

LEUKOCYTIC TRANSFER OF THE IMMEDIATE TYPES OF ALLERGIC REACTION IN MAN. II. THE CORRELATION OF LEUKOCYTIC TRANSFER WITH OTHER SENSITIVITY FACTORS

An Eight-Year Study on One Atopic Donor

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INTRODUCTION

THE passive local transfer of an immediate* wheal type of hypersensitive reaction in man with a suspension of venous blood leukocytes as the sensitizing medium was reported in 1950 by Walzer and Glazer.¹ The donors of the white blood cells were atopic patients, sensitive both clinically and by skin test to pollens and animal danders. The responses elicited by skin tests with allergens at the passive transfer sites were small in size and never exceeded a slight or one-plus (+) reaction. Leukocytic transfer failed in some instances, even though the donor's reaginic titer was high and his skin reactions were pronounced.

The unusual features of the phenomenon were at first attributed to the limitations of the technique. Particularly disadvantageous was the pronounced inflammation which developed at the site of the intracutaneous injection of the leukocytic suspension. To overcome this difficulty, an alternate transfer technique was devised employing an extract of shattered leukocytes as the sensitizing medium. The details of this procedure and a comparison of its merits and limitations with those of the cell-suspension method have been described in a previous communication.² Although the leukocytic extract technique materially reduced the inflammation produced by the sensitizing injection, it did not bring about an appreciable increase in the size of the passive transfer reaction.

In the development of the techniques, it proved advantageous to use some donors and recipients repeatedly. One such donor was D. G., whose history was related briefly in the previous report.² D. G., now 27 years old, has been treated by us for various allergic disturbances for the major part of his life.

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*In this, as in previous reports, the term "immediate" is employed to characterize the time of appearance rather than the immunologic mechanism mediating the allergic reaction. Although the sera of most of the donors studied contained skin-sensitizing antibodies, leukocytic transfer trials were also carried out in persons who manifested immediate whealing responses to tests with allergens but in whom no circulating antibodies could be demonstrated.

Because of our intimate knowledge of his case history and the peculiar pattern of his sensitivities, D. G. was used repeatedly as a donor of leukocytes for transfer studies. From November, 1950, to March, 1959, he served in this capacity on more than fifty different occasions. The findings in his case contributed much toward proving the specificity of the leukocytic transfer reaction.

D. G. was one of a series of donors with different types of immediate hypersensitive reactions being studied with the leukocytic transfer technique. As the studies progressed, the unusual findings in the case of D. G. convinced us of the advisability of presenting these data in a separate communication in advance of the report on the entire donor series. It was felt that the conclusions drawn from the detailed and carefully controlled experiments on D. G. might aid in the analysis and interpretation of the findings obtained in the less complete studies performed on other donors.¹² The fact that the decision to present a separate report on D. G. was reached relatively late in the course of these investigations will account for some of the obvious gaps in the data which appear in this communication.

CASE HISTORY OF D. G.

Because the leukocytic transfer experiments on D. G. dealt exclusively with his pollen sensitivities, only these aspects of his history will be presented in detail. Other phases of his allergic history will be mentioned briefly, but most of the voluminous medical record having no direct bearing on the immunologic problems under consideration will be omitted.

D. G. was born in the borough of Brooklyn, in the city of New York, in March, 1932. At the age of 20 months, he came under our observation with a history of asthma, atopic dermatitis, and allergic rhinitis, all of moderate severity, which started at the age of about 1 year. His family history was positive for atopy. His symptoms and physical signs and his non-immunologic laboratory findings were typical and warrant no detailed description.

Preliminary skin testing by the indirect (serum passive transfer) method^{3a} in May, 1934, and a more thorough study with the same technique, in March, 1936, revealed positive reactions to many allergens. Strongly positive reactions were obtained to pollens, legumes, nuts, seeds, cereals, fruits, and vegetables. Weakly positive transfers were obtained to feathers, wool, house dust, and other household inhalants. The reactions to meats, milk, egg, fish, animal danders, and molds were negative. A regime based primarily upon the elimination of contacts with troublesome inhalants and the avoidance of food offenders as revealed by the indirect tests and by daily diary studies brought about a rapid improvement in the patient's condition. His dermatitis soon ceased to be a problem. The asthma attacks diminished in frequency and severity. Because of the patient's rapid improvement, no hyposensitization treatment was needed. In 1938, when D. G. was 6 years of age, supervision of his case was discontinued.

In March, 1945, at the age of 13, D. G. returned for further observation, primarily because of his allergic rhinitis which had been particularly acute during the previous ragweed season. In the seven-year interval, his asthma attacks had remained infrequent and mild. He had developed a tolerance to most of the previously offending foods, but the ingestion of wheat and chocolate still produced rhinitis. Intracutaneous tests performed directly upon the patient produced weakly positive reactions to cereals, legumes, nuts,

TABLE I. SUMMARY OF POLLEN SKIN REACTIONS, SYMPTOMS, AND TREATMENT IN DONOR D. G. FROM 1944 TO 1959

YEAR	RAGWEED				TIMOTHY			TREES		ENGLISH PLANTAIN	
	SKIN REACTIONS*		SYMP-TOMS	TREATMENT	SKIN REACTIONS*		SYMP-TOMS	SKIN RE-ACTIONS*	SYMP-TOMS	SKIN RE-ACTIONS*	SYMP-TOMS
	0.001 MG.	0.01 MG.			0.001 MG.	0.01 MG.					
1944			+++	None			0				
1945	++	+++	+	Preseasonal	+	±	0	+	0	+	0
1946	++	+++	±	Perennial	+	++	0	±	0	+	0
1947	±	+++	±	Perennial	+	+++	0	+	0	±	0
1948			±	Perennial			0				0
1949	++	+++	±	Perennial	+	+++	0	+	0	+	0
1950	++		±	Perennial	++	+++	0	+	0	+	0
1951			+	Perennial	±	+++	0	+	0	±	0
1952	±		+	Perennial			0	+	0		0
1953	+++		±	Perennial	+++		0	+	0	±	0
1954			±	Perennial			0		0±		0
1955	++		0	Perennial	++		0	+	±	+	0
1956	+	+++	0	Perennial	±	+++	0	±	+	++	0
1957	+	+++	0±	None	±	+++	0	±	±	+++	0
1958	±	±	0	None	+++	+++	0	+	0	++	0
1959	±	+++		None	+++			+		+	

Test injections were made with 0.01 ml. of extract.

Scale of skin reactions:

+++ = Marked.
++ = Moderate.
+ = Slight.
± = Faint.
0± = Doubtful.
0 = Negative.

Scale of symptoms:

+++ = Severe.
++ = Moderate.
+ = Mild.
± = Faint.
0± = Doubtful.
0 = None.

