

The allergens responsible for house dust allergy

I. Comparison of *Dermatophagoides pteronyssinus* and house dust extracts by assay of histamine release from allergic human leukocytes

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*The isolated leukocytes donated by 20 highly dust-allergic individuals living in the Baltimore area showed widely different relative sensitivities to dialyzed extracts of the mite *Dermatophagoides pteronyssinus* (plus growth medium) and a commercial U. S. house dust sample ("U. S. Dust #1"). Most donors, using allergen concentrations determined on a weight basis, were more sensitive to the dust than the mite. There was no significant relationship between sensitivity to dust and mite. Further leukocyte assays with the use of several different dust extracts showed that while there were allergenic differences between extracts, "U. S. Dust #1" was usually the most potent. Immunodiffusion analysis using hyperimmune rabbit anti-mite sera showed that the main antigen shared by the mite and dust extracts was a component of human dander used as part of the mite growth medium. No mite-specific antigens were detectable in the dust extract by this analysis. The anti-mite sera showed little or no ability to block mite-mediated or dust-mediated leukocyte histamine release. For only one donor was blocking of both allergens attained. We conclude that mite components represent only some of the many dust allergens, and that the most potent mite antigens for rabbits are not the major allergens for man.*

Mites of the genus *Dermatophagoides* have been known to be associated with human disease since 1864 when the Russian, Bogdanoff,¹ obtained and identified mites from human subjects afflicted with acariasis. Subsequently, other authors²⁻⁴ confirmed the association of *Dermatophagoides* with human acariasis as well as with scalp dermatitis. Since several species of *Dermatophagoides* have long been known to thrive on human foods and in human habitations, it is not surprising that *Dermatophagoides*-derived antigens should eventually be recognized as causative agents in human allergy and, in particular, as components of the highly complex mixture of antigens responsible for house dust allergy.⁵

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Following extensive investigations, the Dutch workers Voorhorst, Spieksma, and their associates concluded that members of the species of mite *D. pteronyssinus* secrete or excrete an allergen which is identical with the house dust allergen, and that other components of dust were of minor importance as allergens. The results of these and other workers⁵⁻⁹ have further suggested that Dermatophagoides-derived allergens (predominantly from *D. pteronyssinus* and *D. farinae*) are the major allergens in house dust in many parts of the world.

The evidence implicating mite components as allergens in house dust has relied heavily on establishing that: (1) Species of the genus Dermatophagoides are widely found in different house dust samples throughout the world. (2) The relative allergenic activities of dust and mite extracts, measured by skin and bronchoprovocation tests, correlate well in a large number of dust-allergic individuals. (3) The activity of a particular dust extract correlates with the proportion of mites present in the dust sample prior to extraction.

While recognizing that mite allergens are certainly important in dust allergy in many people, we were not completely convinced by the available evidence asserting that Dermatophagoides secrete or excrete antigen(s) which are by far the major allergens in house dust throughout the world. Therefore, we examined the possible allergenic association between dust and mite extracts more quantitatively by histamine release from allergic human leukocytes.

MATERIALS AND METHODS

Selection of subjects

A total of 20 highly symptomatically dust-allergic people (8 men and 12 women, between the ages of 20 and 61 years) were selected for the main part of the study. An additional 10 people, who were either clinically nonallergic or allergic to grass and ragweed allergens, served as negative controls. Several other people reporting mild sensitivity to dust were also studied. The 20 highly dust-allergic subjects were a racially heterogeneous group from widely differing economic surroundings. About half had been residents of the Baltimore area for most of their lives. The others had lived in other parts of the United States and Europe. Nearly all the subjects were highly symptomatically allergic to common inhalant allergens in addition to dust, and over half had bronchial asthma superimposed over seasonal and/or perennial rhinitis (Fig. 1). About a third of the subjects had received limited immunotherapy with dust extracts at some time during their lives.

