

Allergenic and biologic activity of commercial preparations of house dust extract

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The endotoxin, protein nitrogen unit (PNU) content, and allergenic activity of seven different extracts of house dust were determined. The PNU content varied from 1,600 to 44,000 PNU/ml. The endotoxin content as measured by the limulus assay varied from 450 to 500,000 ng/ml. Pyrogenic activity was present by rabbit fever assay and correlated well with the limulus assay. There was no correlation between endotoxin and PNU content of the different dust preparations. Leukocytes from 15 patients with a clinical history of allergy to dust were used for histamine release studies. The allergenic activity did not correlate with the PNU or endotoxin content of the different house dust preparations. The dust extracts could be divided into three groups according to their activities: three extracts which were the most reactive (30% histamine release with 0.12 to 0.39 PNU/ml), two which were about 20 times less active (4.75 to 6.43 PNU/ml for 30% histamine release), and a final group of two which were about 600 times less active. The leukocytes of three out of 15 patients failed to release histamine with one of these least reactive extracts. The reactivity pattern of five of the dust preparations appeared to correlate, suggesting that the different preparations contained the same allergens. Reactivities of two of the other extracts correlated poorly, and the reactivity of the least reactive sample did not correlate with six out of the seven preparations. Therefore, some dust preparations contain significant endotoxin-like material. Wide variations in the endotoxin, PNU content, allergenic activity, and reactivity patterns of different dust preparations were observed.

Allergy to house dust is one of the most frequent diagnoses made by allergists, and for years immunotherapy of these patients has consisted of injections of house dust extracts. Different sources of dust are used for the manufacture of house dust extracts; e.g., some extracts are prepared from vacuum cleaner bags collected from homes, dust collected in carpet-cleaning establishments, or dust collected during the commercial processing of cotton linters.¹ The dust samples are usually washed with ether and then extracted with

water or buffered saline, but the product widely known as Endo dust is processed by a special procedure.² Although a large number of patients are treated with dust extracts, little information is available on the purity or allergenic activity of different preparations of house dust.

The present investigations compare the endotoxin and allergenic activity of different commercial dust preparations. We found wide variation in the allergenic activity of the house dust preparations, and we also discovered that some house dust preparations contained surprisingly large amounts of endotoxin-like materials.

MATERIALS AND METHODS

Dust preparations

The different preparations of dust allergen used in these studies are listed in Table I. The sample from Institute Pasteur was provided by Dr. E. Relyveld and had been partially purified by ammonium sulfate precipitation.³ All

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TABLE I. Characteristics of the different dust preparations

Dust preparation	PNU content (PNU/ml)	Limulus lysate activity (ng/ml)*	Rabbit pyrogenicity		Limulus lysate activity (ng/PNU)	Highest concentration tested for histamine release
			Dilution fail	Dilution pass		
Allergy Labs of Ohio	1,850	450	10 ⁻²	10 ⁻³	0.240	1:100
Hollister-Stier (regular)	4,700	520	10 ⁻²	10 ⁻³	0.110	1:100
Pasteur	15,600	1,000	NT†	NT	0.064	1:100
Pharmacia	1,600	2,000	NT	NT	1.250	1:100
Greer (house dust)	9,100	4,500	10 ⁻⁴	10 ⁻⁵	0.490	1:100
Hollister-Stier (acetone precipitated)	19,300	5,000	10 ⁻³	10 ⁻⁴	0.260	1:20
Center house dust (freeze-dried)	44,000	20,000	10 ⁻⁴	10 ⁻⁵	0.450	1:50
Endo dust	9,900	500,000	10 ⁻⁴	10 ⁻³	50.510	1:20

* Endotoxin equivalents.

† NT: Not tested.

commercially prepared extracts were used before the expiration date on the samples. In order to provide a means of comparing the various extracts, the protein nitrogen unit (PNU) value of each was determined at the Bureau of Biologics by precipitating with phosphotungstic acid and using the Kjeldahl procedure⁴ to determine the nitrogen content of the precipitate.

