

Guanine content and *Dermatophagoides pteronyssinus* allergens in house dust samples

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*The study of the relationship between the guanine content and *Dermatophagoides pteronyssinus* (Dp) allergens in house dust samples is reported. Mattress and carpet dust of bedrooms from 22 different homes constituted the house dust samples. The guanine content was determined by quantitative measurements and the mite allergenicity by two immunochemical assays with a partially purified extract of Dp as internal reference: RAST inhibition and crossed and rocket line immunoelectrophoresis. A large scale range of guanine content was obtained among the 22 house dust samples studied (0.01 to 1.78 mg/0.1 gm of dust). Data of RAST inhibition, analyzed according to parallel line bioassay, demonstrated no significant difference between the slopes of the reference and the house dust sample lines, but a 100-fold variation in the relative Dp potencies was observed. By crossed and rocket line immunoelectrophoresis technique, the presence and the amounts of major Dp allergens (Der p I and Dp 4) were established in most house dust extracts. A significant correlation was found between the guanine content of the house dust samples and their relative Dp potencies ($r = 0.86$) on the one hand, and with their relative content of Der p I and Dp 4, two major Dp allergens ($r = 0.75$ and $r = 0.74$, respectively) on the other hand; in each case, a quantitative relationship was established. These results suggest that the guanine determination could assess mite allergens in house dust and may be a useful tool in large-scale investigations of house dust. (J ALLERGY CLIN IMMUNOL 1989;83:926-33.)*

Mites of the genus *Dermatophagoides* represent the main source of house dust allergens in respiratory allergic diseases.¹⁻³ The identification of allergenic molecules from the mite species *Dp* and *Df* has advanced during the past years, mainly because of the development of immunoelectrophoretic techniques.⁴⁻⁶

Some of the major mite allergens⁷⁻¹⁰ have been purified and characterized. Their measurement in house dust or air is now possible by radioimmunoassay¹¹ or enzyme immunoassay,¹² but these investigations require specific reagents and elaborate techniques.

In 1984, Bischoff and Schirmacher^{13, 14} proposed a colorimetric method for a semiquantitative determination of guanine in house dust samples. The interest of the test is based on the fact that guanine is the main

Abbreviations used

Dp:	<i>Dermatophagoides pteronyssinus</i>
Df:	<i>Dermatophagoides farinae</i>
CLIE:	Crossed line immunoelectrophoresis
RLIE:	Rocket line immunoelectrophoresis
CV:	Coefficient of variation
CARLIE:	Crossed and rocket line immunoelectrophoresis
CIE:	Crossed immunoelectrophoresis
NIBSC:	National Institute for Biological Standards and Control

nitrogenous waste product of chelicerate arthropods, such as *Dp* and *Df* house dust mites.^{15, 16}

Previous studies already questioned to what extent the guanine content of a house dust sample can reflect the level of mite allergens present in the same sample. Preliminary results, based on in vitro tests for mite allergen content, such as RAST inhibition, and on mite counts, demonstrated a certain concordance only between RAST inhibition and the guanine values for different house dust samples.¹⁷⁻¹⁹ A recent study, with

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skin tests in patients selectively sensitized to *Dp*, demonstrated a good concordance between the allergenicity of several house dust extracts and their guanine content.²⁰ In the present work, we specify the relationship between the guanine content and the mite allergenicity of house dust, performing quantitative guanine measurements and assays of *Dp* allergens in dust samples from 22 houses. We estimated the *Dp*-specific relative potency by the RAST-inhibition technique and the relative content of major *Dp* allergens by immunoelectrophoretic analysis.

MATERIAL AND METHODS

House dust samples

Selection of homes. Considering that in high altitude mites are a minor component of house dust and that in homes of allergic patients the bedrooms are the area most contaminated by mites, we chose at random five homes in mountain regions (above 1200 m) and 17 homes occupied by *Dp*-allergic patients attending allergy clinics.

Dust sampling. Mattress and carpet dust from the bedrooms of these 22 homes were collected by vacuum cleaning (1 min/m² of surface area). The paper bags containing dust samples were stored in sealed plastic bags until the raw dusts were sieved through a 0.3 mm screen to obtain a fine dust. These samples were used for the preparation of house dust extracts and for guanine determination.

Allergenic extracts

House dust extracts. A 1 gm sample of fine dust, prepared as described above, was extracted in 10 ml of 3% NaHCO₃ at 4° C for 24 hours on an orbital rotator. After centrifugation, the supernatant was filtered (0.45 µm) and constituted the house dust extract. If it was necessary, the dust extracts were concentrated by lyophilization.

