

Skin window: An aid in the diagnosis of drug allergy

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The skin-window technique was utilized as a means of diagnosis in 22 instances of suspected drug hypersensitivity in 19 patients. Positive reactions, as noted by eosinophil counts of 18 to 97 per cent, were recorded at test sites in all 5 cases of definite drug hypersensitivity in which reactions had occurred within 8 weeks of testing. Positive reactions, as noted by eosinophil counts of 8 to 90 per cent, were recorded at test sites in 10 to 12 cases with probable drug hypersensitivity in which some of the drug reactions had occurred as long as 20 years previously. There were no systemic reactions following the test and only mild local reactions in 2 cases. Negative tests, as noted by eosinophil counts of 0 to 4 per cent, were noted in distilled water controls and in 4 of 5 cases with equivocal drug hypersensitivity. Tests with other drugs being taken by the patients, to which reactions apparently had not occurred, and tests with drugs in nonallergic individuals also gave responses similar to the distilled water controls.

Rebuck and Crowley¹ used the skin-window technique to study inflammatory exudates induced by injury to the skin; they found that the cellular events in the inflammatory reaction induced by skin abrasion commenced with the migration of neutrophilic leukocytes to the site of inflammation. After performing their functions of phagocytosis and enzyme elaboration, they shrank and degenerated. Following this, there was an infiltration of mononuclear cells (histogenetic macrophages and lymphocytes), which later ingested the cytoplasmic fragments of the degenerated neutrophils.¹

Recently, there has been a number of reports in which this technique has been used to demonstrate tissue eosinophilia in hypersensitivity reactions. Eidinger, Raff, and Rose² applied antigens (fish extract, grass, or ragweed) to skin-window test sites of clinically allergic individuals, who exhibited positive intradermal skin reactions of the immediate type to these antigens; they noted

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accumulation of eosinophils greater in number than in control sites at 4 hours. The number of eosinophils gradually increased, so that at 24 hours they were frequently the predominant cell. The eosinophilia was greatest when elicited by allergens clearly associated with the patient's symptoms.³ Hu and colleagues⁴ demonstrated an eosinophilic response by the skin-window technique when individuals with contact sensitivity to rhus antigen were tested with this substance. Weiss, Cichocki, and Blicharski⁵ also reported eosinophilia in the skin-window exudates of several patients with bronchial asthma, eczema, neurodermatitis, and pollinosis. The strongest eosinophil reactions occurred in those with neurodermatitis. Jacob and co-workers⁶ reported a case of iodide sensitivity in which the skin-window site exposed to potassium iodide showed 86 per cent eosinophils; this patient also had a blood eosinophilia as high as 87 per cent. In other cases,^{2, 4} however, tissue and blood eosinophilia did not necessarily coexist.

Although blood eosinophilia may be a response to nonspecific stimulation or a part of a specific allergic reaction, the evidence strongly indicates that tissue eosinophilia goes hand in hand with an allergic reaction. Berger and Lang⁷ and Kline, Cohen, and Rudolph⁸ demonstrated the migration of eosinophils into histologic sections of wheals shortly after the intradermal injection or application of a specific allergen. Eidinger, Wilkinson, and Rose³ noted a striking eosinophilia in the fluid of cantharidin-induced blisters following injection of an allergen.

It is well known that the large majority of drugs are relatively simple chemical compounds of low molecular weight (haptens) which become antigenic only when they become joined with body proteins. Because of this, scratch and intradermal tests with drugs have been inconsistent. Despite this, they have occasionally caused anaphylaxis⁹ and death.¹⁰ The only type of cutaneous test with nonprotein drugs which has often proved of diagnostic value is the patch test in contact dermatitis. The giving of oral or parenteral test doses of drugs to patients is dangerous, because, although quite large doses of a drug may be required to produce a reaction, extremely small doses have caused recurrence of symptoms with severe and even fatal reactions.¹¹

A diagnosis of drug hypersensitivity can be objectively confirmed with certainty only when an antibody can be demonstrated either *in vitro* or by means of the passive-transfer test. Except in those cases in which cellular components of the blood are directly involved, with the tests presently available antibody can be demonstrated only rarely, and then with difficulty, in the vast majority of patients in whom reactions occur. Therefore, the diagnosis of drug hypersensitivity in most cases must be made on the basis of the patient's history.

