Four of these sites demonstrated an intense mononuclear cell infiltration which was identical in all respects to that described for the neg.-pos. reactions to the inhalant antigens. The 1 exception was 1 tuberculin reaction which showed a moderate number (3+) of eosinophils in the intense inflammatory response.

*Manufactured by Parke, Davis & Co., Detroit, Mich.

This resembled the pos.-pos. reaction to an inhalant antigen. This individual was atopic by history and manifested eosinophils in a pos.-neg. reaction to ragweed. He did not have an immediate wheal and erythema reaction to tuberculin. However, biopsies of tuberculin reactions on 2 other atopic individuals did not exhibit any eosinophils.

2. The perivascular reaction was intense (3 to 4+) in each case.
3. The degree of interstitial infiltration was graded as 2+ or greater in all 5 sites.
4. The cellular infiltration extended to the fat layer in each site examined.

DISCUSSION

This data concerning the incidence and characteristics of delayed reactions to inhalant antigens helps to clarify several points.

Although inflammatory reactions occurring for several hours at the site of skin tests are not at all uncommon, most of these reactions fade after 24 hours and are absent when examined at 48 hours. Skin reactions fulfilling the criterion of delayed hypersensitivity as seen in the tuberculin system (that is, 5 mm. or greater in induration at 48 hours) were quite uncommon in the subjects studied and appeared in only 6 per cent of the tests applied.

The incidence of immediate reactions was significantly greater in atopic patients than in nonatopic patients (17 vs. 5 per cent). There was no significant difference in the over-all incidence of delayed reactions between atopic and nonatopic patients (7 vs. 5 per cent). Thus no specific correlation between the occurrence of delayed reactions and the atopic state was demonstrated in this study. This impression was further confirmed by the fact that, for the majority of the antigens studied, there was no significant correlation between the immediate and delayed reactions.

The histologic patterns seen at the test sites 48 hours after the injection of inhalant antigens yield enlightening information about the inflammatory response to such antigens. The reaction at 48 hours usually contained many eosinophils if the site had manifested an immediate wheal and erythema response (pos.-neg. or pos.-pos.). The eosinophils were present uniformly throughout the inflammatory reaction and usually represented from 10 to 50 per cent of the infiltrating cells. Their presence did not correspond to the presence or absence of a visible skin reaction at the time of the biopsy (48 hours) but only to the prior wheal and erythema response. The relative percentage of eosinophils was greater in the pos.-neg. reactions than in the pos.-pos. ones. However, this difference may only reflect a relative difference caused by the greater number of infiltrating mononuclear cells in the pos.-pos. sites.

Occasional exceptions occurred. Two of the 21 biopsies of sites with negative immediate reactions showed a moderate number of eosinophils. These both occurred in a single individual who gave no history of allergic symptoms although he had positive skin tests to grass and ragweed pollen. In addition, 1 of the 5 positive tuberculin reactions contained eosinophils. This individual was atopic by history as noted above. On the other hand, eosinophilic infiltration was not seen in the site of 2 tests which had previously exhibited a wheal and erythema

response. The 2 wheal and erythema responses unaccompanied by evidence of eosinophilic infiltration at 48 hours both occurred in a single individual without a history of allergic symptoms. The other 22 biopsies taken at sites of prior wheal and erythema reaction manifested prominent tissue eosinophilic infiltrations.

Several studies in the past have been carried out to investigate the inflammatory reaction occurring during and after an immediate wheal and erythema response. A study had shown eosinophils in biopsies of skin reactions which had manifested a wheal and erythema response.⁵ Eosinophils were seen as early as 15 minutes after the injection of antigen in that study. One biopsy was performed 22 hours after the injection of dust antigen in a dust-sensitive patient and this was reported to show eosinophils. No biopsies were performed beyond 22 hours in this study.

In our preliminary studies, no eosinophils were seen in 2 biopsies performed at the height of a strongly positive wheal and erythema response (20 minutes after injection); however, an eosinophilic infiltration was prominent 24 hours later. A few eosinophils were present in 2 biopsies performed 8 days after strongly positive wheal and erythema responses to inhalant antigens.