Patient population

Fifteen patients (age range, 25 to 52 years) with a history of allergic symptoms on exposure to dust were used in this study. The characteristics of these patients are presented in Table II. Only one of these patients had previously received hyposensitization therapy. The intradermal skin tests with 0.02 ml of 100 PNU/ml of house dust extract (Greer Labs., Lenoir, N. C.) ranged from 1+ to 4+. Skin-test grading was based on measurements of the wheal with the use of the following criteria: neg, site no different from a diluent control; 1+, <5-mm wheal; 2+, 5- to 10-mm wheal; 3+, 10- to 15-mm wheal without pseudopods; 4+, >15-mm wheal with or without pseudopods or >10 mm wheal with pseudopods. The patients were selected only on the basis of their clinical history. As controls for histamine release studies, blood was obtained also from two nonatopic individuals.

Histamine release

Histamine release from washed leukocytes was performed as previously described.⁵ Serial dilutions of dust allergens were tested at two or three concentrations per log-dilution for their histamine-releasing activity. Reaction mixtures consisted of 0.25 ml of washed leukocytes and 0.25 ml of the dust preparation. After 45 min at 37° C, the tubes were centrifuged and the supernatants analyzed for their histamine content. Cells incubated with buffer alone consistently released less than 10% of their total histamine. All experimental tubes were in duplicate. Histamine release

from whole blood was used to evaluate reactivity of the individual donors to mixed ragweed, mixed grasses, mixed trees, mixed weeds, mixed molds, mixed feathers, and the following animals: cat, dog, mouse, hamster, guinea pig, rat, and rabbit. Tests were performed at 1, 10, and 100 PNU/ml with allergens as described previously.^{6, 7} Histamine release from whole blood correlates closely with release from washed leukocytes.⁶

Limulus lysate and rabbit pyrogenicity assays

For this study limulus amoebocyte lysate lot 179 was purchased from Associates of Cape Cod, Inc., Woods Hole, Mass. The freeze-dried lysate was stored at -20° C and rehydrated with 5.0 ml of sterile, pyrogen-free, distilled water just prior to use. Any unused rehydrated lysate was stored at -20° C and thawed only once.

The endotoxin used was freeze-dried reference endotoxin lot EC-2⁸ containing 1.0 µg of endotoxin per vial. The contents of each vial were rehydrated with 10 ml pyrogen-free water to yield a solution of 0.10 µg/ml of endotoxin. Two serial tenfold dilutions were made of that solution and were followed by a series of twofold serial dilutions made with pyrogen-free water. A new series of endotoxin dilutions was made weekly. The twofold dilutions were stored at 4° C during the week.

The sensitivity of the lysate was determined daily by adding 0.1 ml of reference endotoxin EC-2 dilutions to 0.1 ml of lysate in 10 × 75 mm pyrogen-free tubes as described by Hochstein et al.⁹ After gentle mixing the tubes were left undisturbed for 1 hr in a 37° C noncirculating water bath. After this incubation period the tubes were inverted as they were removed from the water bath. If a solid clot had formed, that dilution of endotoxin was recorded as positive; if no clot had formed, that dilution of endotoxin was recorded as negative.

TABLE II. Clinical information and histamine release data on patients utilized in study

Patient	Age (yr)	Sex	Symptoms		Dust		Ragweed		Trees		Grasses		Weeds		Molds	
			HF	A	ST	HR ₃₀	ST	HR ₃₀	ST	HR ₃₀	ST	HR ₃₀	ST	HR ₃₀	ST	HR ₃₀
H. N.	34	F	+	+	3+	<1	0		0	>100	0		2+	70	0	
J. H.	28	F	+	+	3+	3	4+	<1	0		3+	>100	4+	<1	2+	
M. P.	34	F	+	-	4+	1	0		0		0		0		0	
C. L.	43	M	+	+	2+	3	4+	2	3+	<1	3+	<1	3+	<1	0	
R. B.	37	M	+	+	2+	<1	0		0		0		0		0	
R. G.	28	M	+	+	1+	16	0		0		0		0		0	
E. M.	34	F	+	-	3+	8	0		0		0		2+	>100	2+	3
E. B.	26	M	+	+	4+	<1	4+	<1	3+	7	4+	<1	4+	15	3+	<1
C. F.	39	F	+	+	1+	4	2+	>100	0		0		1+	~200	0	
H. S.	27	M	+	+	2+	3	3+	<1	2+	<1	3+	<1	2+	<1	0	
S. G.	31	M	+	+	3+	14	0		0		0		0		0	
J. S.	37	M	+	+	2+	23	3+	<1	2+	>100	3+	>100	2+	28	0	>100
R. H.	52	M	+	+	1+	>100	3+	<1	3+	2	3+	<1	3+	<1	0	>100
A. H.	25	M	+	+	1+	~200	2+	20	2+	100	2+	20	3+	8	0	
L. M.	50	M	+	+	3+	10	0		0		0		2+	~200	0	