Since drug hypersensitivity may only rarely be demonstrated by other objective tests, we chose to study a number of patients with clinical histories suggestive of drug reactions to determine if the skin-window technique could be a useful aid in the diagnosis of drug allergy.

MATERIALS AND METHODS

Nineteen patients with presumed drug hypersensitivity were tested by the skin-window technique using the suspected drugs as antigens, but tests were

not made until after subsidence of any eruption. Tests with similar drugs were made in nonallergic individuals as controls. Differential blood counts were performed in 11 cases within 2 days of testing, but in 8 instances counts were made between 3 and 13 days prior to testing.

The skin of the forearm, upper arm, or anterolateral surface of the thigh was shaved, cleansed with pHisoHex and 70 per cent alcohol, and then air dried. By the method of Re buck and Crowley,¹ skin windows were produced by scraping the epidermis away from an area 3 to 4 mm. in diameter until fine bleeding points appeared on the scraped area, indicating that the papillary layer of the corium had been reached. Initially, a No. 15 Bard-Parker scalpel blade was used, but later a blunt instrument with a 2.5 mm. tip which had been filed to form a sharp edge was employed; with this instrument, a level surface at the papillary layer of the corium was more easily prepared, and larger and more definitive exudates were achieved. Two drops of concentrated aqueous solutions or suspensions of the drugs to be tested were applied to prepared test sites, and each was covered with a chemically clean, plastic cover slip, which was then secured by cardboard and a circular gauze-adhesive patch. The preparations and concentrations of the drugs used are recorded in Table I. Control sites were similarly prepared at the same time with sterile, distilled water being used instead of a drug. As a result of the injury to the skin produced by the scarification, cells migrated into the area and flattened themselves in a single layer against the undersurface of the cover slip. All slides were removed after 24 hours and allowed to dry. They were then stained with Wright's stain, and differential cell counts were made. The slides were first scanned, and counts were started in areas where eosinophils, if present, predominated. When a sufficient number of cells was present, a total of 500 was counted. The counts were made "blind" without knowledge of whether or not the smear represented a test with a drug suspected of causing a reaction.

It was found to be important that all areas containing cells be examined for eosinophils, because they often were in clumps, located at the periphery, or

Table I. *Preparations and concentrations of drugs used in skin-window tests*

<i>Drug</i>	<i>Preparation</i>	<i>Concentration</i>
Dilantin	Suspension in water	25 mg./ml.
Potassium penicillin	Solution in water	500,000 U./ml.
Penicilloyl polylysine	Solution	1×10^{-5} M.
Novocaine	Solution	2%
Xylocaine	Solution	2%
Aspirin	Suspension in water	75 mg./ml.
Ferrous sulfate	Suspension in water	75 mg./ml.
Iodine	Solution	2%
Bellergal	Suspension in water	1 tablet in 4 ml.
Phenobarbital	Solution in water	15 mg./ml.
Pentothal sodium	Solution	25 mg./ml.
Renograffin	Aqueous solution	60%
Diodrast	Solution	70%
Hypaque	Solution	50%
Pantopaque	Solution in oil	30.5%

associated with debris. Many of the eosinophils had disruption of their cell membranes with scattering of the acidophilic granules (Fig. 1). The other predominant cells were mononuclear in type and consisted essentially of macrophages (many with phagocytized debris) and hypertrophied lymphocytes (Fig. 2). There was also a variable number of neutrophilic leukocytes, many of which showed degenerative changes (Fig. 3). In most of the slides, the acidophilic granules of many of the eosinophils took only a pinkish stain, possibly because of beginning autolysis.

Because of variances in the reliability of the historical evidence of drug hypersensitivity the patients and controls were classified into four groups.

