LEUKOCYTIC TRANSFER OF THE IMMEDIATE TYPES OF ALLERGIC REACTION IN MAN

I. Techniques for the Local Passive Transfer of Atopic Hypersensitiveness Through the Use of Leukocytes and Their Derivatives

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In 1950, Walzer and Glazer[1] reported the passive local cutaneous sensitization of normal subjects by the intracutaneous injection of viable leukocytes obtained from atopic patients who manifested immediate whealing reactions to skin tests with common allergens. The method of Minor and Burnett,[2] slightly modified, was employed to separate the leukocytes from the donor's venous blood. By the addition of bovine fibrinogen, erythrocytic sedimentation was accelerated, leaving the white blood cells suspended in the plasma from which they were separated by centrifugation in Machlett tubes. The leukocytes were washed by centrifugation in changes of Tyrode's solution and were resuspended in fresh solution. Intracutaneous injections of this suspension in the arms of normal recipients provided passive transfer sites for subsequent tests with allergens.

Successful transfers of sensitivity to common allergens, such as ragweed pollen and rabbit dander, were demonstrated with the leukocytes of all but one of six allergic patients with positive skin reactions of average intensity to these allergens.[1] The immediate whealing reaction elicited by the allergen test at the passively sensitized site did not exceed a slight or a one-plus (+) positive. Nevertheless, these small reactions were elicited regularly in certain cases and were proved to be specific. The erythrocytes of the donor lacked the skin-sensitizing properties of his leukocytes.

The paramount difficulty encountered with this technique was the inflammatory reaction which developed in the recipient at the site of the sensitizing injection. This consisted of a central edematous area with superimposed and surrounding erythema. The inflammation was usually late in starting and developed slowly. It did not pass its peak until the fourth or fifth day, so that testing was rarely possible before the sixth to the eleventh day. Even at this time, traces of edema occasionally were still palpable, a circumstance which reduced the responsiveness of the site to the test antigen and made the reading and evaluation of the results more difficult. Not infrequently, the persistence of inflammation precluded any testing of the site.

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Among other difficulties encountered with the leukocytic suspension technique was the fact that the amount of inflammation which developed at the sensitized site varied with the recipient. In addition, attempts to enhance the sensitivity of passive transfer sites by concentrating the leukocytic suspension or by injecting larger amounts of it led to even greater local inflammation.

In the effort to circumvent some of these difficulties, many modifications of the suspension technique were tried. Early in the course of these studies (1951), it became apparent that viable leukocytes were not essential for a successful transfer. Positive results could be obtained with fragmented cells and even with extracts of crushed white blood cells. This finding became more significant when it was noted that the intracutaneous injection of such extracts produced little or no inflammatory reaction. This led to the development of a leukocytic extract technique for passive transfer testing which proved to be quite effective.

With the extract technique, the results previously obtained with the suspension method were confirmed. In many respects, the extract technique was found to present definite advantages over the suspension method, but it also proved to be subject to certain limitations. It could not, for example, replace the suspension technique in studies which required the transfer of normal, functioning leukocytes. It therefore became necessary to develop both techniques simultaneously. The significant findings uncovered during the course of these studies will be presented in this and subsequent articles.

No attempt will be made at this time to review the studies on the cellular transfer of the delayed types of allergic reactions which were originally reported by Landsteiner and Chase[7] and subsequently developed by many others. The immunologic mechanisms involved in the immediate and delayed types of allergic reaction are quite different. The same may be said of the diagnostic procedures which are used for the detection and differentiation of these two groups of hypersensitiveness and for the experimental techniques employed for their passive transfer. Discussions on passive transfer studies in delayed hypersensitiveness will therefore be deferred and will be dealt with in a future report.