Histamine release tested from whole blood with 1, 10, and 100 PNU/ml. Where release was less than 10% the block in this table is left blank. Allergens used have been described previously.^{6,7}

HF: Hay fever; A: asthma; ST: intradermal skin test; HR₃₀: concentrations of allergen in PNU/ml for 30% histamine release. HR₃₀ < 1: histamine release at 1 PNU was greater than 30%. HR₃₀ > 100: histamine release at 100 PNU was between 10% and 30%.

After the limulus-lysate endpoint for each allergenic product tested was determined, equivalent dilutions were injected into rabbits until a pass and fail were noted with dilutions tenfold apart. The rabbits were given a dose of 1.0 ml/kg, and the pass/fail criteria used was that described previously.¹⁰

Calculations

The percent of histamine release was calculated and the ascending part of the curve used to obtain the PNU/ml concentration that released 30% histamine. Statistical analyses were made by Spearman's rank correlations. If the histamine release was completely negative at the highest allergen concentration tested, then the next allergen dose was assumed to be positive in order to obtain a number for use in the statistical calculations.

RESULTS

Limulus lysate and pyrogen activity of dust preparations

A summary of the limulus lysate and pyrogen activities of the dust preparations is shown in Table I. The values are expressed as equivalents of endotoxin standard that would give a similar response in the limulus lysate assay. In most cases at least two different lots of the dust allergens were tested. All samples had limulus-lysate and pyrogen activity, although there was wide variation among the different preparations. There was no correlation between the limulus-

lysate activity and the PNU/ml content of the samples. In general, the results of limulus lysate and rabbit pyrogenicity assays were parallel.

Histamine-releasing activity

The allergenic activities of these dust preparations were evaluated by histamine release with the washed leukocytes of 15 dust-allergic individuals. Serial dilutions of the dust preparations were used to test for histamine release. The ascending portion of the curve was used to determine the PNU/ml concentration that released 30% histamine. A summary of all the data from these 15 patients is shown in Table III and Fig. 1. The cells from two nonatopic individuals did not release histamine at the same concentrations of dust allergen.

It is clear from Table III that the data can be analyzed in two ways. First, the cells of any one of the donors can be used to rank the different dust allergen preparations. For example, using the cells of H. N., the ranking in descending order would be Allergy Lab of Ohio, Hollister Stier-regular, Pharmacia, Hollister-Stier acetone precipitated, Greer, Pasteur, Endo, and Center. The second method of analysis is to compare the reactivity of the different individuals to the panel of dust allergens. The results in this table are arranged with the leukocytes ranked according to their reactivity with the dust preparation from Allergy Labs

Feathers		Cat		Dog		Mouse		Rat		Rabbit		Guinea pig		Hamster	
ST	HR ₃₀	ST	HR ₃₀	ST	HR ₃₀	ST	HR ₃₀	ST	HR ₃₀	ST	HR ₃₀	ST	HR ₃₀	ST	HR ₃₀
2+	60	4+	<1	4+	<1	2+	1	4+	5	2+	11	2+	90	3+	9
2+		3+	4	0		0		0		0		0		0	
1+	>100	3+	<1	2+		4+	<1	3+	<1	3+	36	4+	<1	3+	<1
0		4+	1	3+	>100	4+	<1	4+	<1	4+	<1	4+	<1	3+	<1
1+	3	0		0		0		0		0		0		0	
0		0		0		3+	3	3+	4	2+	>100	4+	<1	3+	<1
1+		3+	8	2+	>100	0		0		1+	>100	1+		2+	70
3+	70	4+	10	3+	25	3+	1	1+	9	3+	17	3+	<1	4+	3
2+	34	2+	28	2+	19	3+	<1	3+	<1	2+	2	0	>100	0	
0		2+	>100	0		2+	21	2+	15	2+	90	3+	<1	2+	40
2+	36	0		0		0		0		0		0		0	
2+		2+	60	3+	72	3+	2	3+	3	3+	3	2+		3+	12
2+	>100	0		0		3+	2	2+	4	2+	41	3+	<1	3+	1
0		0		0		0		0		0		0		0	
0		2+	30	2+	34	4+	1	4+	22	3+	14	2+	>100	2+	9

of Ohio. These analyses are considered in detail in the next two sections.