Studies in several laboratories by the skin window technique have also shown that eosinophils are characteristically present after a wheal and erythema response to an inhalant antigen.^{6, 7} A few eosinophils were present in the early exudates when slides were changed after 4 to 8 hours. The maximum accumulation was noted at 18 to 24 hours after the application of the allergen.

In the instance where delayed reactions to the test antigen occurred (neg.-pos. and pos.-pos.), this response resembled positive tuberculin reactions both grossly and microscopically. Grossly, these delayed reactions presented as well demarcated, firm, indurated areas of at least 5 mm. in diameter. Surrounding erythema was often present and central necrosis occasionally developed.

As has been noted previously, the histologic reaction at a positive tuberculin site was not qualitatively specific, but differed only quantitatively from the histologic response at tuberculin negative sites, which also manifested a mild, superficial mononuclear cell infiltration.^{8, 9} We found a similar qualitative difference between the negative and positive delayed reactions to the inhalant antigens. Hence, the histologic interpretation of negative or positive delayed reactions must take into consideration the effect of dosage of antigen and the time of biopsy.

Studies with inhalant antigens carried out preliminary to this report had shown that delayed reactions tended to be more marked when biopsies were performed 24 hours after antigen injection as opposed to biopsies obtained after 48 hours. We also found that a larger antigen injection would create a more marked inflammatory response. Even when the dosage of antigen and the time of biopsy were standardized, there was no absolute qualitative difference between grossly negative and positive delayed reactions. Both grossly negative and positive reactions manifested perivascular and interstitial mononuclear cell infiltrations, but the reaction was more marked and extended deeper into the dermis when the biopsy was obtained from a grossly positive delayed reaction site. By

taking into consideration the previously mentioned criteria, almost all biopsies could be correctly assigned in a proper category when read in a blind fashion.

The important question as to whether the delayed reactions seen in this study are truly manifestations of delayed hypersensitivity is still not answered. Reactions of similar appearance were induced in some nonatopic volunteers by the injection of inhalant antigens in incomplete Freund's adjuvant.¹⁰ In some instances, the capacity for delayed reactivity could be transferred from these volunteers to nonsensitive recipients by means of white blood cell suspensions.¹¹ Three attempts to transfer the spontaneously occurring delayed reactivity seen in the present study with white blood cell suspensions have been unsuccessful to date.

If the delayed reactivity seen here represents true delayed hypersensitivity, interesting speculations could be raised concerning the immunologic and clinical significance of these findings. In some experimentally induced immune reactions, a transient state of delayed hypersensitivity appears to precede the development of a circulating antibody response.¹² This type of delayed skin reactivity has been called Jones-Mote hypersensitivity by some observers. The follow-up period in the present study has not been long enough to document whether the occurrence of delayed response only (neg.-pos.) to inhalant antigens represents a transient phenomenon preceding the development of an immediate wheal and erythema reaction to the inhalant antigen in question.

The complex antigenic nature of the allergenic extracts used in clinical allergy testing has long been recognized.⁴ By use of better characterized fractions of these allergenic extracts, it would be possible to determine whether the immediate and delayed skin reactions are directed to the same antigenic fraction. The availability of Antigen E, a purified fraction of ragweed antigen, enabled us to perform preliminary studies which may shed some light on this subject. The 2 individuals with pos.-pos. reactions to ragweed antigen (grossly and on biopsy) were tested to Antigen E. Both manifested pos.-neg. reactions to Antigen E. Similarly, 1 subject with a neg.-pos. reaction to ragweed had a neg.-neg. reaction to Antigen E.

It seems likely that the antigenic fraction which induced the delayed component of the reaction in these subjects was not present in Antigen E. Yet, the 2 individuals who had positive wheal and erythema (20 minute) responses to ragweed also had similar positive immediate reactions to Antigen E. Biopsies of the sites 48 hours after Antigen E injection contained eosinophils in both of the subjects. Forthcoming studies using other fractionation components of ragweed and other antigens will add significant information to this subject.

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