*Milligrams of total nitrogen per milliliter of test solution.

and household inhalants, all of which had been positive by indirect testing in childhood. D. G. showed a pronounced reaction to ragweed and weaker positive reactions to other pollens (Table I). On the basis of his history and skin reactions, treatment with ragweed was started at once and continued without interruption until the fall of 1956, when clinical symptoms attributable to this pollen were no longer recognizable.

At the present writing (March, 1959), D. G. is a well-developed 27-year-old adult. He is married and works as an instructor in physical education in the New York City school system. He now resides on Long Island in a home furnished, for the most part, with "allergen-free" materials. Failure to ad-

here to a diet free of foods to which he is still sensitive is undoubtedly responsible for his mild perennial symptoms of allergic rhinitis. His nasal mucous membranes usually appear pale and edematous, and his nasal smears, on repeated examination, have shown a high eosinophil content. He has no pulmonary or cutaneous symptoms, and at this time, he presents no other significant clinical or laboratory findings pertinent to this discussion.

METHODS AND MATERIALS

Pollen Extracts.—The extracts used in these studies were those routinely prepared in the Allergy Laboratory of the Jewish Hospital of Brooklyn.⁴ The stock pollen extracts were made annually, usually in January, and were standardized according to their total nitrogen content. Dilutions were made with buffered saline. The ragweed extracts were made of a mixture of giant and dwarf ragweed pollens. The timothy pollen extract was chosen to represent the grass pollens. Since the potency of the stock extracts tended to fall off during the course of the year, fresh extracts (labeled "SP"), usually made only of dwarf ragweed, were prepared from time to time and employed for testing along with the stock test materials. The six-tree pollen mixture contained equal parts of ash, beech, birch, hickory, oak, and poplar pollen extracts.

TABLE II. POLLEN REAGIN TITRATIONS OF D. G. SERUM FROM 1950 TO 1959

DATE	RECIP- IENT	RAGWEED SERUM DILUTION				TIMOTHY SERUM DILUTION			
		1:16	1:64	1:128	1:256	1:16	1:64	1:128	1:256
11/ 9/50	G.	+±	+			+	±		
4/10/51	W.	+	+		±	+	±		0
3/11/53	K. R.		+		±		+		0±
5/23/56	R.	+	0±		0	+	±		0
5/ 9/57	B.	±	0	0		+±	+	+	
5/ 9/58	K. R.	+	±	0±		+±	+	±	
3/ 9/59	W. A.	+	0		0	++	+		0

Sites prepared with 0.05 ml. of serum.

Sites tested with 0.01 ml. of extract containing 0.01 mg. nitrogen per milliliter.

Scale of skin reactions same as in Table I.

Cutaneous and Reaginic Titrations.—While under treatment, D. G. was tested routinely almost every year with fresh stock pollen extracts. Serial intracutaneous tests were performed with increasing concentrations of timothy, ragweed, English plantain, and the six-tree mixture in order to determine the dilution of each pollen which would produce a marked (+++) reaction (Table I). By means of these annual titrations, pronounced alterations in the degree or pattern of skin sensitivity to pollens were detected.

During the course of the leukocytic studies, titrations for reagins to timothy and ragweed pollens were made at intervals by the method of Coca and Grove.⁵ Cutaneous sites passively sensitized with serial dilutions of the patient's serum were tested with pollen solutions containing 0.01 mg. N/ml. (Table II).

All of the direct and indirect tests made on D. G., as well as the serum titrations for reagins and all leukocytic transfer tests, were performed by the same investigator (K. B.). She also read and recorded the results of all such tests. Hence, the data on this patient were obtained over a period of twenty-five years by one observer. Her extensive experience in serum passive transfer techniques proved of inestimable value in the leukocytic transfer experiments. Since July, 1956, this investigator has prepared all of the leukocytic materials used in the studies on D. G. They had previously been made by a former collaborator (S. S.).²

Leukocytic Transfer Techniques.—The details of the techniques employed in the leukocytic transfer studies on D. G. have been described.² No blood samples for the procurement of leukocytes were drawn for a week following the therapeutic pollen injections which the donor was receiving at four-week intervals. Sites on the outer aspect of the recipient's arm were prepared by the intracutaneous injection into each site of 0.025 ml. of leukocytic suspension (usually 1:10 by volume in Tyrode's solution) or of 0.05 ml. of leukocytic extract (usually 1:4 in Tyrode's solution). From one to four sites were prepared on an arm. These sites were tested when the inflammation produced by the sensitizing injection had apparently subsided. With the extract technique, the interval between sensitization and testing was usually five days; with the suspension method, the interval varied from seven to twelve days. An intracutaneous test with approximately 0.02 ml. of allergenic extract was introduced at the sensitized site and was duplicated as a control on adjacent normal skin. Other controls included tests with buffered saline, physiologic saline, various nonrelated allergens, and special materials.

Recipients in Leukocytic Transfer Studies.—The recipients in the leukocytic transfer studies on D. G. included eighteen presumably nonatopic adults who had been carefully selected from a large number of volunteers because they had shown completely negative reactions to skin tests with concentrated (0.1 mg. N/ml.) ragweed and timothy pollen extracts. Of these recipients, fourteen were used only once or twice. Four others served on seven to fifteen different occasions during periods lasting up to six years.

Reading and Grading Reactions.—The reactions elicited with the leukocytic transfer techniques were graded on the basis of a previously described classification especially designed for this purpose.² Immediately prior to the test with allergen, the sites were read by inspection and palpation to detect any residual inflammation which might have persisted following the sensitizing injection. After testing, readings by inspection and palpation were taken on both the test and control sites.

In the special classification of reactions, which was made necessary by the relatively small size of the leukocytic transfer response to testing, a slight positive or one-plus (+) reaction consisted of a central wheal about 0.5 cm. in diameter with a surrounding erythema. A reaction only slightly less than this was designated as a plus-minus ($\pm$) positive. Erythema on or around the edematous area was not regularly present in this grade of reaction. When the presence of edema was barely perceptible by palpation, the reaction was

read as a negative-plus-minus ($0\pm$). Erythema was not a common component in this grade of reaction. When no change in edema or erythema resulted from the test, the reaction was recorded as negative (0). Pruritus was associated only occasionally with leukocytic transfer reactions.