Variation in activity of dust preparations

The activities of different dust preparations varied widely. The results with two different donors are presented in Fig. 2. With the cells of one of the patients (R. B.) there was no histamine release with Endo dust. With the rest of the dust preparations up to 400-fold differences in reactivity occurred. Therefore, the cells from R. B. would rank the different dust preparations as follows in this descending order of activity per PNU/ml: Allergy Labs, Hollister-Stier regular, Pharmacia, Greer, Hollister-Stier Acetone, Pasteur, and Center. The cells of the second patient (H. N.) reacted with all the dust preparations. However, there was in this case an approximately 1,500-fold variation between the most reactive and the least reactive preparation when they were compared on the basis of their activities per PNU/ml. The preparations in descending order of activity would be Allergy Labs, Hollister-Stier regular, Pharmacia, Hollister-Stier Acetone, Greer, Pasteur, Endo, and Center. Therefore the cells of each individual can be used to rank the activities of the different dust preparations.

The dust preparations can be ranked using each patient's leukocytes. The leukocytes from these patients were most reactive with the dust preparation from Allergy Labs or Hollister-Stier. In contrast, the least reactive would be the Endo and Center preparations. These rankings indicate that certain dust pre-

parations have more activity per PNU (i.e., the most allergenic activity with least amount of extraneous inert material).

Correlations in reactivity with different dust preparations

A second important question is whether the different preparations of dust contain the same allergens. To test this, all the donors were ranked with each dust preparation. Table III is arranged according to the ranking of the patients with the preparation from Allergy Lab of Ohio. Inspection of this table demonstrates that although rankings by different dust preparations have similarities, some rankings are very different. The rankings were compared in pairs by calculating Spearman's rank correlation (Table IV). The best correlation was between the two Hollister-Stier preparations; i.e., between the regular and the acetone-precipitated dust preparations, the Spearman rank correlation coefficient was 0.99. As a group, the following extracts had similar reactivities: Pasteur, Pharmacia, Hollister-Stier, and Allergy Labs. The reactivity of the Greer Dust correlated less with these, and Greer and Center were the only two to correlate with each other. Endo dust correlated very poorly with Center and did not correlate at all with the other preparations.

Reproducibility of histamine release results

There was excellent reproducibility of the histamine release results. The cells from two donors were

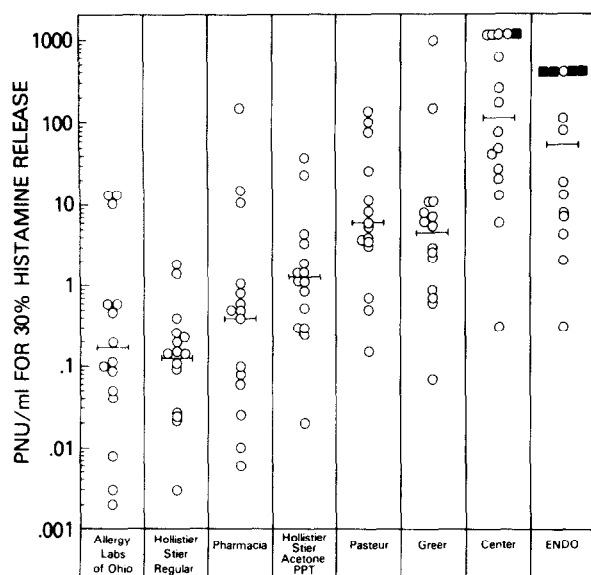


FIG. 1. Concentration of dust preparations required for 30% histamine release. The cells from each individual donor are shown by a circle on the graph. Solid symbols (■) used when histamine release was less than 30% at that concentration of dust. The geometric mean is marked for each allergen preparation.

each tested on two separate occasions 7 mo apart for release with the dust allergen preparations. Separate dilutions of dust were used in these assays. Correlation was close in the concentrations of the allergens required for 30% histamine release (Fig. 3). The correlation coefficient between the two tests was 0.99. The concentration of the allergen required to release histamine, by the second assay was found to be 0.92 ± 0.45 times that required in the first test ($n = 16$, range 0.32 to 1.42 times). Therefore, variations of up to fourfold in PNU/ml concentration required for histamine release could be the result of experimental or donor-leukocyte variation.