***Dp* extract.** The preparation of the partially purified extract from *Dp* mite cultures was done as previously described.²¹ The extract, named *Dp* 80d, was substandardized against the international reference preparation of World Health Organization International Union of Immunological Societies *Dp* (NIBSC 82/518)²² by RAST inhibition and immunoelectrophoretic assays. *Dp* 80d had an allergenic potency of 3.4×10^5 IU/ml, contained 50 µg/ml or 4×10^6 IU/ml of *Der p* I, and 1.5-fold more *Dp* 4 than the *Dp* NIBSC 82/518 extract (content in *Dp* 4 arbitrarily assigned as 1.5×10^5 AU/ml). *Dp* 80d was used as an internal reference extract for the mite-allergen determinations.

Antibodies

Rabbit antiserum to *Dp* 80d was obtained as described by Le Mao et al.⁵

Guanine determination in house dust samples

Quantitative guanine determination is based on the principle of the guanine colorimetric paper test, ACAREX

test (Werner & Mertz, GmbH, Mainz, West Germany),^{13, 14} and was performed as follows: 250 mg of sieved fine dust was washed three times in 20% methanol; the wet dust was extracted by an alkaline alcoholic solvent (20% methanol and 0.16 mol/L of NaOH). After centrifugation, the supernatant, containing the guanine, was mixed with a solution of 16% Na₂CO₃ and then with the freshly prepared coupling solution (sulfanilic acid at 0.25% in 1 N HCl, and NaNO₂ at 5% in distilled water; the two solutions were mixed in a 1:1 ratio). Diazoamino compounds were formed at room temperature, and after 20 mn, the intensity of the yellow color was read at 490 nm. The concentration of guanine in house dust samples was determined by reference to a standard curve with guanine (Merck, Paris, France) at 10 to 70 µg/ml. It was expressed in milligrams per 0.1 gm of house dust, and its CV was calculated as the standard deviation per mean. The guanine content of 0.1 gm of *Dp* mite culture used for producing *Dp* 80d, the internal reference, was 4.35 mg.

RAST inhibition

The experiments were carried out as previously described.²¹ The mite serum pool consisted of equal volumes of serum from 14 nonhyposensitized subjects who were considered to be selectively sensitized to mites, based on the clinical history, positive intradermal tests with a 10⁻⁵ mg/ml *Dp* extract, and negative cutaneous reactions for cat and dog allergens. The paper disks were coated with *Dp* 80d and constituted the mite-reference disks. The assays were performed as follows: 100 µl of serial dilutions of reference extract or house dust extracts were preincubated for 3 hours at room temperature with 100 µl of a fivefold-diluted mite serum pool. Then the mixtures were used for RAST on references disks. All experiments were conducted in quadruplicate or duplicate. RAST-inhibition data were analyzed according to parallel line bioassays designed to calculate the allergenic relative potency²³ that was expressed in international units per 0.1 gm of dust.

Immunoelectrophoretic methods

CIE, CLIE, and RLIE were performed as initially described by Weeke²⁴ and Kroll²⁵ with some modifications.⁵ Furthermore, the techniques were carried out on a gel-bond film, and the procedures used in CLIE and RLIE were combined, as proposed by Dandeu et al.,²⁶ to obtain a method that is named CARLIE. Briefly, for the first dimensional electrophoresis (20 V/cm for 30 minutes), 35 µg of *Dp* 80d extract was applied in the wells of CIE and CLIE (see Fig. 2, *a* and *b*). In the RLIE, 10 µl of each house dust extract concentrated tenfold was placed in the wells (see Fig. 2, *c*). The intermediate gel for CARLIE contained 144 µg of *Dp* 80d extract (Fig. 2, *b* and *c*); 0.3 µl/cm² of a rabbit anti-*Dp* 80d serum was included in the second dimension electrophoresis gels (second dimension electrophoresis, 2 V/cm for 18 hours). CIE and CARLIE films were pressed and stained with Coomassie brilliant blue R 250. The amounts of major *Dp* allergens in house dust extracts were estimated by means of peak area measurements and were quantified relative to the internal reference extract *Dp* 80d.