It frequently became necessary to correct the readings at the test sites to take into account various nonspecific factors, such as the persistence of traces of edema at the sensitized sites or the irritative properties of certain experimental test materials which, in the concentrations used, did not yield completely negative reactions at the control sites. To facilitate corrections of this nature and to preclude bias on the part of the investigators, it seemed advisable to resort to a numerical system of marking reactions, similar to that which had been formulated in a previous study for recording fine differences between skin reactions produced by *direct* testing.⁶ Values from 0 to 12 were assigned to various degrees of response between the completely negative (0) and the maximally positive (+) reactions. The size and induration of the central edematous area represented the main criteria upon which the reactions were graded, with some allowances being made for the amount, intensity, and distribution of the erythema and for pruritus when this element was present. The numerical value of the control was deducted from that of the transfer site, and the remainder was reconverted to one of the aforementioned symbols on the basis of the following scale of equivalence: Numerical values of from 0 to 2, inclusive, were covered by the negative symbol 0; values from 3 to 5, inclusive, by the symbol $0\pm$; from 6 to 9, inclusive, by the symbol $\pm$; and from 10 to 12, inclusive, by the symbol +. The use of a symbol rather than a number as a final value for the reaction made it possible to retain the grading system which had been employed in previous studies, thus providing uniformity in terminology for the entire series.

The reactions resulting from *direct* intracutaneous tests on *donors* and from the *serum* titration tests were graded according to the standards commonly employed in these procedures.³

Interpretation of Results.—A final reading of one-plus (+) which could be elicited on repetition (as either a + or $\pm$ reaction) was considered to be significant. A plus-minus ($\pm$) or weakly positive reaction was deemed significant if it had been adequately controlled and could be elicited repeatedly (though not necessarily invariably) on repetition of the test. A negative-plus-minus ($0\pm$) reaction was too small to be regarded as reliable evidence of transferred sensitivity. Only when it occurred repeatedly and was associated with definite one-plus (+) or plus-minus ($\pm$) reactions elicited by the same test on other sites did it receive any consideration.

POLLINOSIS HISTORY OF D. G.

Although serum passive transfer reactions to timothy and ragweed pollens were already positive in D. G. at the age of 20 months, recognizable hay fever symptoms did not develop until 1944, when he was 12 years old (Table I). In the spring of 1945, preseasonal treatment with weekly sub-

cutaneous injections of increasing doses of ragweed extract was started. At the end of that ragweed season, injections of from 0.025 to 0.1 mg. N were continued at four-week intervals. D. G. responded well to this therapy. In October, 1956, after he had been practically free of ragweed symptoms for two seasons, treatment was discontinued. Symptoms were barely noticeable in 1957, and none were experienced in 1958.

Starting in 1954, when he moved his home to the borough of Queens in New York City, D. G. began to have symptoms of hay fever which started early in May and ended the first week in June. These were caused by tree pollens to which he had shown positive skin reactions for many years. When he again moved his residence in February, 1958, this time to Commack, Long Island, these symptoms became negligible. No leukocytic transfer studies were attempted in connection with the tree sensitivity, since the concentrated tree extracts were found to be nonspecifically irritating in the skin of the recipients.

D. G. has never experienced any symptoms of grass pollen sensitivity, although he has shown markedly positive skin reactions and high reaginic titers to timothy pollen for many years (Table I). Nor did he show evidence of clinical sensitivity to English plantain pollen, to which he also reacted positively by skin test. His reactions to tests with mold spores were always negative.

CUTANEOUS REACTIONS TO TIMOTHY AND RAGWEED POLLENS

The results of skin tests with pollens in D. G. from 1945 to 1959 are recorded in Table I. For the graphic presentation of this feature (Fig. 1), the test readings obtained during this period with 0.001 mg. N/ml. dilutions of pollens were chosen because they had elicited reactions of + or ++ intensity in D. G. Reactions of this size had been found by Bowman⁷ to be more suitable for purposes of comparison than those of larger dimensions.

Direct intracutaneous tests on D. G. in early childhood (1935 to 1938) had revealed weak and approximately equal reactions to grass and ragweed pollen extracts. From 1945 to 1950, ragweed produced the stronger reaction; after 1951, timothy induced the larger response. The cutaneous reactions to both pollens diminished after 1953, but the most recent readings seem to show an increasing reactivity to timothy.

REAGIN TITRATIONS FOR TIMOTHY AND RAGWEED POLLENS

In Table II are listed the titers for timothy and ragweed pollens in the samples of D. G.'s serum taken at intervals during the eight years of leukocytic transfer studies. Because of variations in the recipients and in the age and nature of the test materials, these data are subject to some limitations when employed for comparative studies. Nevertheless, the major fluctuations in the reagin titrations occurring during this period in D. G. are clearly discernible in Fig. 2. For this graphic presentation, the relatively weak (+)

readings elicited on sites prepared with the 1:64 serum dilution were selected for reasons previously noted.

When D. G. was tested by the indirect method in 1932 at the age of 20 months and again at 4 years, reagins for timothy and ragweed were already

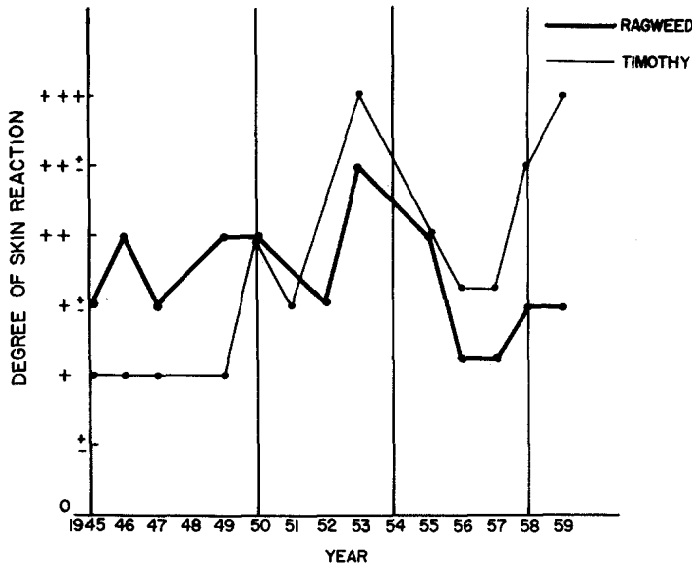


Fig. 1.—Reactions of D. G. to skin tests with pollens from 1945 to 1949. Dilution of test extract, 0.001 mg. N/ml. Amount injected, 0.01 ml.
Scale of positive reactions: + = slight; ++ = moderate; +++ = marked.