Allergen-induced histamine release from the leukocytes of two donors on two occasions were used to rank the preparations of dust. The leukocytes of one donor ranked the different dust preparations identically on both occasions. With the second leukocyte donor there was one change in the rankings between the two tests: the order of the preparations ranked 6 and 7 were reversed. Therefore, because the variation in the reactivity of the different allergen preparations was so great (up to 1,000- to 2,000-fold), experimental variation did not significantly influence the results.

Testing of different lots of allergens

Two different lots of four of the dust allergens were compared for their histamine-releasing activity. Up to eight-fold differences occurred between different lots

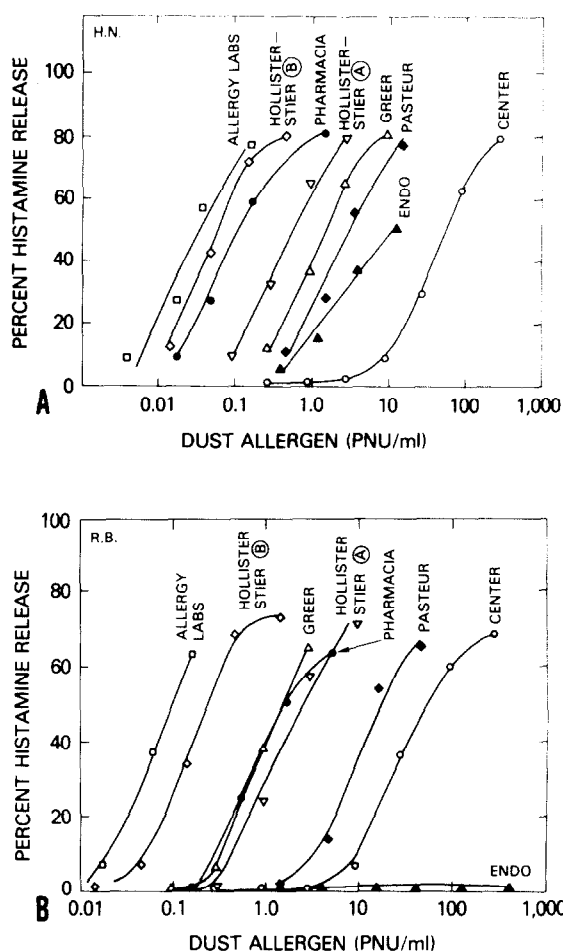


FIG. 2. Histamine release with the leukocytes of two different donors. Serial dilutions (two concentrations per 10-fold change in concentration) were used for histamine release from washed leukocytes. Only the ascending portions of the curves are shown in this figure. A, Donor H. N., B, Donor R. B.

from the same manufacturer $n = 4$, mean = 4.5 ± 2.3 times, range 2.6 to 7.5 times. However the general ranks for these allergens did not change when different lots were tested.

Relationship of dust to mite allergens

The leukocytes from four of the donors were also tested for histamine release with a commercial mite extract. There was wide discrepancy in the reactivities to the mite extract: whereas two of the donors released histamine at low concentrations of the mite allergen, two others did not release histamine even with the highest concentration of mite allergen. For the two reactive individuals in whom the mite preparation released histamine, the mite preparation would have been ranked by one individual as first out of nine allergens and by the second donor as seventh out of