TABLE I. Summary of guanine content and *Dp* allergen assays in house dust samples

House dust samples	Guanine content		Relative <i>Dp</i> potency† (IU/0.1 gm)	Content of ‡	
	Mean* (mg/0.1 gm)	CV %		<i>Der p</i> 1 (<i>Dp</i> 7) (ng/0.1 gm)	<i>Dp</i> 4 (AU/0.1 gm)
1 ○	0.03	13.5	646	142	2064
2 ○	0.06	15	918	142	2064
3 ●	0.07	23.3	8053	1261	6450
4 ○	0.07	9	986	142	2064
5 ●	0.08	14.4	1088	142	2064
6 ●	0.08	23.7	3536	ND	ND
7 ○	0.09	10.5	544	78.5	3354
8 ●	0.10	11.5	2040	309.4	6450
9 ○	0.12	5.9	2550	ND	ND
10 ●	0.13	21.4	16320	785	15480
11 ●	0.14	4.5	1156	78.5	2064
12 ●	0.22	6.6	16830	1737	15480
13 ●	0.22	1.8	5474	1737	15480
14 ●	0.23	5.1	5270	952	8256
15 ●	0.31	8.9	8160	952	2064
16 ●	0.37	9.4	20604	1570	15480
17 ●	0.44	14.8	17374	ND	ND
18 ●	0.48	9.8	18700	1785	15480
19 ●	0.56	4.2	29920	1570	34059
20 ●	1.03	5.7	19210	1094	12900
21 ●	1.52	7.3	42840	2380	51600
22 ●	1.78	1	68000	2380	15480

○, House dust samples from homes at high altitudes; ●, house dust samples from *Dp*-allergic patients' homes.

*Mean of four measurements.

†By RAST inhibition, expressed relative to substandardized *Dp* 80d extract.‡By CARLIE, quantified relative to the two major allergens of *Dp* 80d.

Statistical analysis

Assay data from RAST inhibition were analyzed according to parallel line bioassays²³ with extract *Dp* 80d as the reference. A regression line between log doses and inhibition percentages was established for each assay and for the dust extracts and *Dp* 80d. The deviations from parallelism and linearity of the lines corresponding to a given substance (dust or reference) were tested by means of covariance analysis. In cases of nonsignificant deviations, a common regression line was established between the log doses and the mean inhibition percentages both for the dust and the reference. In cases of nonsignificance of the deviation from parallelism of the two common regression lines (dust extracts and reference extracts) an estimation of the common slope was made, and the potency of the dust extracts was expressed relative to the reference extracts.

Quantitative relationship between guanine content and *Dp* allergen contents were investigated by means of regression analysis.

RESULTS

Guanine content of different house dust samples

The guanine content of 22 house dust samples was determined by quantitative measurements. The pre-

cision of the calibration curve (guanine standard at 10 to 70 µg) was evaluated with 10 identical assays, and the CV did not exceed 9%. The results of the guanine content for the house dust samples that were tested are presented in Table I. The guanine concentration varied according to samples, and the variation ranged from 0.03 to 1.78 mg for 0.1 gm of dust. We found that samples that were obtained from homes in high altitude contain low guanine content. The CV for the total procedure, including extraction of guanine from house dust, was investigated by assaying four subsamples of fine dust from each of the 22 samples. For dusts with a guanine content >0.13 mg/0.1 gm of dust, the CV value was <15%, but the variation was larger when lower guanine concentrations were measured (6% to 24%).

Dp allergen assays

Rast-inhibition studies. The relative potency of house dust extracts with regard to *Dp* allergens was determined by RAST-inhibition assays. Plotted on semilog scale, all the RAST-inhibition data elicited linear response curves ($r > 0.98$). By analysis of covariance, the deviation from parallelism and from lin-