MATERIALS AND METHODS

The Preparation of the Sensitizing Leukocytic Suspensions and Extracts.—Under sterile precautions which were maintained throughout the procedures, from 40 to 60 ml. of venous blood were drawn from the patient into tubes containing heparin in the proportion of 0.4 mg. for each 10 ml. of blood. After mixing gently by twice inverting the tubes, 1 ml. of a freshly prepared Seitz-filtered solution containing 40 mg. of bovine fibrinogen (Fraction I, Armour) was added for each 10 ml. of heparinized blood. The tubes were again inverted twice and the blood was transferred in 10 ml. aliquots to conical centrifuge tubes. Standing at an angle of 60 degrees, these were incubated at 37° C. for about one hour. The supernates were then transferred by capillary

pipette to Machlett capillary-tip tubes and centrifuged at 3,000 r.p.m. for approximately one hour. The supernate was removed and the sedimented leukocytes were resuspended in either 2 ml. of Tyrode's solution or 2 ml. of the recipient's freshly prepared blood serum. This suspension was centrifuged for thirty minutes at gradually increasing speeds up to 3,000 r.p.m. The washing procedure was then repeated with fresh Tyrode's solution or with serum. The sedimented leukocytes obtained at this point were ready for use in either the suspension or the extract technique.

For the *suspension technique,* one part cell sediment was distributed as evenly as possible in nine parts Tyrode's solution. This 1:10 suspension was then drawn up into a sterile syringe and used for the preparation of passive transfer sites on the arms of a recipient.

For the leukocytic *extraction technique,* one part cell sediment and three parts Tyrode's solution were introduced under sterile precautions into a small flat-bottomed vial. Three large sterile beads were added. The vial was sealed with a rubber stopper and was shaken overnight, usually for about sixteen hours, in a rotary machine at a speed of 300 r.p.m. The material in the vial was then transferred to a centrifuge tube and spun at 4,000 r.p.m. for twenty minutes. The clear supernate was drawn up into a sterile syringe and used for the preparation of passive transfer sites on the recipient.

An average yield of 0.028 ml. of sedimented cells was obtained in the earlier studies from each 10 ml. of donor's blood. In later studies, the more effective elimination of erythrocytes reduced the yield to approximately 0.02 ml. from each 10 ml. of blood. More than 85 per cent of the cells in this sediment were viable, as determined by their failure to take a trypan blue stain.

Variations in technique: In the attempt to achieve better transfers, many modifications of the techniques were tried. For washing the leukocytes, human nonreaginic serum was substituted for Tyrode's solution. The serum was most frequently obtained from the recipient on the day of the experiment. When two recipients participated in the same experiment, the serum of one was used for both. Human serum proved to be advantageous for the washing of the leukocytes only in those experiments in which the preservation of the cells was an important consideration. Otherwise, it produced results no more satisfactory than those obtained with Tyrode's solution. Washing the cells in Tyrode's solution containing 0.13 per cent gelatin provided leukocytes in a better state of preservation but did not improve the results of passive tranfer.

As a menstruum for the sensitizing leukocytic suspension, human serum proved inferior to Tyrode's solution. Serum tended to increase the hyporesponsiveness of the sensitized sites.[3] Moreover, its occasional use in recipients other than the one who provided the serum increased the possibility of introducing unrecognized immunologic factors at the sensitized sites.

Washing the cells by slow centrifugation four times instead of twice slightly reduced the size of the transfer reaction in some instances,[1] but not in others.

In the studies on the influence of time and temperature factors in the techniques, leukocytes and their derivatives were stored for twenty-four, forty-eight, and occasionally for seventy-two hours in the refrigerator with little perceptible effect on their sensitizing properties. Similarly, standing at room temperature for periods up to twenty-four hours did not noticeably influence the sensitizing properties of sterile leukocytic preparations. On the other hand, leukocytic suspensions stored in the incubator for more than a few hours produced marked delayed inflammatory reactions which rendered the passively sensitized sites unfit for testing.

The fact that the sensitizing principles of the leukocytic preparations were not affected by refrigerator or room temperatures during the first twenty-four-hour period eliminated the need for haste in the extraction of white blood cells. This proved fortunate, since the procedures which are commonly recommended for rapid fragmentation of leukocytes were not found to be applicable in these studies. For example, the triple alternate freezing (−70° C. in a dry ice and alcohol bath) and thawing (37° C.) of the leukocytes yielded, on centrifugation, a slightly hemorrhagic supernate which caused considerable inflammation and discoloration of the injected sites in the recipient. Other common methods of demolishing the leukocytes also proved impractical in these studies because of the small amounts of cells which were being employed.