Allergenic extracts

The main part of the study was performed with the use of one batch of Hollister-Stier's Acetone Precipitated (AP) Dust Extract and a sample of the mites *D. pteronyssinus* kindly supplied by Dr. F. T. M. Spieksma, University Hospital, Leiden, Holland. These materials will be referred to as "U. S. Dust #1" and "Dutch Mite" to conform with the nomenclature to be used in a subsequent paper.¹⁰ A limited amount of data was also collected with the use of randomly selected lots of house dust extract from three other U. S. commercial sources ("U. S. Dust A," "U. S. Dust B," and "U. S. Dust C") and a "Dutch Dust" extract given to us by Dr. R. Voorhorst, Leiden.

The various U. S. commercial dusts were highly heterogeneous mixtures of components obtained mainly from carpet sweepings from different parts of the country. The "Dutch Dust" was a standard extract of house dust from houses in Leiden, Holland. This material has been used for much of the research reported by Voorhorst, Spieksma, and their associates. The "Dutch Mite" specimen consisted of heat-killed mites together with human dander (80 per

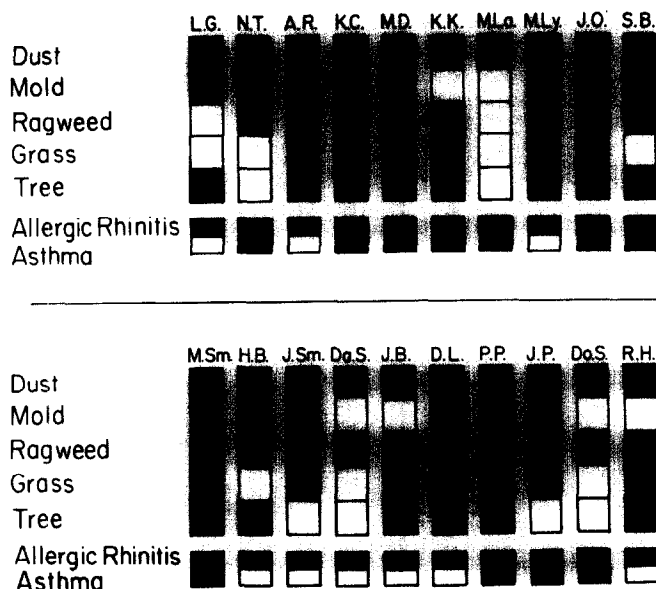


FIG. 1. Specific allergies of 20 highly dust-sensitive individuals used for histamine release experiments with "U. S. Dust #1" and "Dutch Mite." Solid squares indicate that the individual had positive symptoms and a positive wheal-and-flare skin reaction to the allergen. Open squares indicate both negative symptoms and skin reactivity.

cent) and powdered yeast (20 per cent) used as the growth medium. It had been produced in Holland by Dr. Spijksma under conditions described by Voorhorst and associates.⁶

Preparation of allergens for testing

Known volumes of the dust extracts were extensively dialyzed against 0.002M NH_4HCO_3 at pH 7.8 and finally distilled water; they were then lyophilized and weighed. The dry mite (plus medium) sample was defatted with ether and extracted overnight at 4° C. with ten times its weight of 0.125M NH_4HCO_3 at pH 7.8. The extract was centrifuged, and the supernatant dialyzed, lyophilized, and weighed in the same way as the dust extracts.

Antisera

Anti-mite sera were prepared by immunizing 2 rabbits with dialyzed "Dutch Mite" emulsified in complete Freund's adjuvant. The rabbits each received one injection of 5 mg. of antigen followed, at weekly intervals, by 3 separate injections of 1 mg. of antigen. They were bled out 10 days after the last injection. Four rabbit antisera prepared against *D. farinace* (which had been grown by Dr. G. Wharton, Ohio State University, Columbus, Ohio, on Gainesburger dog food) were given to us by Dr. H. Baer, D.B.S., Washington. A pooled human anti-dust serum, from allergic individuals who had been extensively immunized with dust extract, was prepared from sera supplied by Dr. C. May, CARH, Denver.