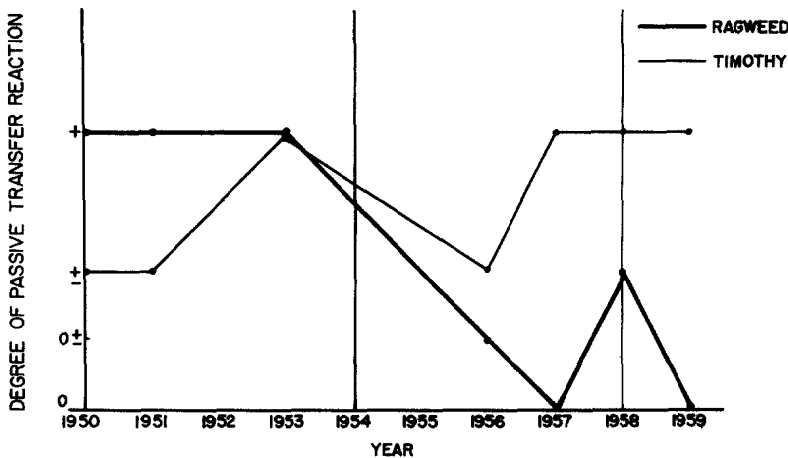


Fig. 2.—Titers of pollen reagins in D. G.'s serum from 1950 to 1959. Serum dilution, 1:64. Test extract, 0.01 mg. N/ml.
Scale of reactions: + = slight positive; ± = faint positive; 0± = doubtful positive; 0 = negative.

present in his serum, the latter in slightly greater concentration than the former. In 1950 the antibody titers for both pollens were greater than in childhood, with ragweed still the stronger of the two (Table II and Fig. 2).

In 1953, the readings with the 1:64 serum dilution were equal for the two pollens, but the 1:256 dilution tests still seemed to indicate a slight superiority for ragweed. In 1956 both reagin titers were diminishing, but at this time the timothy readings were greater than those for ragweed and they remained so thereafter. In the most recent titrations, the ragweed antibodies seem to be diminishing while those for timothy are gradually increasing.

ANNUAL ESTIMATES OF POLLEN SYMPTOMS

Estimates of the severity of the pollen symptoms in D. G. during each pollen season are listed in Table I along with his skin reactions for the same year. Because of the many specific and nonspecific factors which can influence hay fever symptoms, it proved to be extremely difficult to grade this component effectively. Fig. 3 represents a crude attempt to chart the severity of the patient's pollen symptoms from 1944 to 1958, inclusive.

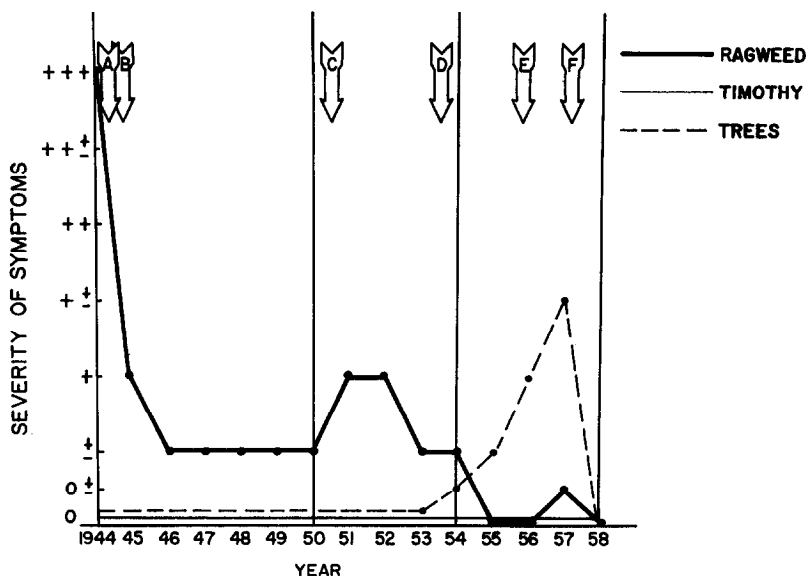


Fig. 3.—Severity of pollen symptoms in D. G. from 1944 to 1958. *A*, Preseasonal treatment with ragweed started in March, 1945. *B*, Perennial treatment with ragweed started in October, 1945. *C*, Changed summer residence in June, 1951. *D*, Moved his home to borough of Queens in New York City in April, 1954. *E*, Ragweed treatment discontinued in October, 1956. *F*, Moved his home to Commack, Long Island, in February, 1958.

Scale of symptoms: +++ = severe; ++ = moderate; + = mild; ± = faint; 0± = doubtful; 0 = none.

The ragweed pollen indices for New York City, computed annually in this laboratory from the daily ragweed pollen counts taken in this area,⁸ were reviewed to determine whether any seasons of unusually heavy pollination had occurred from 1950 to 1958 which might have unduly influenced the severity of pollen symptoms in D. G. While the leukocytic transfer studies on D. G. were in progress, these indices seemed to have remained relatively constant. Annual variation in pollination was therefore considered insign-

nificant as a factor affecting the severity of this patient's symptoms during the course of these studies.

As a result of his ragweed therapy which had started early in 1945, D. G. experienced only mild symptoms that season and in subsequent years. In 1951 he started spending his leisure time during the summer months at Freehold, New Jersey, a community with a relatively high pollen index for ragweed.⁹ For two ragweed seasons he was not quite as comfortable there as he was at his home in Brooklyn. In 1953 symptoms became mild again, even when he was at Freehold, New Jersey. In the spring of 1954 he married and changed his residence to the borough of Queens in New York City, where the pollen index was slightly higher than at his former home in Brooklyn. He nevertheless experienced only slight ragweed symptoms that fall and became practically symptom-free in 1955 and 1956. Because of this pronounced improvement, his ragweed treatment was discontinued in October, 1956. The next year very faint symptoms were occasionally noticed at the peak of the season in September, but the patient was completely free of ragweed symptoms in 1958.

The tree symptoms which D. G. began to experience in 1954 during most of May and the first week in June became progressively stronger each year (Table I and Fig. 3) until he moved away from this area in February, 1958. In the spring of 1958 he had negligible tree symptoms.

LEUKOCYTIC TRANSFER STUDIES ON D. G.

Leukocytic transfer experiments were started on D. G. in November, 1950. For several months only the suspension technique was used. After the extract method was developed, this became the preferred procedure. Throughout 1952 and 1953 the cells of D. G. were used repeatedly in a variety of studies. Then, except for an occasional trial in 1954, he was not tested again until April, 1956, when he was recalled in connection with another leukocytic transfer investigation. It was at this time that the loss of his leukocytic transfer potential for ragweed was discovered. Realizing the significance of this finding, we resumed leukocytic transfer tests with pollens.

Only part of the findings in the many studies made with D. G.'s cells are listed in Table III. Included there are the results of those experiments in which the standard techniques were employed or in which these procedures were modified in only minor detail. The experiments are listed in chronological order, with the suspension and extract techniques represented in separate sections of the table. The results obtained at each of the tested sites are recorded in Table III and are plotted in Fig. 4. As a result of the careful screening of recipients, the control reactions to pollens were almost always negative; for this reason, these readings were not listed in the tables. In the occasional instance when the control reaction was not completely negative, the reading at the transfer site was corrected accordingly. The listings in Table III, therefore, represent the final readings after all adjustments for nonspecific factors had been made.