Extraction of the white blood cells by shaking could be completed in less than sixteen hours. At times, it was possible to obtain positive results after only eight or twelve hours of shaking, even though the cells had not yet been completely demolished. Nevertheless, an overnight shaking period of sixteen hours proved both convenient and effective, since it was impossible to complete both the procurement and extraction of the leukocytes in a single working day. The extended shaking period not only assured complete demolition of the cells, but also permitted the performance of the active phases of the procedure during routine laboratory working hours.

*Passive Local Sensitization of the Recipients.**—

1. *Choice of recipients:* These were carefully selected nonatopic young adults with normal skins who, on preliminary testing, had shown completely negative reactions to concentrated extracts of the allergens employed in these studies as well as to a representative series of common allergenic offenders. Some recipients with particularly suitable skins were used repeatedly at appropriate intervals. This proved helpful in studying the effects of modifications in the technical procedures. It is noteworthy that the repeated injections of viable cells from the same or different sensitive donors produced no evidence of the development of generalized cutaneous sensitivity in the recipient.

2. *The preparation of cutaneous sites on the recipient:* In the earliest studies,[1] passive transfer sites were prepared by the intracutaneous injection of 0.05 ml. of a 1:13 suspension of leukocytes. Many of these sites developed

*For detailed description and discussion of the techniques employed in routine (indirect testing) and special passive transfer procedures, the reader is referred to previous discussions on this subject by Walzer and Bowman.[4]

marked inflammation and were unfit for testing. This difficulty was partially overcome by reducing the volume of the sensitizing injection to 0.025 ml. and increasing the concentration of the leukocytic suspension from 1:13 to 1:10. With this smaller sensitizing dose, the sites recovered their normal appearance and responsiveness much more rapidly.

Because the leukocytic extract failed to produce the pronounced inflammatory response observed with the cell suspension, the injection of 0.05 ml. of extract into each site proved feasible. Sites were sensitized on the outer aspects of the recipient's arms, not more than four sites being prepared on each arm. The marking of the sites and instructions to the recipient concerning the preservation of the markings and the avoidance of particular allergenic contacts were the same as those employed in routine passive transfer procedures.[4]

The Testing of the Sites.—

1. *Interval:* The inflammatory reactions which developed at the sites of injection of leukocytic suspensions prevented skin testing before the sixth day, and frequently further postponement was necessary. If the sites failed to regain their normal appearance by the fourteenth day, no testing was done. Because the inflammatory factor was a minor consideration with the extract technique, sites prepared in this manner were usually suitable for testing within a few days. However, in keeping with the principles recommended for all passive transfer procedures,[4] testing was rarely performed before the fifth day. This interval allowed the sensitized sites to recover most of the responsiveness which had been impaired by the trauma of the sensitizing injection.[3]

2. *Allergens:* The allergens employed for testing were those prepared in the Allergy Laboratory of the Jewish Hospital of Brooklyn for routine use in the Allergy Clinic.[5] They were standardized on the basis of their total nitrogen content. The ragweed pollen extracts were made annually in January from mixtures of giant and dwarf ragweed. Because of the tendency for these extracts to lose some of their potency with aging, fresh pollen extracts (labeled SP in the tables) were often prepared and used instead of, or in addition to, the stock extracts. These special (SP) ragweed extracts were usually prepared from dwarf ragweed pollen only.

3. *Testing:* About 0.02 ml. of allergenic extract was used for testing a passive transfer site. The allergen concentration was the highest which could be employed without producing a nonspecific positive reaction on the *normal* skin of the recipient. This concentration naturally varied with the allergen and the nature of the recipient's skin. With pollens, a solution containing 0.1 mg. nitrogen per milliliter proved to be suitable for most recipients. Occasionally tests were made with extracts containing 0.05 or 0.2 mg. of nitrogen per milliliter but the results obtained with these concentrations did not differ materially from those produced by the 0.1 mg. tests.