Skin testing

All dust-allergic and nonallergic individuals were serially skin tested intradermally (0.05 ml. injected) using the extract of *D. pteronyssinus* at 0.1, 1.0, and 10 μg per milliliter and "U. S. Dust #1" at 0.5 and 5 μg per milliliter until a strong (2+) reaction or the highest antigen concentration was attained. All solutions used for skin testing and subsequent leukocyte histamine release assays were prepared on the basis of dry weights of nondialyzable solids per milliliter of solution. The diluent was physiological Tris-buffered saline containing 0.03 per cent (w/v) human serum albumin as a stabilizer.

TABLE I. Yields of nondialyzable solids from mite and dust extracts

Extract	Concentration	Nondialyzable solids ($\mu\text{g}/\text{ml.}$)
"Dutch Mite"	1:10 (w/v)*	6,240
"U. S. Dust #1"	10,000 PNU/ml.	930
"U. S. Dust A"	1:10 (w/v)*	480
"U. S. Dust B"	10,000 PNU/ml.	760
"U. S. Dust C"	1:10 (w/v)*	1,680
"Dutch Dust"	100%†	3,000

*Weight per volume (w/v) concentrations expressed in grams of original solids per milliliter of extracting fluid.

†Sample stated to contain "1,000 Noon equivalents per ml."

Leukocyte assays

Washed leukocytes from the volunteer blood donors were used for quantitative determination of the allergenic reactivities of "U. S. Dust #1" and "Dutch Mite," using a micromodification¹¹ of the basic leukocyte histamine release assay.¹² The relative allergenicities of these 2 extracts were determined in different people from the ratios of mite and dust concentrations which elicited 50 per cent histamine release from the cells. The relative allergenic potencies of the other dust extracts were determined in a similar manner.

In order to investigate whether mite allergens were present in "U. S. Dust #1" extract, we employed a modification of the allergen neutralization ("blocking" antibody) assay of Lichtenstein and associates,¹³ using extensively dialyzed* rabbit anti-mite sera to neutralize "Dutch Mite" and "U. S. Dust #1" allergens. Concentrations of the allergens sufficient to elicit 60 to 80 per cent histamine release from a donor's leukocytes were preincubated for 30 minutes at 37° C. with a series of different dilutions of the antisera. These mixtures, and appropriate allergen plus normal rabbit serum controls, were then tested for their capacity to elicit histamine release from standard suspensions of the leukocytes. Percentage inhibition of histamine release due to presence of antiserum was calculated as previously described.¹³ The relative capacity of an antiserum to inhibit mite-mediated versus dust-mediated histamine release was determined from the ratio:

Reciprocal dilution of anti-mite serum inhibiting 50 per cent of dust-mediated
histamine release

Reciprocal dilution of anti-mite serum inhibiting 50 per cent of mite-mediated
histamine release

Similar assays were also performed with the use of human anti-dust sera.

Immunodiffusion analysis

Dust, mite, and mite growth medium (human dander) antigens were compared by immunodiffusion analysis in agar against rabbit anti-mite and anti-dust sera, by use of the micro-Ouchterlony technique.

RESULTS

The yields of nondialyzable components from the dust and mite extracts are presented in Table I. In the absence of any meaningful immunologic standardization, it was decided to compare the biologic activities of these extracts on the basis of concentrations of high molecular weight, nondialyzable components per milliliter.

*Extensive dialysis of rabbit serum is essential to remove histamine released from rabbit platelets on clotting of blood.