TABLE III. RESULTS OF LEUKOCYTIC TRANSFER TESTS ON DONOR D. G. FROM 1950 TO 1959

SUSPENSION TECHNIQUE										EXTRACT TECHNIQUE										
DATE	EX- PERI- MENT NO.	RE- CIPI- ENT	TIMOTHY		EX- PERI- MENT NO.	RE- CIPI- ENT	LEUKO- CYTIC EXTRACT DILUTION	TIMOTHY		DATE	EX- PERI- MENT NO.	RE- CIPI- ENT	LEUKO- CYTIC EXTRACT DILUTION	TIMOTHY		ANTIGEN DILUTION (MG. N/ML.)	RAGWEED			
			ANTIGEN DILUTION (MG. N/ML.)	REAC- TION				ANTIGEN DILUTION (MG. N/ML.)	REACTION					SITE 1	SITE 2		ANTIGEN DILUTION (MG. N/ML.)	REACTION	SITE 1	SITE 2
11/10/50	S1A	S.C.	0.1 SP	0	S1B	G.	0.1 SP	0		4/10/51	E5A	S. W.	1:6			0.1		+		
2/13/51	S2A	G.									E5B	W.	1:6	0	0	0.2		+		
3/ 6/51	S3	G.								5/ 1/51	E7	S. W.	1:6	0	0	0.1		+		
3/20/51	S4A	G.												0	0	0.1		+		
3/24/51	S4C	G.								6/18/51	E11	W.	1:6			0.2		+		
5/23/51	S8	B.														0.1		+		
																0.1 SP		+		
																0.1 SP		0±		
										3/ 4/52	E12A	S. W.	1:6			0.1		+		
											E12B	W.	1:6			0.1		+		
										3/26/52	E15A	F.	1:6	0	0	0.1		+		
											E15B	W.				0.1		+		
										4/ 7/52	E17	G.	1:5	0	0	0.2		+		
										4/16/52	E19	S. W.	1:5			0.1		0±		
																0.1		+		
										4/21/52	E20	G.	1:5	0	0	0.1 SP		+		
																0.2 SP		0±		
										5/27/52	E28	G.	1:4			0.1		+		
																0.1		+		
																0.2		+		
																0.1 SP		+		
																0.2 SP		+		
										6/ 9/52	E29	S. W.	1:4			0.1		0±		
																0.1 SP		+		
																0.2 SP		+		

11/ 3/54 S43 K. R. 0.1 0	0.1	+	±	4/ 8/53 E35 K. R. 1:4 0.1 0	0.1	0	0.1	±
4/10/56 S52 R.	0.1	0		4/30/53 E37 K. R. 1:4 0.1 0	0.2	0	0.2	+
				10/22/53 E38 K. R. 1:4 0.1 0	0.1	0	0.1	+
							0.1 SP	+
				4/10/56 E52A R. 1:4 0.1 0±	0.1	0±	0.1	0
				E52B S. 0.1 0±	0.1	0±	0.1	0
				5/16/56 E53A K. R. 1:4 0.1 SP 0	0.1	0	0.1	0
				E53B F. U. 1:4 0.1 0±	0.1	0±	0.1	0
				5/23/56 E54A F. 1:4 0.1 0±	0.1	0±	0.1 SP	0±
					0.1 SP	0±		
				5/19/57 E55A K. R. 1:4 0.1 0±	0.1	0±	0.1	0
				E55B S. I. 0.1 0	0.1	0	0.1	0
				10/20/57 E56 K. R. 1:4 0.1 SP 0±	0.1	0±	0.1 SP	0
							0.2	0±
3/ 8/59 S57A K. R. 0.1 0±	0.1	0						
0.2 0±	0.2	0						
S57B I. 0.1 0±	0.1	0						

Sites were prepared with 0.025 ml. of leukocytic suspensions in Tyrode's solution (1:10) or with 0.05 ml. of leukocytic extract.
Sites were tested with 0.02 ml. of antigen.
Scale of reactions is described in text.
SP = Freshly prepared extract.

Averages were computed for all of the readings obtained during each calendar year with each pollen. With these data, the leukocytic transfer results with the two pollens over the eight-year period were plotted in Fig. 4. The line between the $\pm$ and the $0\pm$ zones represents the point below which it becomes exceedingly difficult to gauge accurately any fine differences in the degree of reaction. For this reason, the data below this line were considered of limited statistical value, particularly as a basis for comparative analysis.

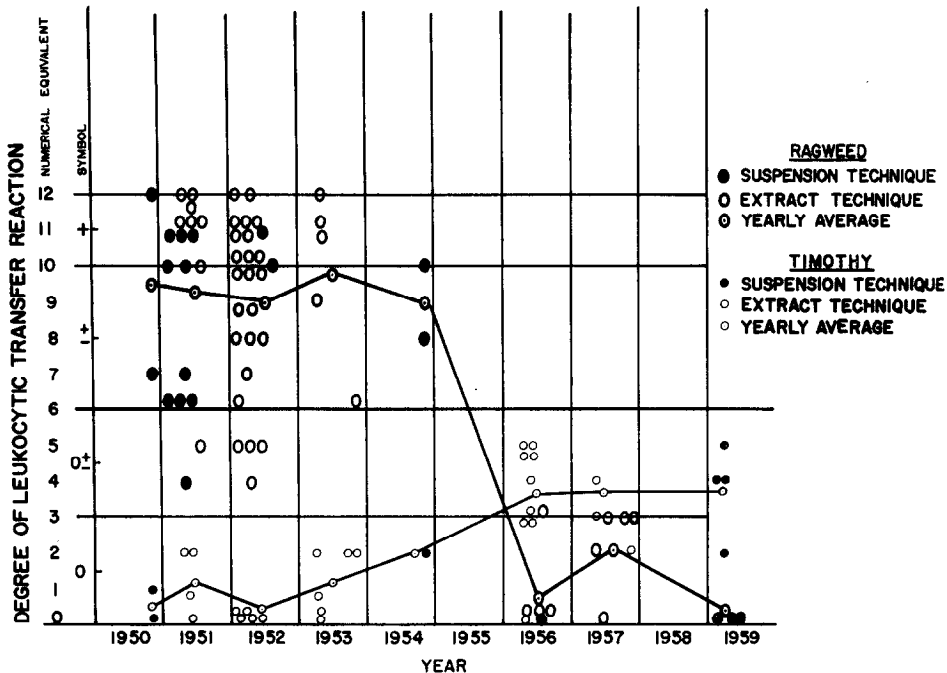


Fig. 4.—Results of leukocytic transfer tests with pollens in D. G. from November, 1950, to March, 1959. Yearly averages derived from results of all tests with both techniques. For scale of reactions, symbols, and numerical equivalents see discussion of "Reading and Grading Reactions" in text.