4. *Controls:* A control test, an exact duplicate of that made on the sensitized site, was made on the adjacent normal skin of the recipient. If enough

suitable transfer sites were available, one site was usually tested with diluting fluid and another with a suitable concentration of an unrelated antigen, that is, a member of a biologic group different from the one under consideration and one to which the donor failed to react either clinically or by skin test. In each instance, the tests were duplicated on adjacent normal skin.

In some of the earlier experiments, additional control tests were made on transfer sites prepared with leukocytic preparations obtained from nonsensitive donors. In other studies, sites prepared with erythrocytes from sensitive donors were tested. Since these special types of control tests uniformly yielded negative results, they were eventually discontinued.

The Reactions at the Transfer Sites.—The positive reactions elicited by tests with allergens at leukocytic transfer sites have always been small and have rarely exceeded the equivalent of a slight or one-plus reaction. With the extract technique, these reactions usually reached their maximum in about fifteen to twenty minutes and closely resembled those elicited in routine passive transfer testing. They consisted primarily of a central edema or wheal and variable amounts of erythema. Because of the small size of the reactions, the erythema was frequently faint and limited to the central edematous area. In the larger reactions, a secondary areola of solid or broken erythema was usually present. With the suspension technique, the reactions tended to develop somewhat more slowly and to show smaller areolas than those elicited with the extract technique. Pruritus infrequently accompanied positive reactions and, when present, was always minimal.

The Reading and Recording of Reactions.—Because slight inflammation frequently persisted at the sensitized sites, especially at those prepared with leukocytic suspensions, readings were routinely made on the transfer sites by both inspection and palpation *before* the allergens were introduced. Readings were taken again about twenty minutes after testing at both test and control sites. The amounts of edema and erythema were read and recorded separately. Pruritus was also noted when it occurred. Because edema tends to be absorbed more slowly in specific reactions than in nonspecific cutaneous responses, the reactions to the tests were frequently studied for periods up to an hour.

Grading of Reactions.—Because the small reactions obtained with the leukocytic transfer technique were more significant than similar responses in other passive transfer techniques, it became necessary to devise a new and more precise scale for the grading of weak positive reactions. For this purpose, reactions between the completely negative (0) response and the definite slight positive (+) response were divided into two grades, negative plus-minus (0±) and plus-minus positive (±). A description of the various grades of reactions, their symbols, readings, and significance, appear in Table I.

The slight positive or one-plus (+) reaction consists of a central wheal about 0.5 cm. in diameter with a surrounding erythema and is only occasionally accompanied by a faint pruritus. Reactions slightly less than this were

called plus-minus (±) positives. Erythema was not regularly present in this grade of reaction, and pruritus was rare. When the presence of edema was barely perceptible, the reaction was considered to be negative plus-minus (0±). A surrounding erythema was usually absent in this grade of reaction. When no change in edema or erythema was observed following the test, the reaction was recorded as negative (0).

TABLE I. THE GRADING OF IMMEDIATE PASSIVE TRANSFER REACTIONS OBTAINED WITH THE LEUKOCYTIC TECHNIQUES

READING	SYMBOL	DESCRIPTION	INTERPRETATION
Negative	0	Site unchanged	Negative
Negative—plus-minus	0±	Barely perceptible edema	Too weak to be regarded as significant
Plus-minus positive	±	Definite but small amount of edema, often with faint erythema on or around it	A weak positive reaction which is significant if it is adequately controlled and can be confirmed on repetition of the test on another site
Slight positive	+	Wheal at least 0.5 cm. in diameter with definite areola	A definite and significant positive transfer reaction

Evaluation of Results.—The evaluation of results of the passive transfer tests was based upon the comparison of the reactions at the sensitized sites with those elicited by the various control tests. Edema or wheal formation represented the primary basis of comparison. Erythema and pruritus, when present, were recognized as additional but secondary and less reliable criteria of positive reactions.