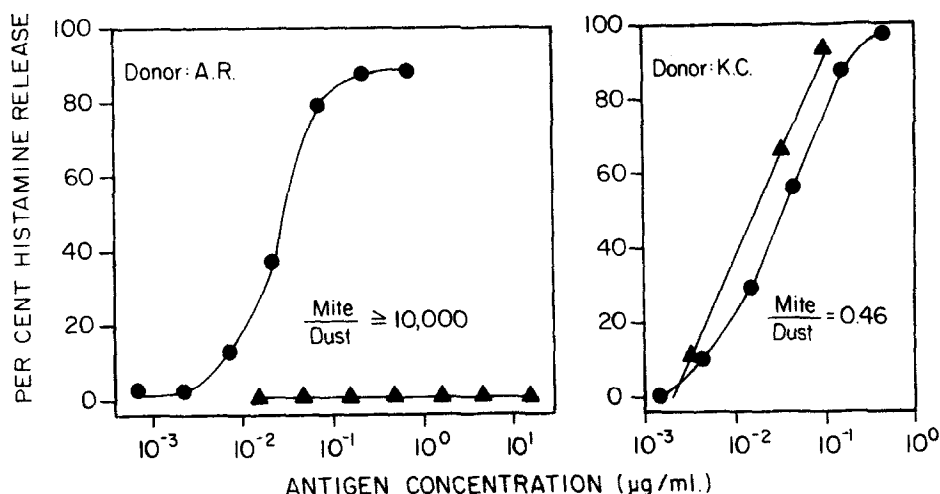


FIG. 2. Histamine release curves to "U. S. Dust #1" (●—●) and "Dutch Mite" (▲—▲) for the leukocytes of 2 donors that showed radically different relative sensitivities to the 2 extracts.

All of the 20 individuals reporting allergy to dust had strongly positive (2+ or greater) wheal-and-flare skin tests to "U. S. Dust #1" at 0.5 μg per milliliter. A few mildly dust-sensitive people not selected for the study had positive skin tests only at 5 μg per milliliter. The 10 nonallergic controls had negative skin reactions at both concentrations of allergen. One of the 20 dust-allergic individuals (A. R.) had a negative skin test to "Dutch Mite" at 10 μg per milliliter, but all the others gave positive reactions of different intensity to the mite at 10 μg per milliliter or lower.* All of the 10 nonallergic controls and most of the mildly dust-sensitive group had negative skin tests to mite at 10 μg per milliliter.

Significant leukocyte histamine release to "U. S. Dust #1" was obtained by the use of leukocytes from all the 20 individuals who showed substantial dust sensitivity by skin test as well as by symptoms. Leukocytes from nonallergic and mildly allergic individuals showed no significant histamine release to "U. S. Dust #1" or "Dutch Mite" extracts. Among the highly dust-allergic population sample, the individuals' relative degrees of leukocyte sensitivity to "U. S. Dust #1" and "Dutch Mite" varied over a 100,000-fold range. Fig. 2 shows leukocyte histamine release curves to "Dutch Mite" and "U. S. Dust #1" for 2 donors at the extreme ends of this range. Most of the donors' leukocytes were more sensitive, on a weight basis, to the dust than to the mite extract. Furthermore, there was no significant relationship between leukocyte sensitivity to dust and the mite/dust sensitivity ratio (Fig. 3).

The above results suggested that mite was unlikely to be a major dust allergen in our highly dust-allergic population sample. However, due to the antigenic complexity and lack of uniformity between house dust extracts, it was

*Quantitative skin test results with reference to mite, dust, and other allergens will be dealt with in detail in a subsequent paper.¹⁰