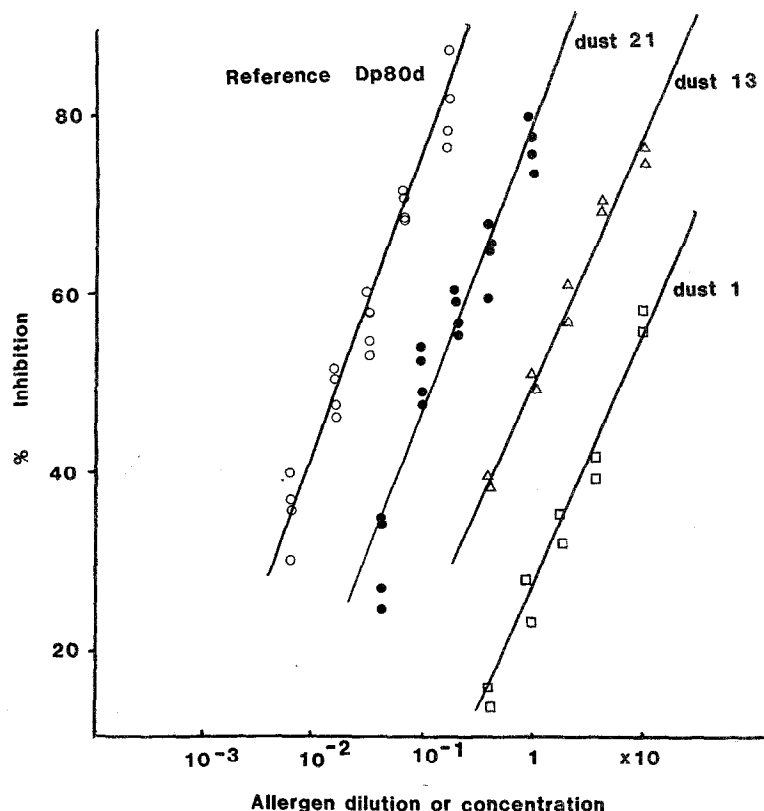


FIG. 1. Example of *Dp* 80d RAST-inhibition regression lines for different house dust samples.

earity of dose-response curves corresponding to the reference extract and to house dust extracts was not significant and allowed us to establish the corresponding common regression lines as illustrated in Fig. 1. Comparison of the slopes of reference and house dust dose-response lines did not reveal significant differences ($p > 0.05$). The relative potency results are presented in Table I. They were calculated with *Dp* 80d extract, the internal reference preparation whose relative potency was 3.4×10^5 IU/ml. We observed a 100-fold variation in potency between the different house dust extracts.

Immunoelectrophoretic studies. The investigation of major *Dp* allergens in house dust extracts was performed by the CARLIE technique. The typical pattern obtained when *Dp* 80d and house dust extracts reacted with *Dp* 80d antiserum is illustrated in Fig. 2. Without intermediate gel (Fig. 2, a) we could observe the CIE reference *Dp* 80d for which we previously identified several allergens.⁵ In front of each house dust extract (Fig. 2, c), we observed peaks which fused with lines corresponding to the allergens identified in *Dp* 80d (Fig. 2, a). The presence of major allergens *Dp* 7 (*Der p* I) and *Dp* 4 could be demonstrated in most house dust extracts. We could also note the presence of *Dp* 6 allergen. The amounts of mite allergens vary according to the house dust extract; the content of *Der p* I and *Dp* 4 are presented in Table I.

Relationship between guanine concentration and *Dp* allergens in house dust extracts

The relationship between mite allergenicity and the guanine content of house dust samples was determined by linear regression analysis; regression line and linear correlation coefficient are presented in Table II. Significant correlation were found between the guanine content and the various parameters assessing *Dp* allergens. The best correlation was obtained between relative *Dp* potencies, as measured by RAST inhibition, and guanine content (Fig. 3).

DISCUSSION

Since guanine, an essential nucleic component, is also an important constituent of excreted nitrogenous waste in mites, as *Dp* and *Df* living in house dust, it could serve as an indicator of house dust-mite activity. Its concentration in dust samples reflects the quantity of mite pellets; these fecal pellets are known to be a major source of allergens.²⁷⁻²⁹ The correlation between guanine content and mite allergenicity in house dust samples has been roughly studied with a semiquantitative test (ACAREX test), skin testing, mite counts, and RAST-inhibition experiments.¹⁷⁻¹⁹

In this work, we performed quantitative guanine determination, as well as identification and quantification of *Dp* allergens in house dust samples from 22 homes supposed to contain various mite numbers.