The evaluation of results with the extract technique differed very little from the procedure employed in routine passive transfer tests[4] and presented no unusual difficulties. With the suspension technique, however, evaluation was more complicated. The reading at a sensitized site was accepted without correction only when the site was completely normal at the time of testing and all the controls were completely negative. Otherwise the amount of site edema which preceded testing or which developed at a control site had to be subtracted from the reading on the sensitized site. All readings in the text and tables represent the final results after all corrections have been made.

Interpretation of Results.—In the interpretation of results, the slight positive (+) reaction was considered to be definitely significant. A plus-minus positive (±) was deemed to be somewhat less reliable than the slight positive (+), but was considered to be valid when it could be confirmed by repetition on fresh sites and when its controls were adequate. Since the negative plus-minus (0±) reaction might well be the result of technical variation, it was not considered to be significant in itself and was usually grouped with the negative reactions.

PASSIVE TRANSFER EXPERIMENTS WITH LEUKOCYTES FROM DONOR D. G.

Most of the donors studied were atopic patients with immediate positive whealing reactions to tests with common allergens. Not all these reactions

were clinically significant. Atopic reagins in low or average titer were present in these patients' sera. Some cases illustrating nonatopic forms of hypersensitiveness in which immediate whealing reactions occur were also tested. These studies will be the subject of a subsequent report.

In studying the effects of variations in technique, we found that it was advantageous to use the same donor and recipient repeatedly. For this reason, D. G., an atopic young male adult who, since infancy had been under the observation of one of the authors, was selected to provide the leukocytes for many of these studies. Only a few relevant details of his history are presented here, since a complete report of this case will appear in another communication.

Clinical History of D. G.—This 24-year-old man has a strong family history of atopy. As an infant, he had eczema for several months. Since then he has at various times manifested symptoms of allergic rhinitis, asthma, urticaria, and alimentary disturbances. Skin testing by the indirect method in childhood and by the direct intracutaneous technique in subsequent years revealed many positive reactions to foods and inhalants. In recent years, hay fever has been the predominating allergic symptom and practically all the leukocytic transfer studies on this patient were done in relation to his pollen allergy.

D. G. developed ragweed hay fever symptoms at the age of 12 (1944). Preseasonal treatment with ragweed was started and continued on a perennial basis, doses being given at monthly intervals. For many years the skin reaction to timothy pollen was strongly positive, but no clinical sensitivity to grass pollens developed. At the age of 21 (1953), the patient's serum showed moderate reaginic titers to both pollens by the method of Coca and Grove.[6] Serum dilution 1:64 gave a one-plus (+) transfer reaction to tests with ragweed and timothy pollen extracts containing 0.01 mg. nitrogen per milliliter. With serum dilution 1:256 and the same test extracts, plus-minus (±) reactions were obtained to both pollens, although the response to timothy was slightly weaker than that to ragweed.

Passive Transfer Studies With Suspensions of Leukocytes of Donor D. G.—From November, 1950, to April, 1951, the successful transfer of ragweed sensitivity was repeatedly demonstrated with suspensions of leukocytes of donor D. G. Some representative examples of the results obtained in those studies are listed in Table II. In most experiments, the leukocytes were washed and suspended in Tyrode's solution. The sensitizing injection, in the early trials, consisted of 0.05 ml. of a 1:10 leukocytic suspension but in later studies the sensitizing dose was reduced to 0.025 ml. for reasons previously noted. (See Materials and Methods.) Several recipients were used, most of them repeatedly (Tables II, III, and IV). The interval between the preparation and testing of sites usually lasted from six to eleven days. Stock or freshly prepared (SP) timothy and ragweed pollen extracts containing 0.1 mg. nitrogen per milliliter were used for testing.

1. *Passive transfer tests with ragweed pollen extracts:* With the leukocytic suspension technique, positive passive transfer reactions to ragweed

pollen extracts were obtained fairly regularly with the cells of D. G., although the degree of reactivity varied with the recipient. Occasionally there was some variation in response at different sites on the same recipient, which could be attributed to the difficulty in obtaining a uniform suspension of the leukocytes. The largest transfers to ragweed were one-plus (+) reactions, but weaker positives (±) were quite common. Negative (0) or negligible (0±) reactions to ragweed were infrequent with this donor's cells when the standard technique and suitable recipients were employed.