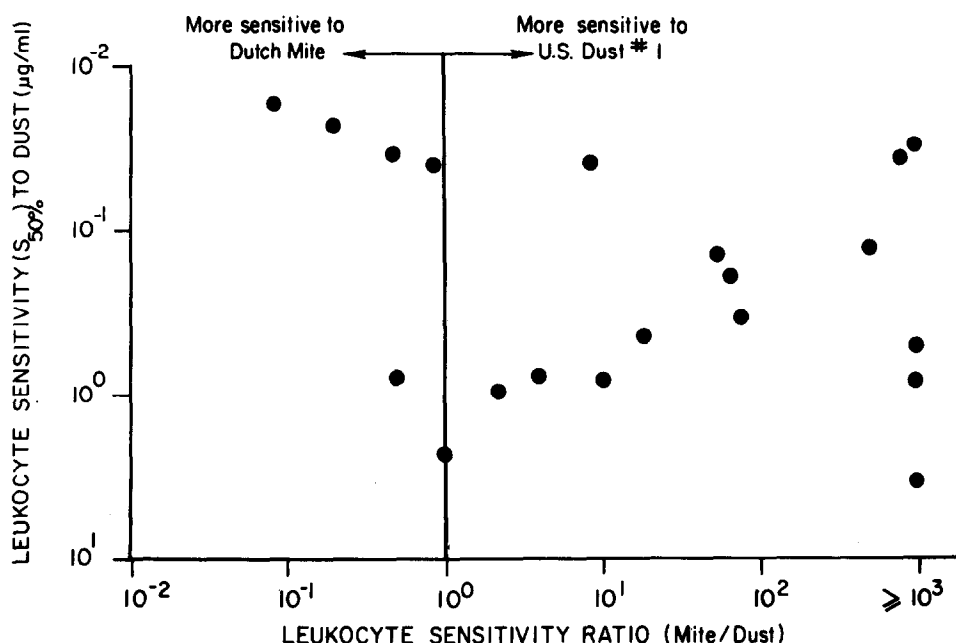


FIG. 3. Comparison, in 20 highly dust-sensitive donors, of leukocyte sensitivity to "U. S. Dust #1" versus leukocyte sensitivity ratio ("Dutch Mite"/"U. S. Dust #1"), determined at the antigen concentrations inducing 50 per cent histamine release from the cells.

TABLE II. Relative sensitivities of selected donors to different dust extracts

Donor	Leukocyte sensitivity ($\mu\text{g/ml.}$)		Leukocyte sensitivity ratios relative to "U. S. Dust #1"			
	"Dutch Mite"	"U. S. Dust #1"	A/#1	B/#1	C/#1	Dutch/#1
L. G.	1.4×10^{-2}	1.7×10^{-2}	5.3	0.21	>1,000	$\geq 500^*$
J. P.	2.1×10^1	9.8×10^{-1}	3.3	23	51	$\geq 100^*$
G. R.	ND†	ND	1.0†	3.6†	17†	ND
D. L.	16% @ 2×10^1	8.2×10^{-1}	10	2.9	400	ND
J. Sc.	ND	$< 5 \times 10^{-1}$	>10	>2.0	>6,000	ND
C. H.	1.2×10^2	2.0×10^{-1}	ND	ND	ND	$\geq 100^*$
R. H.	0% up to 1×10^2	3.4×10^0	ND	ND	ND	$\geq 12^*$

*Results with Dutch Dust are probably meaningless with most donors since cell lysis, resulting in nonspecific histamine release, occurred using antigen concentrations of about 10 μg per milliliter and above.

†ND = Not determined.

‡Since results using "U. S. Dust #1" were not available, the sensitivity ratios were calculated on the assumption that Dust A/Dust #1 = 1.0.

necessary to investigate this point further with the use of other preparations of house dust extract. A few dust-sensitive donors' cells were therefore tested with a number of dust extracts from U. S. and Dutch sources. The results (Fig. 4 and Table II) showed that: (1) Each donor varied considerably in sensitivity to the different extracts (a 100-fold to 1,000-fold difference was usually found between the most potent and least potent extracts). (2) The sensitivity ratios for each dust extract relative to "U. S. Dust #1" varied from donor to donor. "U. S. Dusts #1, A and B" showed the highest allergenic activity by leukocyte