TABLE III. Dust allergen preparation

PNU/ml for 30% histamine release								
Patient	Allergy Labs of Ohio	Hollister- Stier regular	Pharmacia	Hollister- Stier acetone ppt.	Pasteur	Greer	Center	Endo
H. N.	0.002	0.003	0.006	0.02	0.17	0.07	3.0	0.3
J. H.	0.003	0.029	0.080	0.33	0.52	0.60	180.0	>400.0*
M. P.	0.008	0.020	0.025	0.26	0.74	0.90	49.0	80.0
C. L.	0.040	0.11	0.060	0.90	4.1	7.0	620.0	125.0
R. B.	0.050	0.13	0.70	1.2	8.4	0.73	22.0	400.0
R. G.	0.090	0.025	0.010	0.31	3.8	11.0	1200.0	>400.0*
E. M.	0.10	0.15	1.07	1.8	5.4	5.6	>1000.0	7.9
E. B.	0.11	0.13	0.40	1.2	6.2	2.4	27.0	13.0
C. F.	0.20	0.090	0.50	0.53	3.2	3.1	6.0	7.0
H. S.	0.50	0.27	0.10	4.4	12.0	6.0	44.0	2.0
S. G.	0.53	1.57	10.7	24	78.0	10.9	262.0	>400.0*
J. S.	0.60	0.21	0.50	1.5	3.9	7.0	13.0	19.0
R. H.	10.3	0.39	0.82	3.9	28.0	150.0	>1000.0*	>400.0*
A. H.	12.0	1.9	160.0	39.0	104	>910.0	>1000.0	>400.0
L. M.	12.5	0.24	14.5	1.5	140	2.7	77.0	4.4
Geometric mean	0.18	0.12	0.39	1.23	6.43	4.75	113.3†	53.9†

* <15% release at this concentration.

† When the histamine release was completely negative at the highest allergen concentration tested, the next allergen dose was taken for use in these calculations.

nine allergens. In contrast, the two nonreactive individuals would have ranked the mite allergen either as eighth or ninth out of nine preparations, respectively.

Relationship to other dander allergens

The leukocytes of these 15 donors were also tested for histamine release with a number of pollens, molds, mixed feathers, and animal allergens (cat, dog, mouse, rat, hamster, rabbit, and guinea pig) (Table II). To any one of these allergens only seven to 11 of the donors were reactive. Therefore the reactivity to dust could not be explained by a single reactivity with one of these allergens. Although it is possible that the dust preparations contain some danders, feathers, and mites, the contribution of these factors to reactivity of dust cannot be determined from the present results. The results in Table I indicate that as a group these patients were reactive to a number of pollens, feathers, molds, and animals.

DISCUSSION

The present results demonstrate that extracts of house dust contain variable amounts of endotoxin as demonstrated by limulus-lysate or rabbit pyrogenicity assays. There is no correlation between the allergenic activity and the endotoxin content. Therefore, changes could be initiated in the collection or manu-

facture of these preparations to decrease their endotoxin content. The presence of bacteria and bacterial products in house dust has been previously suspected. Furness and Maitland found large numbers of gram-negative bacteria in cotton fibers used for the manufacture of mattresses.¹¹ Rabbit pyrogenic material was found in house dust extract that by cross-tolerance tests was found to be *Escherichia coli* endotoxin.¹² Some purifications of house dust have resulted in the separation of the active allergens from the endotoxin contaminants.¹³

Histamine release with house dust preparations has been reported previously.^{5-7, 14-16} The present experiments indicate that histamine release from washed leukocytes can be used to assess the activity of dust preparations. When cells from the same donor were tested on two occasions, the results were reproducible.

The leukocytes of allergic individuals could be used to rank the different dust preparations according to their allergenic activities, i.e., the PNU/ml concentration required to release 30% histamine (Table III). The ranking of the different dust preparations with the cells of the 15 donors were very similar. However, there was up to a 10,000-fold difference in the concentration of dust required to release histamine from the cells of the most vs least sensitive donor. The

TABLE IV. Spearman's rank correlation (r) for leukocyte histamine release with different dust preparations

	Hollister-Stier regular	Hollister-Stier acetone ppt.	Pasteur	Pharmacia	Allergy Labs	Greer	Center
Hollister-Stier acetone ppt.	0.99*						
Pasteur	0.90*	0.88*					
Pharmacia	0.82*	0.83*	0.81*				
Allergy Labs	0.81*	0.86*	0.81*	0.79*			
Greer	0.62†	0.64†	0.53	0.39	0.63†		
Center	0.44	0.46†	0.44	0.36	0.29	0.68‡	
Endo dust	0.04	0.00	0.05	0.05	0.12	0.28	0.47†

Levels of significance were tested with Student's t test for 13 degrees of freedom.