Results of Leukocytic Tests With Ragweed Pollen.—From November, 1950, to May, 1953, positive leukocytic transfers with ragweed pollen were obtained quite regularly with both techniques. In a single trial with the suspension technique in November, 1954, the result was still positive. When tests were resumed in April, 1956, after an interval of about seventeen months, only negative results were obtained with ragweed, although the same methods, the same technicians, and in some cases the same recipients were used. All subsequent trials with both techniques failed to produce another positive transfer to ragweed.

Results of Leukocytic Tests With Timothy Pollen.—At no time from November, 1950, to March, 1959, was a definite (+ or $\pm$) transfer obtained with timothy pollen with either technique (Table III and Fig. 4). At the beginning, the results of tests with this pollen were completely negative. In recent years, however, zero-plus-minus ($0\pm$) reactions have been obtained repeatedly. Although reactions of this size may not be considered significant, their rela-

tively recent appearance and their repeated occurrence warrant continued observation of this reaction in the future.

CORRELATION OF LEUKOCYTIC TRANSFER WITH OTHER SENSITIVITY FACTORS

The negative result of the leukocytic transfer test for ragweed in April, 1956, came as a surprise, and explanations for this loss of transfer potential were sought. Upon investigation, it became apparent that at this time D. G. was also experiencing changes in his clinical symptoms, reagin titers, and

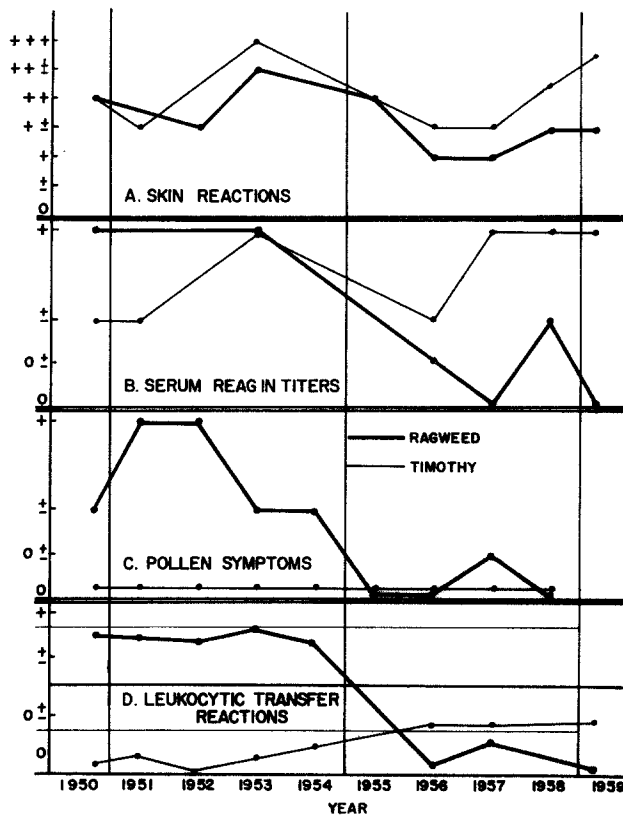


Fig. 5.—Correlation of skin reactions (A), serum reagin titers (B), severity of pollen symptoms (C), and leukocytic reactions (D), in D. G. from November, 1950, to March, 1959. (For symbols and scales see corresponding Figs. 1 to 4.)

cutaneous reactions to pollens. In view of these developments, the various data for the entire period were reviewed and the relationship of leukocytic transfer potential to other sensitivity factors was studied. The most important features of this analysis are presented herewith.

A. Relationship of Leukocytic Transfer to the Degree of Cutaneous Sensitivity.—

Ragweed: In the case of D. G., the results of the leukocytic transfer tests with ragweed might seem to bear some relationship to the intensity of the

cutaneous sensitivity to this pollen (curves *A* and *D*, Fig. 5). Positive leukocytic transfer tests with ragweed occurred only during the early years (1950 to 1954) while the skin reactions to this pollen were pronounced. Later, when the skin reactions became weaker, the transfer potential for this pollen was lost.

Timothy: The results of leukocytic transfer with timothy bore no relationship to the degree of cutaneous reactivity to this pollen. No definite transfers were obtained, despite the fact that for the major part of the eight-year period the skin reactions to timothy exceeded those to ragweed (Figs. 1 and 5, *A*).

B. Relationship of Leukocytic Transfer to Reaginic Titers.—

Ragweed: A comparison of the graphs representing the results of leukocytic transfer tests and of reaginic titrations over the eight-year period revealed a greater similarity between them than had been evident in the preceding comparison involving cutaneous sensitivity (Fig. 5, curves *B* and *D*). During the early years of this study, when the reaginic titers for ragweed were high, positive leukocytic transfers with this pollen were obtained regularly. With the decline in these titers seen in the later phases of the investigation, only negative results were obtained.

Timothy: As was the case with cutaneous sensitivity, the reaginic concentrations of the serum for timothy could not be correlated with the results of leukocytic transfer with this pollen. Despite the presence of serum antibody titers to timothy which were as high as or higher than those for ragweed during the major part of the eight-year period, a definite leukocytic transfer with timothy was never demonstrated.

C. Relationship of Leukocytic Transfer to Clinical Sensitivity.—

Ragweed: From 1950 to 1954, inclusive, the patient experienced typical symptoms of ragweed hay fever, although they were ameliorated to a considerable degree by therapy with this pollen. During these years the leukocytic transfer tests were regularly positive (Fig. 4 and Fig. 5, *C* and *D*). In 1955 and 1956 ragweed symptoms disappeared almost completely, although the pollen exposure factors remained unaltered. This clinical change was occurring in D. G. at about the time his leukocytes were losing their power to transfer sensitivity to this pollen. Ragweed symptoms were negligible in the fall of 1957 and were completely absent in 1958. The leukocytic transfer tests remained negative during this period.

Timothy: Throughout the entire eight-year period not a single definite positive leukocytic transfer was obtained with timothy, and at no time did D. G. manifest clinical symptoms of hay fever or asthma caused by this grass pollen.

The results of the leukocytic transfer test in D. G. were therefore unique in that they paralleled the clinical status of the patient with respect to both pollens. This was in obvious contrast to the findings obtained with direct and serum transfer tests in this patient. The latter tests could be correlated to a limited extent with symptomatology in the case of ragweed but not at all in the case of timothy.

THE SPECIFICITY AND NATURE OF THE LEUKOCYTIC TRANSFER REACTION

Because of the peculiar properties of the leukocytic transfer reaction, particularly its diminutiveness and variability in size at different sites on the same recipient, the need for adequate controls was imperative. Many routine and special methods of control were therefore employed.

The tests on leukocytic transfer sites were routinely duplicated on normal adjacent skin in the same manner as the control tests performed in serum passive transfer techniques.^{3(a)} Any transfer test in which the control reaction was for any reason greater than a $0\pm$ was considered to be unacceptable for consideration.