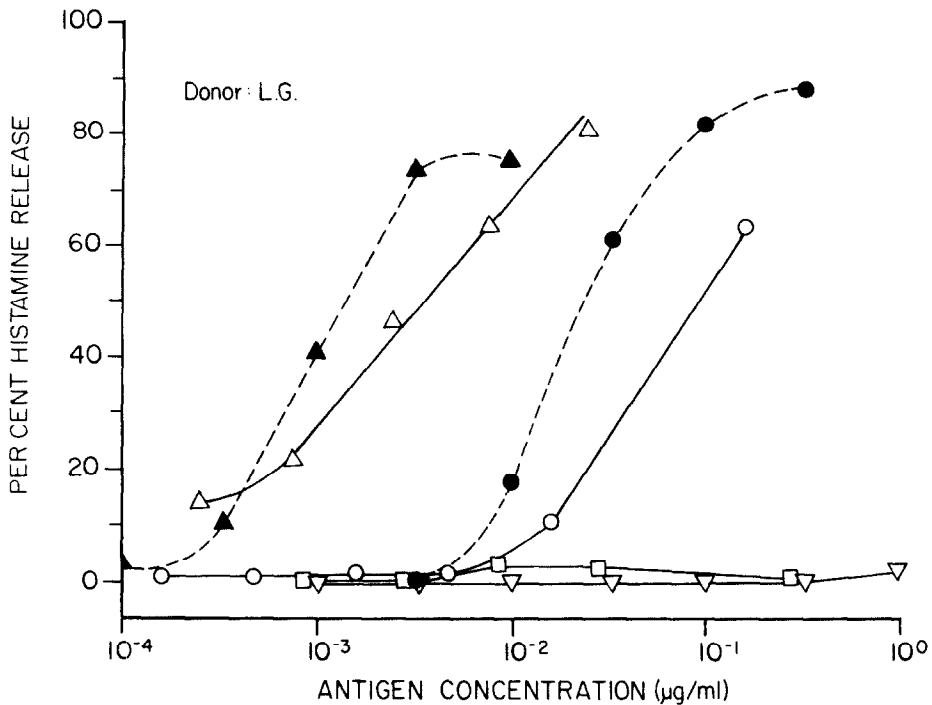


FIG. 4. Histamine release curves to "U. S. Dust #1" (●—●), "Dutch Mite" (▲—▲), "U. S. Dust A" (○—○), "U. S. Dust B" (△—△), "U. S. Dust C" (□—□), and "Dutch Dust" (▽—▽) for the highly mite-sensitive Donor L. G. Note the wide variation in sensitivity to the different dust extracts and the insignificant histamine release to "Dutch Dust" over a wide range of antigen concentrations.

histamine release assay in all subjects tested; "U. S. Dust C" showed the greatest donor-to-donor variations.

In the 4 donors tested, "Dutch Dust" induced cell lysis (resulting in non-specific histamine release) at antigen concentrations in the region of, or lower than, the concentrations required to induce specific secretory histamine release. Thus "Dutch Dust" appears to be virtually or completely inactive with regard to inducing antigen-mediated histamine release from these donors' leukocytes.

Immunodiffusion analysis of "Dutch Mite," "U. S. Dust #1," and human dander extracts against anti-Dutch Mite sera revealed a major line of identity between all 3 extracts (Fig. 5), indicating the presence of a common human dander antigen. This result is not surprising in view of the high human dander content of the growth medium in "Dutch Mite." Human dander antigens were also detected in all the other dust extracts by similar immunodiffusion analyses. However, no antigens common only to mite and dust were detectable by this method.

Our tests for the presence of shared *allergens* in mite and dust extracts, by the use of various hyperimmune rabbit anti-mite sera to neutralize allergic histamine release by mite and dust, were negative except with cells from the 3 donors with highest sensitivity to mites. In 2 of these, only marginal inhibi-