Group I was comprised of 5 patients in whom the reactions appeared to be clearly related to a single drug and were observed by us or documented in hospital records. In this group, all of the skin-window tests were made within 8 weeks of the reaction.

Group II was comprised of 10 patients whose histories strongly indicated probable drug reactions, but in some instances more than one drug was possibly implicated, and, in some, several years had passed since the alleged reaction. One patient classified in Group I also had a history of a prior drug reaction to another drug, and in relation to this was also classified in Group II. Another patient gave a history of 2 distinct reactions to separate drugs. Therefore, 10 patients with 12 separate reactions were classified in this group.

Group III was comprised of 5 patients whose histories of drug reactions were equivocal.

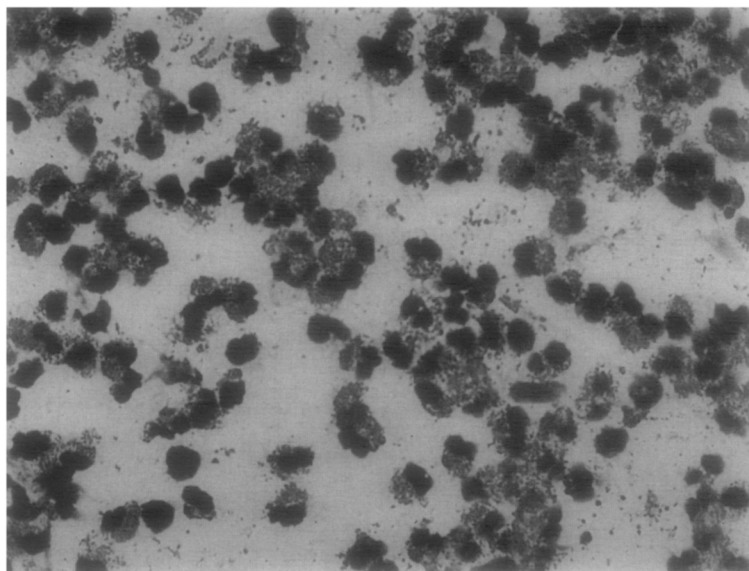


Fig. 1

Eosinophilic inflammatory reaction in skin window at 24 hours (Case 2). Almost the entire field consists of eosinophils, some with disruption and scattering of granules. (Original magnification $\times 800$.)

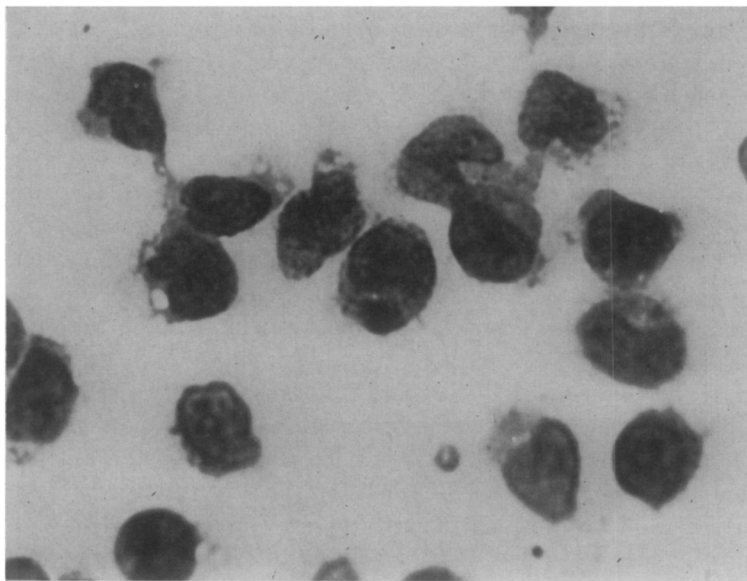


Fig. 2

Field of mononuclear cells at 24 hours. Note the macrophage with the ingested portion of another cell. (Original magnification $\times 2500$.)