TABLE II. PASSIVE TRANSFER EXPERIMENTS WITH LEUKOCYTIC SUSPENSIONS OF DONOR D. G.

EXPERIMENT NO.	DATE OF BLEEDING AND SENSITIZATION	SENSITIZATION OF SITE		INTERVAL (DAYS)	TESTING OF SITE		INTERPRETATION OF RESULTS
		RECIPIENT	AMOUNT INJECTED (ML.)		ALLERGEN	REACTION OBTAINED‡	
S 1a	11/ 9/50	SC	0.05	11	Ragweed SP*	+	Positive
					Timothy SP	0	Negative
S 1b		G	0.05	7	Ragweed 50†	±	Weak positive
					Ragweed SP	+	Positive
					Timothy SP	0±	Negative
					Diluting fluid	0±	Negative
S 2a	2/13/51	G	0.05	6	Ragweed 51	+	Positive
		G				±	Weak positive
						+	Positive
S 2b		G	0.05	5	Ragweed 51	±	Weak positive
S 3	3/ 6/51	G	0.025	7	Ragweed 51	+	Positive
			0.05			±	Weak positive
S 4a	3/20/51		0.025	10	Ragweed 51	±	Weak positive
S 4c	3/24/51	G	0.025	6	Ragweed 51	+	Positive

*SP = Recently prepared extract.

†Ragweed 50 = Stock extract of ragweed pollen prepared in January, 1950.

‡Corrected readings:

Sites tested intracutaneously with 0.02 ml. of pollen extract containing 0.1 mg. introgen per milliliter.

Leukocytes washed with Tyrode's solution.

Sensitizing suspensions made of one part leukocytic sediment and nine parts Tyrode's solution.

2. *Passive transfer tests with timothy pollen extracts:* Although the reaginic titers for timothy and ragweed pollens were virtually equal in the serum of D. G., positive passive transfers to timothy were never elicited with his leukocytes by any technique. As the studies progressed, it became apparent that this failure did not rest on any inadequacies of method, but on some immunologic consideration. The fact that D. G. had no clinical symptoms of timothy hay fever, despite his strongly positive skin reaction and high reaginic titer for this pollen, prompted a more intensive investigation into the relationship between leukocytic transfer and clinical symptoms. These studies will be described in detail in a subsequent communication.

Passive Transfer Studies With Extracts of Leukocytes of Donor D. G.— When the passive transfer of positive skin reactions by means of extracts of leukocytes was successfully accomplished in April, 1951, studies on donor D. G. were continued with emphasis on this new technique. Representative

examples of the results obtained with the extract technique in this case are presented in Table III. Tyrode's solution or the serum of the recipient was used for the washing and extraction of leukocytes. In the earlier trials, one part cell sediment was shaken in five parts diluent (recorded as a 1:6 sensitizing extract in the tables). In later studies, a more concentrated extract (1:4) was successfully employed. Of this, 0.05 ml. was injected at each cutaneous site. The interval between site preparation and testing was usually from five to nine days.

TABLE III. PASSIVE TRANSFER EXPERIMENTS WITH LEUKOCYTIC EXTRACTS OF DONOR D. G.