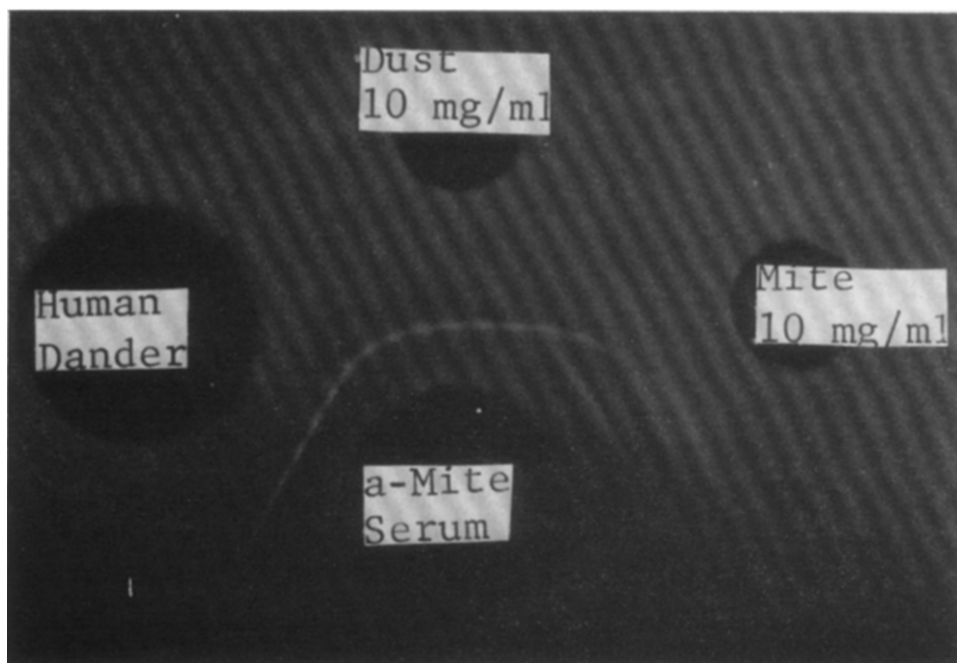


FIG. 5. Comparison by immunodiffusion analysis of a freshly collected human dander extract, "U. S. Dust #1" and "Dutch Mite" against a rabbit anti-Dutch Mite serum (Serum No. 1). Note the presence of a shared human dander antigen.

tion of mite-mediated release was obtained at high serum concentrations with the use of the most potent anti-mite sera available, i.e., the 2 anti-Dutch Mite (*D. pteronyssinus*) sera. Clear-cut neutralization of both mite- and dust-mediated histamine release was obtained only in the individual with highest sensitivity to mites (Fig. 6). For this donor (L. G.), inhibition of dust-mediated histamine release occurred at 10- to 18-fold higher serum concentrations than were necessary to inhibit mite-mediated release. The 4 antisera prepared from extracts of the mite *D. farinae* were somewhat less potent than the 2 anti-Dutch Dust sera in blocking mite-mediated and dust-mediated histamine release from the cells of L. G. Pooled human anti-dust serum was only marginally effective in blocking dust-mediated and mite-mediated release in the same donor by our leukocyte assay.

DISCUSSION

In our population sample, we found that more than half of the people were more sensitive, on a weight basis, to the non-dialyzable components of a potent dust extract ("U. S. Dust #1") than to those of an extract of the mites *D. pteronyssinus* plus media (referred to as "Dutch Mite"). While it is impossible at present to determine which of these high molecular weight components might be allergens, we feel that comparisons based on concentrations of these substances are probably more meaningful than those based on "weight per volume"

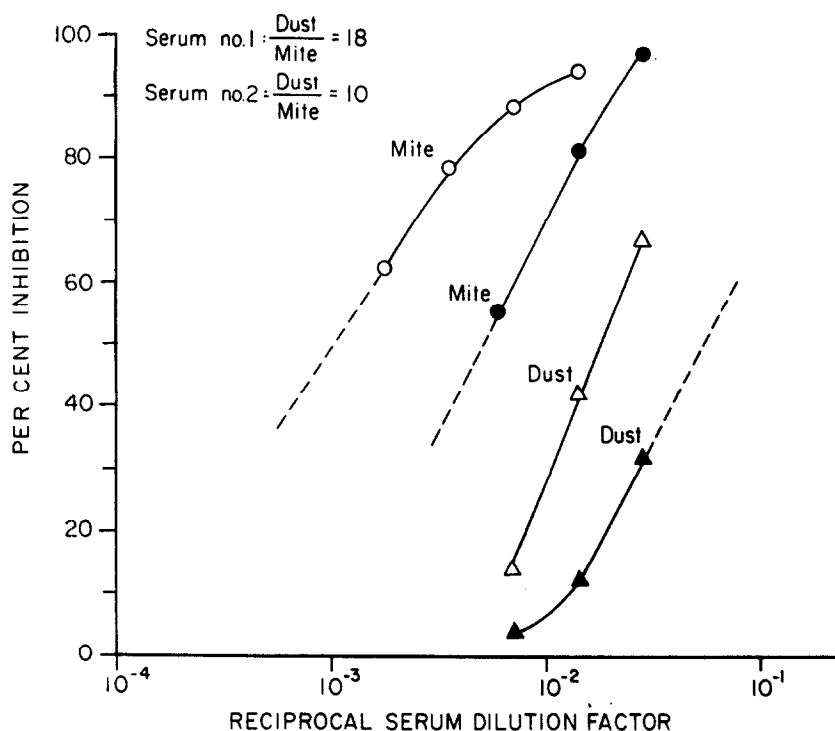


FIG. 6. Inhibition of Dutch Mite-mediated and U. S. Dust #1-mediated histamine release by 2 rabbit anti-Dutch Mite sera, using cell donor L. G. Curves for Serum No. 1 are represented by open symbols and for Serum No. 2 by closed symbols; inhibition of mite-mediated release is shown by circles and of dust-mediated release by triangles. Inhibition ratios were calculated at the points of 50 per cent inhibition.