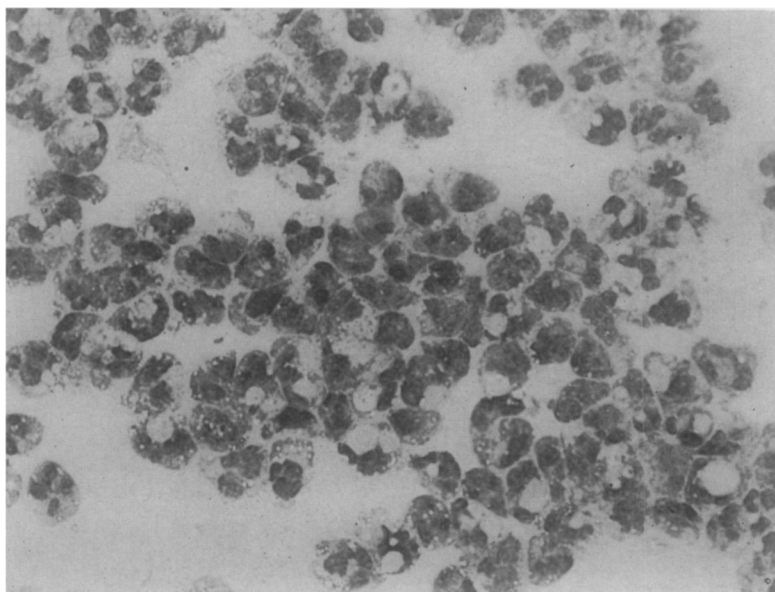


Fig. 3

Cellular infiltrate at 24 hours showing neutrophilic leukocytes, many of which have undergone degeneration. (Original magnification $\times 800$.)

Group IV was comprised of 9 individuals with no history of allergy or drug hypersensitivity.

In Group I to III, all the drugs known to have been taken by the patient at the time of reaction were tested, and in some cases drugs closely related chemically were also tested. Since in many of these cases drugs tested were clearly not related to reactions, they served as additional tested controls. Thus, 19 patients with 22 suspected drug reactions were tested with 47 drugs, and 9 control individuals were tested with 20 drugs.

Because of the possible danger of anaphylactic reactions following application of drugs to the skin-window sites, all patients were carefully observed for at least 30 minutes following the application of the drug.

RESULTS

In the tested controls (Table II) and distilled water controls (Tables III, IV, and V), eosinophil counts were between 0 and 4 per cent.

In Group I, eosinophil counts ranging between 18 and 97 per cent were observed at test sites of the probable sensitizing drugs in each case. In one patient a related drug gave a positive test, but in 9 other tests with related or other drugs being taken by the patient, the results were similar to those in the controls (Table III).

In Group II when testing for the drugs most likely to have caused the reactions, eosinophil counts ranging between 8 and 90 per cent were noted at 10 of 12 test sites, and 0 and 3 per cent, respectively, in the other two sites. At 12 other test sites where related or other drugs being taken by the patient were applied, eosinophil counts ranged between 0 and 6 per cent (Table IV).

In Group III, eosinophil counts at test sites of 8 possible drugs in 5 patients ranged from 0 to 7 per cent (Table V).

In one patient (Case 2) with an eosinophilotoxic response of 97 per cent to penicillin, vesicles appeared over the intact skin adjacent to the denuded window site; they disappeared in 3 days.

Another patient (Case 5a) with a 36 per cent eosinophilotoxic response to iodine developed a 2 by 2 cm. wheal surrounding the skin-window site. This subsided in one day.

Hu¹ also reported that in his subjects with Rhus sensitivity, vesiculation occurred in the intact skin surrounding the skin-window at about the same time that eosinophils appeared in the inflammatory exudate.

Except for some localized mild pruritus at the skin-window site and transient burning when the drug was applied, there were no systemic reactions or other adverse effects from this procedure.