Among the special control studies on D. G. was the attempt to transfer sensitivity to ragweed by the use of his erythrocytes processed in the same manner as his leukocytes.¹ Only negative results were obtained. Leukocytes from nonsensitive donors did not transfer sensitivity to ragweed or to any other common atopen.¹ In the case of D. G., the leukocytic transfer tests were negative with allergens, such as animal danders, to which he manifested no sensitivity either clinically or by skin test.

To rule out a nonspecific hyperresponsiveness of the type occasionally seen at serum-sensitized sites,¹⁰ leukocytic transfer sites were tested with histamine. No abnormal responsiveness to this nonspecific stimulant was noted. For similar reasons, leukocytic transfer sites were repeatedly tested with physiologic and buffered saline, with negative results.

Dilution control tests¹ were performed repeatedly with the serum and plasma of D. G. to rule out the possibility that these media were contaminating the leukocytic preparation in sufficient concentration to produce positive transfer reactions. The results of these tests were uniformly negative.

The possibility that serum reagents might be absorbed or adsorbed by the leukocytes and in this way might be responsible for the positive transfer reactions was investigated in several ways. In early experiments¹ with the leukocytic suspension technique, cells obtained from various donors were washed four times instead of twice. Although this extra manipulation sometimes reduced the size of the transfer reaction, it did not eliminate it except in some instances in which the reactions were doubtful at the outset. When, at a later date, similar experiments were done with D. G. leukocytes and improved techniques were employed, washing the cells four times with the recipient's serum or with Tyrode's solution failed to affect the results of the transfer tests.²

In another study, comparisons were made of the transfer properties of D. G. cell preparations which were obtained after shaking periods of variable duration. Leukocytes which had been isolated and washed in the usual manner were shaken (without beads) in Tyrode's solution for two, four, and fifteen hours, respectively (Table IV). Following centrifugation, the supernates and sediments of each cell preparation were used for sensitization of sites on recipients. At the sites prepared with the two- and four-hour preparations, only faint evidences of sensitivity were elicited. Most of the results

consisted of 0± reactions, with only an occasional ± among them. In the two- and four-hour experiments, the average of the transfer reactions was perhaps slightly larger for the residues than for the supernates. After fifteen hours of shaking, however, the supernates yielded sites which produced definite (+) reactions to ragweed, while the results with the residues were negligible (0±).

TABLE IV. TRANSFER TESTS WITH CELL RESIDUES AND SUPERNATES AFTER VARIED PERIODS OF SHAKING

EXPERI- MENT NO.	DATE	RECIPI- ENT	DILU- TION	TEST ANTIGEN (MG. N/ML.)	RESULTS OF TRANSFER TEST WITH						
					NORMAL SUSPEN- SION	2-HOUR		4-HOUR		15-HOUR	
						SUPER- NATE	RESI- DUE	SUPER- NATE	RESI- DUE	SUPER- NATE	RESI- DUE
ES 7	5/1/51	S. W.	1:10	R	0.2	+					
				R	0.2						0±
			1:5	R	0.1					+	
				R	0.2					+	
				T	0.1					0	
				T	0.2					0	
				BS						0	
ES 8	5/23/51	G.	1:10	R	0.1		0				
				R	0.2		0±				
		B. R.	1:10	R	0.1		0±		0±		
				R	0.2		0±		0±		
				R	0.1	0±					
				R	0.2	+					
ES 10	6/13/51	S. T.	1:5	R	0.1			0			
				RSP	0.1			0±			
				RSP	0.2			±			
				TSP	0.1			0			
		G. E.	1:5	R	0.1			0±			
				RSP	0.1			±	±		
				RSP	0.2			0±	0±		
ES 11	6/18/51	W. E.	1:5	RSP	0.1					+	0±
				RSP	0.1					+	0±

R and T = Stock extracts of ragweed and timothy pollens prepared in January, 1951.

SP = Freshly prepared extracts.

BS = Buffered saline.

Sites were prepared with 0.025 ml. of cell suspensions and residues and with 0.05 ml. of supernates.

Sites were tested after seven- to ten-day intervals with 0.02 ml. of antigen.

Additional indications that the transferred substance was an integral property of the leukocyte and not merely an absorbed or adsorbed serum component were provided in another way. Studies were instituted to determine whether it was possible to passively sensitize leukocytes. Cells derived from nonsensitive donors were suspended in the serum of D. G. or in sera with high reaginic titers from other donors clinically sensitive to various types of allergens. After immersion of the cells for one or more days at icebox or room temperatures,* they were used in leukocyte transfer experiments. No definite (at least a ±) positive transfer reaction was ever obtained at sites prepared with such materials.¹²

These findings were in accord with the results of the investigations of Harris and his co-workers,¹¹ who injected antigen into the pad of the rabbit's

*The intracutaneous injection of incubated cells produced marked inflammation of the sites which precluded their use for transfer tests.

hind foot and then at intervals studied the antibody content of the components of the lymph obtained from the efferent lymphatic of the popliteal node. They found that antibody tended to pass from the lymphocytes into the surrounding lymph but not in the reverse direction. They also demonstrated that viable lymphocytes of normal rabbits, when suspended in fresh homologous lymph fluid of high antibody titer for a period of twenty-four hours at room or refrigerator temperature, did not readily absorb or adsorb antibodies from the fluid surrounding them.

An additional challenge to the concept that leukocyte contamination by serum reagins could account for the transfer phenomenon arose from the fact that the white blood cells of D. G. did not transfer sensitivity to timothy as they did to ragweed. It is significant that no such selectivity was exhibited by this donor's serum reagins in the sensitization of his skin, which responded equally well to tests with both pollens (Table I and Fig. 1). The same held true for the sensitizing power of his serum reagins in the skins of the normal recipients on whom the reagin titrations were done (Table II and Fig. 2). Hence, there is little reason to assume that these serum reagins would selectively sensitize the donor's leukocytes, particularly in the light of the aforementioned experiments in which attempts to passively sensitize human and rabbit leukocytes proved unsuccessful. The available evidence preponderantly supports the conclusion that the mediating principle in the leukocytic transfer phenomenon is a derivative of the cell itself and is not a contaminating serum antibody.

Before leaving the subject of controls, it would not be amiss to point out that, because D. G. served as a donor more than fifty times over an eight-year period and provided more than 350 leukocytic transfer sites, his tests provided unique opportunities for checking the specificity of the reactions obtained. In the early years of the study, the consistently positive leukocytic transfer with ragweed and the equally consistent negative results with timothy became such a regular pattern that any sudden deviation from it attracted attention. The fact that, after four years of positive results with ragweed, the leukocytes of D. G. lost their power to transfer this sensitivity was also significant. Since the experimental methods, materials, technicians, and even some of the recipients had remained the same, it was reasonable to attribute this transition to immunologic changes in the donor. The alterations in his skin reaction, reagin titer, and symptomatology that were developing at about the same time supported this interpretation of the findings. Under the circumstances, the consistently negative leukocytic transfer results with ragweed after 1954 served to accentuate, even more decisively than before, the significance of the positive reactions which had been obtained so regularly in the preceding years.