extraction or "phosphotungstic acid precipitable nitrogen per milliliter"* which have been used widely in the past.

Comparison of the leukocyte sensitivity ratios (mite/dust) in different donors shows that relative sensitivities to "Dutch Mite" and "U. S. Dust #1" vary over at least a 100,000-fold range (Figs. 2 and 3). Furthermore, there is no significant relationship between sensitivity to dust and the mite/dust ratio. The most probable explanation is that there are potent allergens in dust other than those derived from mites of the genus *Dermatophagoides*, toward which different dust-allergic subjects express widely different relative sensitivities. Indeed, considering the extreme heterogeneity of dust extracts, and the established fact that different allergic individuals show widely different sensitivities (by skin test or leukocyte assay) to different highly purified grass and ragweed pollen allergens¹⁴⁻¹⁶ one might anticipate such a result a priori.

Leukocyte histamine release assays in which a number of different dust extracts were used showed that different donors had different *relative* sensitivities

*Phosphotungstic acid is known to precipitate low molecular weight nitrogenous compounds in addition to proteins, which makes it an unsatisfactory means of estimating protein content. Furthermore, neutral carbohydrate and possibly other potentially allergenic components are not detected by protein nitrogen determination.

to the different extracts (Table II), demonstrating that there are allergenic differences between different dust extracts; however, for most donors "U. S. Dust #1" had the highest allergenic activity. It is of interest to note that the leukocytes of the 4 dust-sensitive donors tested with a standard extract of Leiden Dutch House Dust supplied by Dr. Voorhorst were virtually or completely unresponsive. One of the 4 donors (L. G., Fig. 4) tested with "Dutch Dust" was highly sensitive to *D. pteronyssinus* ("Dutch Mite") extract which is claimed by the Leiden and many other workers⁵⁻⁹ to contribute the major dust allergen. Subsequent assay of "Dutch Dust" and "Dutch Mite" extracts by quantitative skin testing¹⁰ has shown that most of our highly mite-sensitive patients are only weakly sensitive to "Dutch Dust" extract on the basis of micrograms of nondialyzable solids injected.

Immunodiffusion analysis (Fig. 5) revealed that the main antigen common to the dust and mite extracts was a human dander component which had constituted part of the growth medium for the mites. This antigen, in its native form, is unlikely to be a *major allergen* common to the 2 extracts. However, human dander may well contribute minor allergen(s),¹⁷ which contaminate the mite extract and are also present in dust.

Certain individuals were found to have similar leukocyte sensitivities to the "U. S. Dust #1" and "Dutch Mite" extracts. In order to demonstrate whether this result was due to a common allergen in the extracts, we tried to neutralize mite- and dust-mediated leukocyte histamine release with potent anti-mite sera known to give several precipitin lines against mite (cf. Fig. 5). In only the most mite-sensitive individual did we observe neutralization of both mite and dust allergens. However, such allergen neutralization required serum concentrations several hundred times those required for neutralization in comparable pollen-anti-pollen systems (unpublished observations). These results suggest that at least some mite allergen(s) are common to "U. S. Dust #1" without being sufficiently strong antigens for the rabbit, due to either their low intrinsic immunogenicity and/or their relatively low concentration in the crude "Dutch Mite" extract used for immunization.

We conclude from these studies that components of mites of the genus *Dermatophagoides* represent only some of the many possible house dust allergens and that a proportion of highly dust-sensitive North Americans are virtually or completely insensitive to mite allergens.

Our data are consistent with the view that hypersensitivity to dust is multifactorial and that no single component can be used to standardize dust extracts.

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