DISCUSSION

Speirs^{12, 13} in studies of the peritoneal fluid of sensitized mice believed that an eosinophilic response preceded the formation of new antibody, and that there was a quantitative relationship between the number of eosinophils present and amount of antibody produced. On the other hand, Litt^{14, 15} reported that eosinophils followed the union of antigen with antibody. He indicated that since

Table II. *Skin-window tests in nonallergic individuals*

<i>Individual</i>	<i>Drugs tested</i>	% <i>Eosinophils</i>		<i>Interval*</i>
		<i>Test</i>	<i>Blood</i>	
1	Dilantin	0	2	0
	Aspirin	3		
2	Xylocaine	0	1	0
	Phenobarbital	0		
3	Ferrous sulfate	0	3	2
	Dilantin	0		
4	Phenobarbital	0	2	3
	Aspirin	0		
5	Dilantin	0	1	0
	Aspirin	0		
6	Dilantin	0	0	0
	Aspirin	0	0	
7	Penicillin	1	10	2
	Novocaine	2		
	Dilantin	2		
	Phenobarbital	2		
	Ferrous sulfate	1		
8	Dilantin	4	4	0
	Aspirin	1		
9	Novocaine	0	1	0

*Interval, days between blood counts and test.

Table III. *Skin-window tests in cases of obvious drug hypersensitivity (Group I)*

<i>Case</i>	<i>Past history of allergy</i>	<i>History</i>	<i>Reaction-test interval</i>	<i>Drugs tested</i>	% <i>Eosinophils</i>				<i>Remarks</i>
					<i>Test</i>	<i>Control</i>	<i>Blood</i>	<i>Interval*</i>	
1	0	Dilantin therapy 2 months; maculopapular rash 1 day following respiratory infection and penicillin	5 weeks	Dilantin Penicillin Penicilloyl polylysine	26 0 —	4	13	1	Patch positive Patch negative Intradermal negative
2	0	Urticaria within a few hours of intravenous penicillin	25 days	Penicillin	97	0	5	1	
3	+	Maculopapular rash 1 week after institution of Dilantin therapy	16 days	Dilantin Phenobarbital	18 2	4	3	13	
4	+	Exfoliative reaction and urticaria 1 month after institution of Dilantin therapy	8 weeks	Dilantin Pentothal Iodine Hypaque Pantopaque	42 4 4 2 1	3	7	0	Patch negative Patch negative Patch negative Patch negative Patch negative
5a	+	Exfoliative dermatitis 1 day following arteriography with Diodrast	19 days	Diodrast Renograffin Hypaque Iodine	81 4 3 36	2	1	2	2 × 2 cm. wheal about skin window

*Interval, days between blood counts and tests.

Table IV. *Skin-window tests in cases of probable drug hypersensitivity (Group II)*

Case	Past history of allergy	History	Reaction-test interval	Drugs tested	% Eosinophils				Remarks
					Test	Control	Blood	Interval*	
5b	0	Exfoliative dermatitis following Dilantin, 1957	8 years	Dilantin	90	2	1	2	
6	0	Urticaria following penicillin injection, 1948	17 years	Penicillin Penicilloyl polylysine	30 2	0	3	5	Endermal positive
7	0	Angioedema same day as injection of Novocaine, 1955	10 years	Novocaine Xylocaine Penicillin	14 1 4	1	2	0	Scratch negative Scratch negative Scratch negative
8	0	Aspirin and penicillin, 4 days later, angioedema	9 weeks	Aspirin Penicillin Penicilloyl polylysine	18 0 0	0	1	1	Angioedema definitely induced by aspirin later
9	+	Urticaria and arthralgias 6 days following reinstitution of ferrous sulfate	2 weeks	Ferrous sulfate Aspirin Penicillin	19 0 0	0	1	4	Urticaria disappeared following discontinuation of ferrous sulfate
10	0	Urticaria following penicillin injection, 1945	20 years	Penicillin Penicilloyl polylysine	8 1	0	2	1	
11a	0	Urticaria following penicillin injection, 1949	16 years	Penicillin Penicilloyl polylysine	67 6	2	3	3	Scratch positive Endermal positive
11b	0	Vomiting after ingesting aspirin on 2 occasions, 1952	13 years	Aspirin	13	2	3	3	
12a	+	Urticaria following penicillin injection, 1960	5 years	Penicillin Penicilloyl polylysine	0 2	0	0	2	Endermal negative
12b	+	Anaphylactoid reaction during injection of Renograffin	3½ years	Renograffin Iodine	68 0	0	0	2	
13	0	Urticaria following penicillin injection, 1955-62	3 years	Penicillin Penicilloyl polylysine	25 0	3	4	5	Endermal negative
14	+	Urticaria fourth day of penicillin therapy	2 months	Penicillin	3	0	5	0	