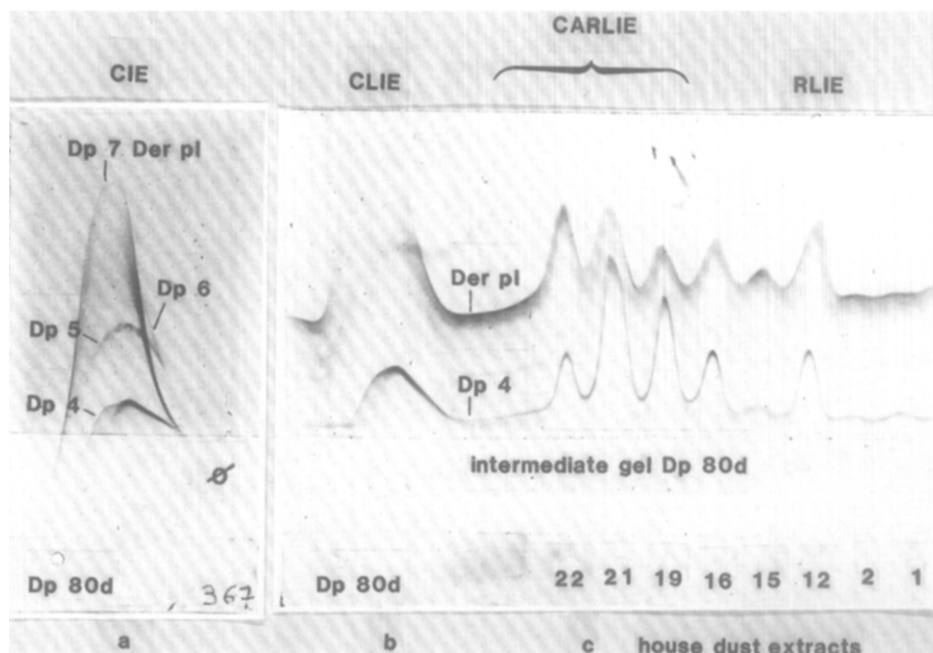


FIG. 2. Combination of CIE and CARLIE to detect major *Dp* allergens in several house dust extracts obtained from house dust samples with different guanine contents. Antigens, *Dp* 80d (35 μ g) and house dust extracts (10 μ l, tenfold concentrated); antibodies, rabbit serum pool against *Dp* 80d (0.3 μ l/cm²); intermediate gel, *Dp* 80d, 144 μ g.

The quantitative guanine measurement is based on a chemical reaction in which a yellow guanine azodye results from attack by the diazonium ion of the amino group and nitrogen atoms of the guanine nucleus.³⁰ The colorimetric reaction was demonstrated to be specific for guanine since other metabolic products, such as uric acid from insects, do not exhibit a yellow color under the same reaction conditions.¹⁴ The CV of the calibration curve is 9%, but for some house dust samples, we observed a higher CV (samples 3, 6, 10). The preparation of house dust samples could be a source of variability in this analysis.¹² Indeed, despite sieving through a 300 μ m screen to remove large particles and fibers, the fine dust could have remained heterogeneous (presence of sand or other small materials), and dust samples with the same given weight may have had a different composition. A large-scale range of guanine content was obtained among the house dust samples studied; we noted low guanine content for samples collected in high altitude, which confirms previous studies.²⁰

The specific assays of *Dp* allergens, performed on extracts prepared from house dust samples, were done in two ways: the evaluation of relative *Dp* potency and the identification and the determination of the relative content of major *Dp* allergens.

The relative potency was studied by the RAST-inhibition technique. This assay method³¹ presents the

advantage of quantifying allergens that have elicited an IgE response in allergic subjects. It is the most widely used method for measuring the total allergenic activity of an allergenic extract.^{22, 32, 33} The source of IgE antibodies and the quality of the reference extract are important. To work with high levels of *Dp*-specific IgE antibodies, we used a serum pool obtained from *Dp* selectively sensitized patients. For the reference extract, we chose the partially purified extract from *Dp* mite culture *Dp* 80d. This internal reference has been previously characterized by immunochemical methods.^{5, 21} It is a preparation that contains two major allergens (*Dp* 7 [*Der p* I] and *Dp* 4) and whose allergenic potency could be expressed in terms of the international units defined for the *Dp* NIBSC 82/518 international reference. The parallelism between inhibition lines suggests a large identity between the qualitative mite composition of the house dust extracts and of the *Dp* reference, whereas the quantitative differences in the allergenic activity of the house dust extracts demonstrated a roughly 100-fold variation.

These results were completed by the identification and measurements of the relative content of major *Dp* allergens by immunoelectrophoretic analysis of house dust extracts with the anti-*Dp* 80d serum. We used the CARLIE technique, which is a combination of the CIE, CLIE, and RLIE methods. This technique, in which *Dp* 80d was used in the first-dimension elec-