*p < 0.001.

†p < 0.02.

‡p < 0.01.

TABLE V. Summary of activity measurements of different dust preparations

Preparation	Rank geometric mean (1-8)	PNU/ml for 30% histamine release geometric mean	Activity ratio by histamine release*	Limulus lysate activity/PNU (ng/PNU)	Endotoxin content at 30% histamine release concentration (ng/ml)
Hollister-Stier (regular)	1.6	0.12	1.0	0.11	0.013
Allergy Labs of Ohio	1.7	0.18	1.5	0.24	0.04
Pharmacia	2.6	0.39	3.3	1.25	0.49
Hollister-Stier (acetone ppt)	3.8	1.2	10.0	0.26	0.31
Greer	5.3	4.8	40.0	0.49	2.35
Pasteur	5.5	6.4	53.0	0.064	0.41
Center	7.3	113.0	942.0	0.45	50.9
Endo dust	6.9	54.0	450.0	50.51	2728.0

*Calculated from the value of PNU concentration (PNU/ml) for 30% histamine release.

results indicate that there is more "allergenic" material and less extraneous matter in the dust preparations ranked higher by this analysis.

It has been recognized a priori that house dust is a heterogeneous mixture of allergens from different sources. Over the years, the major source of allergen in house dust has been postulated to be feathers, molds, animal danders, mites, kapok fibers, cotton dust, or even human dander.¹⁻¹⁷ Therefore, it would be important to know whether dust preparations from different manufacturers contain similar arrays of allergens. Spearman rank correlations used to test this question indicate that there were five different preparations with similar patterns of activity (Hollister-Stier regular and acetone precipitated, Pasteur, Pharmacia, and Allergy Labs of Ohio). This correlation was present even with $\times 50$ differences in activities

per PNU/ml. Therefore, the presence of extraneous matter in the allergen preparations does not influence this analysis, although if the dust preparation were a mixture of allergens, the relative concentration of the different allergens in the extract would influence the results. Experimental support for this conclusion is the fact that two dust preparations from a single manufacturer demonstrated over 10-fold differences in activity, although by Spearman rankings they correlated very closely.

The results with mite antigens support the conclusion that mite sensitivity is the important allergen in only a small group of dust-reactive individuals.¹⁵⁻¹⁹ This observation also correlates with the finding that some dust samples obtained from various areas of the United States contain relatively few and frequently no mites.^{20, 21} Endo dust was the least reactive prepara-

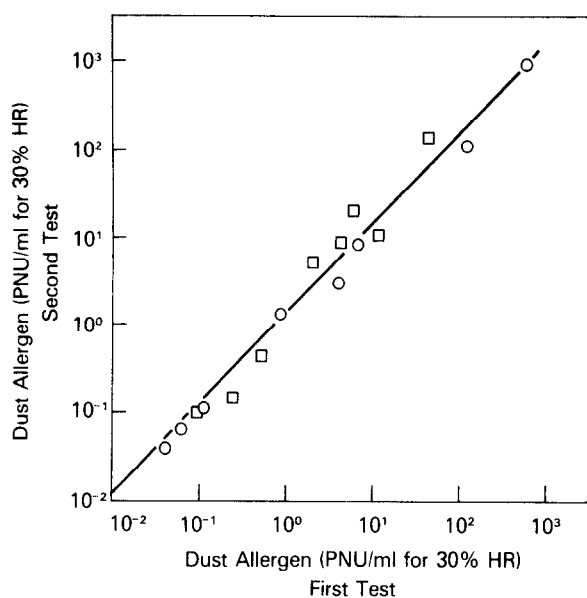


FIG. 3. Reproducibility of histamine release with dust allergens. Leukocytes from two different donors (○□) were used on two separate occasions to determine the concentration required for 30% histamine release. The least-square regression line was found to be $y = 0.60x + 1.43$ with a correlation coefficient (r) of 0.99.

tion tested, and only a fraction of the individuals allergic to dust reacted with Endo.

Our results indicate that some house dust preparations have significant amounts of endotoxin. There is wide variation in both allergenic activity and reactivity patterns of different dust preparations (Table V). Part of this could be attributable to the heterogeneous mixture of allergens in dust, with different commercial preparations containing varying amounts of the different components.

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