

- sociated with hydrochlorothiazide therapy. *Chest* 1981;79(4):482.
20. VonArsdel PP. Adverse drug reactions. In: Middleton E, Reed CE, Ellis EF, eds. *Allergy, principles and practice*. 2nd ed. St. Louis: The CV Mosby Co, 1983:1392.
 21. Beermann B, Groschinsky-Grind M. Clinical pharmacokinetics of diuretics. *Clin Pharmacokinet* 1980;5:221.
 22. Mitchell JR, Potter WZ, Hinson JA, Jollow DJ. Hepatic necrosis caused by furosemide. *Nature* 1974;251:508.
 23. Mandell GL, Sande MA. Antimicrobial agents: sulfonamides, trimethoprim-sulfamethoxazole, and agents for urinary tract infections. In: Goodman AG, Goodman LS, Rall TW, Murad F, eds. *Goodman and Gilman's the pharmacological basis of therapeutics*. 7th ed. New York: MacMillan Publishing Co, 1985:1095.
 24. Rieder J. Metabolism and techniques for assay of trimethoprim and sulfamethoxazole. *J Infect Dis* 1973;128:567.
 25. Frisch JM. Clinical experience with adverse reactions to trimethoprim-sulfamethoxazole. *J Infect Dis* 1973;128(suppl):607.
 26. Carrington DM, Earl HS, Sullivan TJ. An in vitro assay for human IgE to sulfonamides. *J ALLERGY CLIN IMMUNOL* 1986;77(1):224.

The prevalence of indoor allergens in the Baltimore area: House dust-mite and animal-dander antigens measured by immunochemical techniques

P. Lind, Ph.D.,***** P. S. Norman, M.D.,** M. Newton, B.Sc.,**
H. Løwenstein, Ph.D.,* and B. Schwartz, Ph.D.*****
Copenhagen, Denmark, and Baltimore, Md.

Mattress dust samples from 42 homes in Baltimore, Md., were analyzed by ELISA and rocket immunoelectrophoresis (RIE) for major excrement allergens (P, [equal to Dp 42], Df 6, and Dm 6) of house dust mites, Dermatophagoides pteronyssinus, D. farinae, and D. microceras, by RIE for cat major epithelial allergen (cat Ag 1) and horse major epithelial antigen (horse Ag 11), and by counter-current immunoelectrophoresis (CCIE) for cat and dog antigens. Dp 42 was detected by ELISA in 24 samples (57%) and Df 6 in 39 samples (92%). All samples were negative to Dm 6. Recorded levels varied from 50 ng of allergen per gram of dust (detection limit) to 15,000 ng/gm. A combined content of Dp 42 and Df 6 >700 ng/gm was associated with a positive reaction in RIE. Concentrations of Dp 42 and Df 6 did not correlate ($r_s = 0.21$; $p > 0.05$). Cat and dog antigens were frequently detected by CCIE (77% and 63%, respectively). CCIE titer to cat correlated well with quantitation of cat Ag 1 by RIE ($p < 0.01$). Horse antigen was detected in few homes (5%). None of the specific allergen determinations correlated with levels of human serum albumin determined by RIE. (J ALLERGY CLIN IMMUNOL 1987;80:541-7.)

Information about patients' exposure to allergens plays an important role in diseases like rhinitis, conjunctivitis, and asthma. In the case of strictly seasonal allergies like hay fever, it is often possible to make

deductions about the causative, exogenous agent by recording the coincidence of symptoms with the presence of airborne pollens.¹ For several indoor allergies, e.g., house dust mites, which prevail to varying extent throughout the year, measurement of allergen levels in the patients' homes may be a helpful supplement to the diagnostic tests. When allergen elimination measures are introduced, there is often a need to monitor the decrease of allergen exposure. Thus, in the case of allergy to animal danders, removal of a cat or dog does not always lead to the anticipated relief from symptoms. Considerable levels of allergen may be found in the dust of habitations in which there is no pet.²

From *the Protein Laboratory, University of Copenhagen, **School of Medicine, The Johns Hopkins University, Baltimore, Md., ***Diagnoselaboratoriet, ALK, Copenhagen, and ****ALK Research Group, Copenhagen, Denmark.

Received for publication Dec. 30, 1985.

Accepted for publication March 24, 1987.

Reprint requests: Peter Lind, Ph.D., The Protein Laboratory University of Copenhagen, Sigurdsgade 34, DK-2200 Copenhagen N, Denmark.

Abbreviations used

Dp 42, Df 6, Dm 6:	Major fecal allergens of <i>Dermatophagoides pteronyssinus</i> , <i>D. farinae</i> , and <i>D. microceras</i>
Cat Ag 1:	Major allergen of cat epithelium
Horse Ag 11:	Major allergen of horse epithelium
HSA:	Human serum albumin
RIE:	Rocket immunoelectrophoresis
CCIE:	Counter-current immunoelectrophoresis

Microscopic examinations and counting of mites have been extensively used in studies of the mite fauna^{3,4} and as parts of allergen elimination trials.⁵⁻⁷ However, for routine purposes, techniques that are less tedious and demanding of taxonomic expertise would be preferred.

Recent advances in allergen purification⁸⁻¹⁰ have led to production of immunochemical reagents suitable for laboratory assays of mite- and animal-dander allergens in house dust.

In this study we present data on the occurrence and quantity of important indoor allergens in mattress dust, collected from a normal population of a metropolitan area (Baltimore, Md.) with such immunochemical techniques.

MATERIAL AND METHODS

Selection of homes

It was considered that the employees (approximately 5000), professional and nonprofessional, of the Johns Hopkins University and Hospital represented a reasonable cross section of home locations in the Baltimore area. From a computerized list of employees, 50 names were chosen at random, and a letter was sent, requesting voluntary cooperation in allowing the collection of a mattress dust sample. In the event of no reply, a second letter was sent. If there was no reply to the second letter or a refusal, the next person on the list was chosen. Fifty households were selected.