EXPERIMENT NO.	DATE OF BLEEDING	PREPARATION OF LEUKOCYTIC EXTRACT: CELLS WASHED AND EXTRACTED WITH*	PREPARATION OF LEUKOCYTIC EXTRACT: CELL EXTRACT†	SENSITIZATION OF SITE: DATE	SENSITIZATION OF SITE: RECIPIENT	INTERVAL (DAYS)	TESTING OF SITE: ALLERGEN (MG. N/ML.)	TESTING OF SITE: REACTION OBTAINED	INTERPRETATION OF RESULTS
E 5a	4/10/51	T	1:6	4/11/51	S	7	Ragweed 51 0.1	+	Positive
E 5b				4/11/51	W	9	Ragweed 51 0.1	+	Positive
							Timothy 51 0.1	0±	Negative
							Peanut 0.05	0	Negative
E 15a	3/26/52	S	1:6	3/27/52	F	5	Ragweed 52 0.1	+	Positive
								±	Weak positive
							Timothy 52 0.1	0	Negative
E 15b				3/27/52	W	8	Ragweed 52 0.1	+	Positive
E 17	4/ 7/52	S	1:6	4/ 8/52	G	7	Ragweed 52 0.1	+	Positive
							Ragweed SP 0.1	+	Positive
							Timothy 52 0.1	0	Negative
E 19	4/16/52	S	1:6	4/17/52	S	7	Ragweed SP 0.1	+	Positive
							Ragweed 52 0.1	±	Weak positive
							Timothy 52 0.1	0	Negative
E 20	4/21/52	S	1:6	4/22/52	G	6	Ragweed 52 0.1	±	Weak positive
							Ragweed SP 0.1	+	Positive
							Timothy 52 0.1	0	Negative
E 28	5/27/52	S	1:4	5/28/52	G	8	Ragweed SP 0.1	+	Positive
							Ragweed 52 0.1	±	Weak positive
E 35	4/ 8/53	T	1:4	4/ 9/53	K	8	Ragweed 53 0.1	+	Positive
							Timothy 53 0.1	0	Negative
E 37	4/29/53	T	1:4	4/30/53	K	7	Ragweed 53 0.1	+	Positive
							Timothy 53 0.1	0	Negative

*T = Tyrode's solution; S = serum of recipient; SP = recently prepared extract.

†Sensitizing extract prepared by shaking one part cell sediment in three or five parts diluent to form approximately a 1:4 or 1:6 extract. Average shaking time, sixteen hours. Amount injected into each site, 0.05 ml.

As with the cell suspension technique, positive transfer reactions to ragweed were regularly obtained with the extract method. These reactions varied from + to ± in intensity, with less tendency to disparity than had been

TABLE IV. COMPARATIVE PASSIVE TRANSFER TESTS WITH THE TWO TECHNIQUES USING IDENTICAL BLOOD SAMPLES OF DONOR D. G.

EXPERIMENT NO.	DATE OF BLEEDING	CELL PREPARATION			SENSITIZATION OF SITES		INTERVAL (DAYS)	TESTING OF SITE		INTERPRETATION OF RESULTS
		CELLS WASHED WITH*	SUSPENSION (DILUENT AND DILUTION)	EXTRACT (DILUENT AND DILUTION)†	DATE	AMOUNT INJECTED (ML.)		ALLERGEN (MG. N/ML.)	REACTION OBTAINED	
S 7	5/1/51	T	T 1:10		5/ 2/51	0.025	7	R51 0.2	+	Positive
E 7	5/1/51			T 1:6	5/ 2/51	0.05	7	R51 0.2	+	Positive
S 29	6/9/52	S	S 1:10		6/ 9/52	0.025	11	R52 0.1	+	Positive
								RSP 0.1	+	Positive
		S	S 1:10					RSP 0.1	+	Positive
		S	S 1:10					RSP 0.2	±	Weak positive
E 29	6/9/52	S		S 1:4	6/10/52	0.05	9	R52 0.1	±	Weak positive
								RSP 0.1	+	Positive
								RSP 0.2	+	Positive

*T = Tyrode's solution; S = serum of recipient; SP = recently prepared extract; R = ragweed pollen extract.
†One part cell sediment shaken in three or five parts diluent to form approximately a 1:4 or 1:6 extract.
All tests performed on one recipient, S. W.

observed with the suspension technique. As had been the case in studies using the suspension technique, passive transfer reactions with timothy pollen were also consistently negative when the extract technique was employed.