*Interval, days between blood counts and tests.

immune complexes are rapidly cleared from the blood, one should look for eosinophils at the sites of allergic lesions, where they may be found regularly and persist for long periods. He believed that the union of antigen and antibody formed an immune complex. This or an intermediate possessed eosinophilotactic

Table V. *Skin-window test in cases of questionable drug hypersensitivity (Group III)*

Case	Past history of allergy	History	Reaction-test interval	Drugs tested	% Eosinophils				Remarks
					Test	Control	Blood	Interval*	
15	+	Probable psychogenic urticaria	1 week	Aspirin	0	0	1	9	
16	0	Indefinite reaction to Diodrast, 1952	14 years	Diodrast Iodine Renograffin	0 0 0	0	5	1	Renograffin given without reaction following test
17	0	"Allergic" vasculitis, cause undetermined	18 months	Aspirin Penicillin	3 0	0	1	9	Spontaneous improvement
18	+	Probable psychogenic urticaria	1 week	Bellergal†	7	0	1	?	
19	0	Fever of unknown origin, while taking Dilantin	1 week	Dilantin	1	1	4	6	

*Interval, days between blood counts and tests.

†Ergotamine tartrate, bellafoline, and phenobarbital.

qualities. Litt¹⁶ has further concluded that eosinophils probably phagocytize antigen-antibody complexes.

There have been other explanations for tissue eosinophilia. Several workers^{8, 17, 18} have noted that eosinophils respond chemotactically to histamine which is released at the site of many antigen-antibody reactions. Kovacs¹⁹ suggested that eosinophils may participate in the neutralization of histamine since they contain spermine, a biogenic amine with antihistaminic activity. Cohen and Sapp²⁰ suggested that nonimmunological mechanisms may be "eosinotactic," but that they then require macromolecular complexes of critical qualities.

Thus, it seems likely that the migration of eosinophils into the cellular exudate during the development of a positive skin-window test is provoked by the formation of antigen-antibody complexes.

The slightly lower average eosinophil counts in Group II, as compared to Group I, may be explained on the basis that many of the tests were made years after the original drug reaction, and antibody titers may have been low. The two negative tests in Group II and the five negative tests in Group III may be explained on the basis that the history of the drug reaction was inconclusive, or that the wrong drug was used as antigen in the test.

In Case 18, the eosinophil count at the test site was 7 per cent while it was 0 at the control site; yet this must be considered a mild falsely positive reaction because the suspected reaction (urticaria) was later judged to be of psychogenic origin. It is possible, of course, that the patient had a mild drug reaction in addition, and the positive test was indicative of a response to a poor antigen (Bellergal).

Degranulation of eosinophils was noted in several of the positive tests. This phenomenon has not previously been observed by others and may have

represented unusual reactions to specific compounds. Unfortunately, tests with varying concentrations of drugs were not made to clarify this point. Basophilia also was noted in several of our positive tests, as had also been reported by Fowler and Lowell.²¹

Shelley²² noted negative basophil degranulation tests during the week or two following a severe allergic reaction. It was felt that this was due to the removal of available reactive antibody from the circulation during the reaction. Of the five negative reactions in Group III, three were tested within one week.

Since corticosteroids, given systemically, cause eosinopenia and abolish skin-window eosinophilia,³ it is inadvisable to perform the test while the patient is receiving these compounds. Care must be exercised in the performance of skin-window tests in patients with neurodermatitis or exfoliative dermatitis because of the well-known fact that these individuals often exhibit blood and tissue eosinophilia.

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