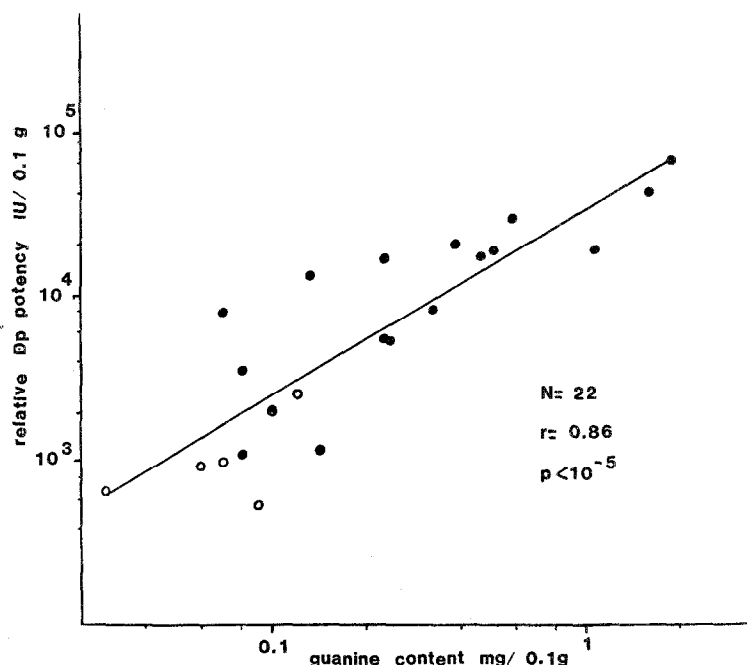


FIG. 3. Regression line between guanine content and relative *Dp* potency in 22 house dust samples.

TABLE II. Linear regression data from the analysis of house dust samples

Relationship	Sample size	Regression line $Y = aX + b$	Correlation coefficient	p
Guanine content (X) vs relative <i>Dp</i> potency (Y)	22	$Y = 1.15 X + 4.55$	0.86	10^{-5}
Guanine content (X) vs relative <i>Der p</i> I content (X)	19	$Y = 0.80 X + 3.32$	0.75	10^{-3}
Guanine content (X) vs relative <i>Dp</i> 4 content (Y)	19	$Y = 0.69 X + 4.32$	0.74	10^{-3}

All relationships were determined by the use of log-transformed data.

trophoresis, as well as in the intermediate gel and in which different house dust extracts were placed in the rocket wells, allows a direct identification and a relative quantification of mite antigens in house dust extracts, knowing the allergogram of the mite extract (*Dp* 80d).⁵ The presence of the two major allergens, *Dp* 7 and *Dp* 4, in the house dust extracts was clearly demonstrated and corroborates the qualitative allergenic similarity between the house dust extracts and *Dp* 80d extract.

The mite assays have to be able to measure allergens derived from *Dp* and *Df* mite species, since house dusts contain either or both species. In our RAST inhibition and CARLIE experiments, we used *Dp* 80d extract, whose cross-allergenic reactivity with the equivalent extract from *Df* has been demonstrated.⁵ Thus, we measured the allergens derived from the two

mite species. The content in *Der p* I in house dust extracts could be considered as the content in antigen P_1 equivalent.¹¹

Our study demonstrated a positive correlation between the guanine content of house dust samples and, as well their relative *Dp* potencies ($r = 0.86$) as their relative content of *Der p* I and *Dp* 4, two major *Dp* allergens ($r = 0.75$ and $r = 0.74$; respectively). We observed a better correlation coefficient when the *Dp* allergens were assayed by RAST-inhibition experiments measuring the total mite fecal allergens of the house dust extracts.

Some occasional discrepancies were noted in a few results. For house dust samples with more guanine than would be expected from their mite allergenicity (i.e., sample 20), it can be assumed that the origin of the guanine is not fecal pellets but other biologic

sources, such as other mite species, spiders, or birds. To explain why a house dust with a low guanine content may reveal a high relative potency (samples 3 and 10), a first explanation could be the heterogeneous composition of these two samples previously noted and leading to a variability of the weight of the fine dust. Another explanation would be that the guanine was used by microorganisms living in mattresses, such as bacteria under moist conditions, as suggested for uric acid, the most common form of nitrogenous waste excretion in insects.³⁴ These hypotheses have to be tested.

The study of the relationship between guanine determination and mite allergenicity in house dust required quantitative and specific parameters. Our study has fulfilled this aim with RAST-inhibition experiments, CARLIE analysis, and quantitative guanine measurements. A good correlation was found, confirming previous studies. The guanine determination, which is an indirect method of evaluating mite allergens in house dust, appears as a more convenient and available technique than the immunochemical methods and could be used in large-scale investigations.