Comparison of Results Obtained With Both Techniques Using a Single Blood Sample.—In the experiments recorded in Tables II and III, the blood samples of D. G. were taken on different days and processed by either the suspension or extract technique with frequent variations in both procedures. In only a few instances was it feasible to run both techniques simultaneously on aliquots of the same blood sample. The results of such comparative studies on cells of D. G. are presented in Table IV. These comparisons revealed no distinct superiority of either technique in demonstrating the transfer of ragweed sensitivity in the case of D. G.

A COMPARISON OF THE TWO TECHNIQUES

Comparative tests on specimens obtained from other donors, which are being reported elsewhere, tended to confirm the impressions gained from the

TABLE V. A COMPARISON OF THE LEUKOCYTIC SUSPENSION AND EXTRACT TECHNIQUES

	SUSPENSION TECHNIQUE	EXTRACT TECHNIQUE
1. The nature of the transferred material	A suspension of viable leukocytes	The extracted products of shattered leukocytes
2. Time required for processing transferred material	Approximately 8 hours	At least 24 hours
3. The concentration of the transferred material	1 part leukocytic sediment suspended in 9 parts of diluent (1:10)	One part leukocytic sediment shaken in 3 parts diluent (1:4)
4. The amount injected at each cutaneous site	0.025 ml.	0.05 ml.
5. The amount of inflammation developing at the site of injection	Varies from little to much	Varies from none to little
6. The variability in site inflammation:		
(a) in different recipients	Considerable	Slight
(b) at different sites on same recipient	Slight	Negligible
7. The average interval between preparation and testing of sites	From 6 to 11 days	From 5 to 9 days
8. The reading and interpretation of test results	Rendered difficult by the preceding site inflammation	Presents no unusual problem
9. The maximal passive transfer reaction obtained	One plus (+)	One plus (+)

studies on D. G. that the two methods seemed equally effective for the localized transfer of the atopic type of sensitivity. As the work progressed, various features developed in connection with both techniques which served to limit their usefulness in certain types of experiments. The most important of these considerations are summarized briefly and tabulated in Table V.

From a comparative analysis of the two techniques, it becomes obvious that the suspension method is irreplaceable where the studies require the transfer of viable cells. Moreover, the preparation of sensitizing materials in the suspension method is a shorter procedure. In most other respects, however, the extract technique seems to have more advantages. Its greatest asset is that it provides a passive transfer test site which, from its inception, usually passes through no pronounced inflammatory phase and at the time of testing tends to be relatively normal in its appearance and responsiveness. These site qualities are especially important in a passive transfer procedure in which the maximal reaction obtained does not exceed a one-plus (+) positive. The fact that sites prepared with the extract technique can be tested and read in practically the same manner as are serum-sensitized sites in routine indirect testing[4] will unquestionably appeal to the technician or clinician who is already well versed in passive transfer procedures.

It is advisable, nevertheless, to qualify these early impressions on the comparative merits of the two procedures by the consideration that so many of the studies which prompted them were made on the cells from a single donor. Tests with the cells of other atopic donors have tended to support these preliminary impressions. However, the total number of patients studied, the diversity of atopic manifestations, and the types of allergens investigated are still relatively limited. Moreover, the allergic mechanisms which are characterized by immediate positive skin reactions and may be regularly transferred by means of leukocytes are still to be defined. Furthermore, the evidence is by no means complete that the leukocytic extract will transfer *every* sensitizing principle which may be carried by viable leukocytes. For these reasons, a much larger and more diversified series of comparative studies will be needed before final conclusions can be reached as to the comparative values of the two techniques reported here.

SUMMARY

The local passive transfer of the atopic wheal type of skin reaction, which had previously been effected with suspensions of viable leukocytes, has now been accomplished by the use of extracts of fragmented white blood cells. The suspension and extraction techniques have been described in detail. They have thus far been found to be equally successful for passive transfer except in those experiments in which the preservation of cell viability is a prerequisite, in which case the suspension technique is obligatory. The relative merits and disadvantages of both procedures have been reviewed. The absence of a pronounced inflammatory response at passive transfer sites prepared with leukocytic extracts minimizes most of the difficulties encountered in the testing and reading of sites prepared with leukocytic suspensions.

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