LEUKOCYTIC TRANSFER AND CLINICAL SENSITIVITY

The findings in the case of D. G. do not represent the first or only instance in our experience in which the clinical status of the patient was more ac-

curately reflected by his leukocytic transfer reactions than by his skin test or serum transfer reactions. The first example of this had been noted in the initial report on leukocytic transfer.¹ It concerned an 11-year-old asthmatic boy (V. P.) who exhibited, among other sensitivities, pronounced positive skin reactions and moderate serum reagin titers for ragweed pollen. He showed no symptoms of ragweed hay fever, however, and his leukocytes failed to transfer this sensitivity. Included in that same report were the results of studies on four other patients who were clinically sensitive to ragweed. Positive leukocytic transfers to this pollen were obtained in these patients, although in some cases the skin reactions and reagin titers were no greater than those of Patient V. P.

In another instance,¹² Patient B. K., a young man, showed definite skin reactions to timothy and ragweed pollens but experienced hay fever symptoms only during the grass season. His leukocytes transferred only the timothy sensitivity. In this instance, however, the patient's skin reactions for the grasses were somewhat stronger than those for ragweed. Nevertheless, the case is worthy of note in that it illustrates a positive leukocytic transfer to timothy pollen, a finding never demonstrated with the cells of D. G.

The studies on the relationship of leukocytic transfer to clinical sensitivity will be continued in the hope that they may shed additional light on the factors which determine the "clinical" and "nonclinical" significance of positive skin reactions.

THE SENSITIZING PRINCIPLE

The exact nature of the sensitizing principles involved in the leukocytic transfer experiments on D. G. is still uncertain. The substance bears obvious resemblances to the skin-sensitizing antibody, the atopic reagin. The localization of sensitivity to the injected cutaneous area and the immediate whealing response of these sites to tests with related allergens are among the typical features of reaginic passive transfer phenomena. Some of the peculiarities of the leukocytic transfer reaction, such as size limitation and variability in site response, may rest on technical considerations rather than on the properties of the sensitizing principle. It would, however, be premature to assume at this time that differences do not exist between the atopic reagin as it appears in the serum and the leukocytic transfer antibody. These questions will be discussed at greater length in a future communication.¹²

In D. G. there may have been some relationship between the height of his serum reagin titer for ragweed and the success of the leukocytic transfer tests with this pollen. Such a correlation seemed to apply in other cases as well, but it was by no means a constant finding.¹² Positive leukocytic transfers were obtained in some cases in which the serum reagin titers were comparatively low. Moreover, the failure of D. G.'s leukocytes to transfer timothy sensitivity demonstrated conclusively that serum reagin concentration was not the decisive factor in the leukocytic transfer phenomenon.

MISCELLANEOUS CONSIDERATIONS

The fact that specific treatment with pollen affects the related skin reactions and reagin titers during the first years of therapy and, to a diminishing degree, as treatment continues is well known.^{13, 14} There has been no indication, however, that the perennial ragweed therapy, which was started on D. G. in 1945 and predated his leukocytic transfer studies by five years, was influencing the results of the leukocytic transfer tests in his case. Positive leukocytic transfer reactions to various types of allergens have been demonstrated in other donors who have never received any form of hyposensitization treatment.¹²

Because of the importance of the results obtained in the studies on D. G., it may be worth while to consider briefly whether his was a typical example of atopic sensitivity to pollens. To those of us who have had the opportunity of observing this patient throughout most of his life, D. G. presented no unusual immunologic findings if one excepts those brought out in the leukocytic transfer studies. Among the more or less commonly observed features of his pollen history were the fluctuations in his cutaneous reactions to pollens, the changes in his skin reactions conforming roughly to the alterations in his serum reagin titers, the lack of conformity between the cutaneous and reagin findings on the one hand and the patient's symptomatology on the other, the presence of "clinical" and "nonclinical" positive skin reactions to pollens in the same patient, and the conversion of some positive "clinical" reactions to a "nonclinical" state (for example, ragweed) and vice versa (for example, tree pollens). The well-known inadequacies of reaginic data as a guide for the clinical management of pollinosis have again been illustrated in this case. The degree to which the leukocytic transfer results obtained in this donor will be reproducible in other forms of atopy and in other sensitivities with immediate positive skin reactions, including those which are not ordinarily associated with circulating skin-sensitizing antibodies, will be discussed in a future communication.¹²

The failure to demonstrate positive leukocytic transfer reactions in several cases of pollinosis was recently reported by Freedman, Fisher, and Cooke.¹⁵ For the preparation of the leukocytic materials and for the sensitization and testing of transfer sites, they employed techniques that were quite different from those which we have found successful.² In the reading and interpretation of the results of their tests, Freedman and his associates state: "Any reaction in which, on visible inspection, there was a wheal obviously larger than the control and in which the area of erythema extended beyond the borders of the wheal was considered to constitute a positive reaction. Any lesser reaction was regarded as inconclusive evidence of a positive transfer." Positive reactions were graded from one-plus (+) to four-plus (+++). With these criteria, it is obvious that these investigators, even with adequate techniques, would have disregarded the small but significant reactions that we obtained in our leukocytic transfer studies.^{1, 2}

SUMMARY

In an eight-year study on D. G., an atopic patient with hay fever, an attempt was made to correlate the results of leukocytic transfer tests with his skin reactions, reagin titers, and symptoms. During the early years of observation, while D. G. was having ragweed hay fever and his skin reactions and serum reagin titers for this pollen were pronounced, his reactions to leukocytic transfer tests by both the suspension and the extract techniques were regularly positive. Then as the immunologic status of the patient changed and his skin reactions, reaginic titers, and hay fever symptoms diminished, the leukocytic transfer tests for ragweed became negative. Throughout the major part of this observation period, D. G. showed skin reactions and serum reaginic titers for timothy grass pollen which were as great as or greater than those for ragweed, but his leukocytes never transferred this sensitivity and he never experienced any symptoms attributable to this pollen. Hence, the leukocytic transfer tests more accurately reflected the status of D. G. with respect to his clinical sensitivities to *both* pollens than did his cutaneous tests or reaginic titers.

The transfer principle in the leukocytic preparations bore a close resemblance to the atopic reagin. All findings pointed to the sensitizing substance as a derivative of the leukocytes rather than as a serum component absorbed or adsorbed by these cells.

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