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REFERENCES

- Voorhorst R, Spiekma-Boezeman MIA, Spiekma FThM. Is a mite (*Dermatophagoides* sp.) the producer of the house dust allergen? *Allerg Asthma* 1964;10(6):329.
- Maunsell K, Wraight DG, Cunningham AM. Mites and house dust allergy in bronchial asthma. *Lancet* 1968;1:1267.
- Pauli G, Bessot JC, Guisard G, Roegel E, Oudet P. Importance clinique des acariens chez les asthmatiques allergiques à la poussière domestique. *Rev Fr Allergol* 1972;12:141.
- Lind P, Lowenstein H. Identification of allergens in *Dermatophagoides pteronyssinus* mite body extract by crossed radio immunoelectrophoresis with two different rabbit antibody pools. *Scand J Immunol* 1983;17:263.
- Le Mao J, Dandeu JP, Rabillon J, Lux M, David B. Comparison of antigenic and allergenic composition of two partially purified extracts from *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* mite cultures. *J ALLERGY CLIN IMMUNOL* 1983;71:588.
- Krilis S, Baldo BA, Sutton R, Basten A. Antigens and allergens from the common house dust mite *Dermatophagoides pteronyssinus*. Part I. Demonstration of multiple allergens by immunochemical and biologic analysis. *J ALLERGY CLIN IMMUNOL* 1984;74:132.
- Chapman MD, Platts-Mills TAE. Purification and characterization of the major allergen from *Dermatophagoides pteronyssinus* antigen P₁. *J Immunol* 1980;125:587.
- Stewart GA. The isolation and characterization of the allergen Dpt 12, from *Dermatophagoides pteronyssinus* by chromatofocusing. *Int Arch Allergy Appl Immunol* 1982;69:224.
- Dandeu JP, Le Mao J, Rabillon J, David B. Antigens and allergens in *Dermatophagoides farinae* mites. II. Purification of Ag 11, a major allergen in *Dermatophagoides farinae*. *Immunol* 1982;46:679.
- Lind P. Purification and partial characterization of two major allergens from the house dust mite *Dermatophagoides pteronyssinus*. *J ALLERGY CLIN IMMUNOL* 1985;76:753.
- Platts-Mills, TAE, Heymann PW, Chapman MD, Hayden ML, Wilkins SR. Cross-reacting and species-specific determinants on a major allergen from *Dermatophagoides pteronyssinus* and *D. farinae*: development of a radioimmunoassay for antigen P₁ equivalent in house dust and dust mite extracts. *J ALLERGY CLIN IMMUNOL* 1986;78:398.
- Lind P. Enzyme-linked immunosorbent assay for determination of major excrement allergens of house dust mite species *D. pteronyssinus*, *D. farinae*, and *D. microceras*. *Allergy* 1986;41:442.
- Bischoff E, Schirmacher W. Farbnachweis für allergenhaltigen Hausstaub. *Allergol* 1984;11:446.
- Bischoff E, Schirmacher W. Farbnachweis für allergenhaltigen Hausstaub. *Allergol* 1985;8:35.
- Baker WE, Warton GW. Digestive system: an introduction to acarology. New York: Macmillan, 1952:27.
- Keilin J. The biological significance of uric acid and guanine excretion. *Biol Rev* 1954;34:265.
- Bischoff E, Schirmacher W, Schober G. Farbnachweis für allergenhaltigen Hausstaub. *Allergol* 1985;8:97.
- Van Bronswijk JEMH. Guanine as a hygienic index for allergologically relevant mite infestations in mattress dust. *Exp Appl Acarol* 1986;2:231.
- Van Bronswijk JEMH, Bischoff E, Schirmacher W, Berrens L, Schober G. A rapid house dust allergen test: preliminary results. *J Med Entomol* 1986;23:217.
- Pauli G, Tenabene A, Bessot JC, Hoyet C. Intérêt du dosage de la guanine dans la poussière de maison pour la quantification des allergènes des acariens. *Rev Fr Allergol* 1986;26:163.
- Le Mao J, Weyer A, Pauli G, Lebel B, David B. Studies on *Dermatophagoides pteronyssinus* allergens: measurement of the relative potencies of *D. pteronyssinus* extracts by in vivo and in vitro methods. *J ALLERGY CLIN IMMUNOL* 1980;65:381.
- Ford A, Seagroatt V, Platts-Mills TAE, Lowenstein H. A collaborative study on the first international standard of *Dermatophagoides pteronyssinus* (house dust mite) extract. *J ALLERGY CLIN IMMUNOL* 1985;75:676.
- Finney DJ. Statistical method in biological assay. 3rd ed. London and High Wycombe: Charles Griffin, 1978:69.
- Weeke B. Crossed immunoelectrophoresis. In: Axelsen NH, Kroll J, Weeke B, eds. A manual of quantitative immunoelectrophoresis. Oxford: Blackwell Scientific, 1973:47.
- Kroll J. Quantitative immunoelectrophoresis applied to the analysis of elution profiles in column chromatography. In: Peeters H, ed. Protids of biological fluid. Oxford: Pergamon Press, 1972:529.
- Dandeu JP, Rabillon J, David B. Specific house dust allergens are not mythic ones [Abstract]. *J ALLERGY CLIN IMMUNOL* 1987;79:132.
- Tovey ER, Chapman MD, Platts-Mills TAE. Mite faeces are a major source of house dust allergens. *Nature* 1981;289:592.
- Stewart GA, Butcher A, Lees K, Ackland J. Immunochemical and immunoenzymatic analysis of extracts of the house dust mite *Dermatophagoides pteronyssinus*. *J ALLERGY CLIN IMMUNOL* 1986;77:14.
- Arlian LG, Bernstein IL, Vyszenski-Moher DL, Gallagher JS, Martin B. Investigations of culture medium-free house dust mites. IV. Cross antigenicity and allergenicity between the house dust mites, *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*. *J ALLERGY CLIN IMMUNOL* 1987;79:467.
- Shapiro R. Chemistry of guanine and its biologically significant derivatives. *Prog Nucleic Acid Res* 1968;8:73.

31. Yman L, Ponterius G, Brandt R. RAST-based allergen assay methods. In: Regamey RH, Hennesen W, Perkins FT, eds. Development of biological standard, vol 29. Basel: S Karger AG, 1975:151.
32. Gjessing B, Jager L, Marsh DG, Lowenstein H. The international collaborative study establishing the first international standard for timothy (*Phleum pratense*) grass-pollen allergenic extract. J ALLERGY CLIN IMMUNOL 1985;75:258.
33. Tovey E, Vandenberg R. Mite-allergen content in commercial extracts and bed dust determined by radioallergosorbent tests. Clin Allergy 1979;9:253.
34. Van Bronswijk JEMH, Sinha RN. Interrelations among physical, biological, and chemical variates in stored grain ecosystems: a descriptive and multivariate study. Ann Entomol Soc Am 1971;64(4):789.

The formation of eosinophil and neutrophil chemotactic activity during a pollen season and after allergen challenge

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Heat-stable (HS) and heat-labile (HL) neutrophil chemotactic activities (NCAs) have been demonstrated in serum after allergen challenge of subjects with asthma. In this investigation, we have studied the possible occurrence of similar activities in 20 atopic individuals on natural exposure to allergen, that is, during the birch-pollen season. Since eosinophil accumulation is a hallmark of an ongoing allergic inflammation in the respiratory tract also, the possible production of eosinophil chemotactic activity (ECA) was examined in serum after allergen challenge and at natural exposure to pollen. Both HL-NCA and HL-ECA were produced to a significant extent ($p < 0.001$) during the season, with the peak of activities occurring simultaneously with the peak pollen count. HL-ECA was produced after allergen challenge of subjects with asthma in the laboratory, as has been demonstrated for NCA previously. The activity of the HS-NCA was unaltered during season. Gel-filtration studies of the major HL-NCA and HL-ECA indicated a molecular weight for both activities of 100 to 150,000, and the activities produced during season cocromatographed with the HL-NCA and HL-ECA produced after allergen challenge in the laboratory, suggesting that all these activities are due to one and the same molecule. The results suggest that the heat-labile chemotactic activity found in serum of atopic subjects and subjects with asthma after allergen exposure may be involved in the attraction of eosinophils and neutrophils to the site of allergic inflammation. (J ALLERGY CLIN IMMUNOL 1989;83:933-9.)

NCA has been identified in serum of patients with asthma after inhalation of their respective allergen.¹⁻⁴ Basically, two activities can be distinguished, based

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Abbreviations used

NCA:	Neutrophil chemotactic activity
HS-NCA:	Heat-stable NCA
HL-NCA:	Heat-labile NCA
IAR:	Immediate asthmatic response
LAR:	Late asthmatic response
ECA:	Eosinophil chemotactic activity
HL-ECA:	Heat-labile ECA
CV:	Coefficient of variation

on their physicochemical properties. One activity resists heating for 30 minutes at 56° C, has a molecular weight of approximately 650,000, and occurs in serum minutes after challenge, concomitant with the IAR to