Collection and shipment of samples

At a convenient time during the months of March, April, and May, 1982, the house was visited, and a bed was selected for sampling. A vacuum cleaner containing a fresh disposable bag was used to vacuum the top of the mattress for 1 minute. The bag was taken to the laboratory, and the dust was emptied into a sealable plastic bag and stored at -20°C until shipment. When all the samples had been collected, they were packed without refrigeration and sent by air to the laboratory in Copenhagen. Since the package

went astray in shipment, it is estimated that the samples stayed at ambient temperature for about 4 weeks. Because the mites were killed by placing in a freezer and because the antigens for which the tests were being made are quite stable, no artifacts should have been introduced.

Preparation of samples

On arrival, the samples were weighed, and 42 samples were extracted 1:10 wt/vol in 0.005 mol/L of ammonium hydrogen carbonate for 3 hours at 20°C . The rest of the bags contained amounts of material too small ($<200\text{ mg}$) to be processed. Two milliliters of the 1:10 extracts were stored at -20°C . The remaining volumes of 37 samples were freeze-dried. Reconstitution of freeze-dried extracts took place in 1/20 of the original volume. These preparations are referred to as 2:1 extracts in the following.

Standard preparations. Preparations were (1) freeze-dried, dialyzed, whole mite-culture extracts of *D. pteronyssinus*, *D. farinae*, and *D. microceras*, containing 64 μg of Dp 42, 86 μg of Df 6, and 69 μg of Dm 6, respectively, per milligram of extract,¹⁰ (2) freeze-dried, dialyzed, cat-dander extract (ALK Laboratories, Copenhagen, Denmark, batch No. 209), containing 16 μg of Ag 1 as described by Ohman et al.¹¹ (equal to our previously described Ag 4¹²), per milligram of extract, according to the producer's certificate of analysis, (3) freeze-dried, dialyzed, dog-dander extract,¹³ (4) freeze-dried, dialyzed, horse-dander extract,¹³ containing approximately 250 μg of Ag 11 per milligram of extract, and (5) HSA, purchased from Behringwerke, Germany.

RIE. Five or 15 μl of the 2:1 ($n = 37$) or 1:10 extracts ($n = 5$) and four concentrations of standard antigen were applied in RIE, which was performed according to the method of Lowenstein.¹⁵ The rabbit antibodies used were the following: (1) mite: a 1:1 mixture of monospecific antibodies to Dp 42 and to Dm 6,¹⁰ (2) cat: oligospecific rabbit antibodies to Cat Ag 1, obtained from polyspecific anticat-dander antibodies by immunoabsorption on CNBr-activated Sepharose 4B, coupled with a pooled Sephadex G-75 fraction (molecular weight $>60,000$) of a cat-pelt extract. This antibody elicited a strong, sharply delineated precipitate with Ag 1 in RIE and to a weak, fuzzy precipitate with a second antigen, easily allowing quantitation of Ag 1, (3) horse: monospecific rabbit antibodies to horse Ag 11, raised by immunization with purified Ag 11,¹⁴ and (4) HSA: antibodies to HSA, purchased from Dakopatts, Copenhagen, Denmark. The lowest measurable quantities of antigen were 2 $\mu\text{g}/\text{ml}$ of Dp 42/Dm 6, 0.2 $\mu\text{g}/\text{ml}$ of cat Ag 4, and 1.5 $\mu\text{g}/\text{ml}$ of horse Ag 11.

CCIE. Five microliters of the 2:1 ($n = 32$) or 1:10 extracts ($n = 10$) and twofold dilutions were applied in CCIE according to the method of Lind et al.¹⁶ Antibodies to cat- and dog-dander antigens were polyspecific antibodies, rendered specific by immunoabsorption on a CNBr-activated Sepharose 4B column coupled with human-serum protein. These antibodies elicited no reaction with human-serum or human-dander extract in CCIE. The antigen content was expressed as a titer (reciprocal of the last twofold dilution eliciting a visible precipitate).

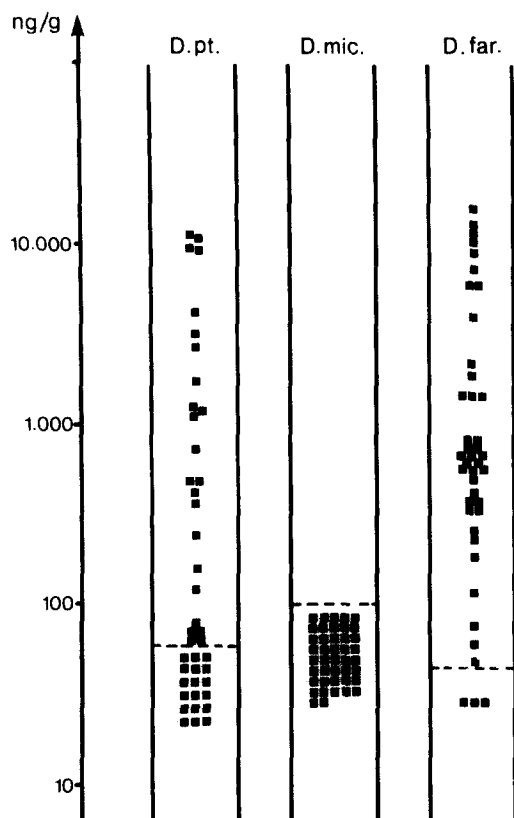


FIG. 1. Levels of house dust-mite allergen in 42 mattress samples, extracted 1:10 wt/vol and analyzed by ELISA. D.pt, Dp 42 (equal to P₁); D.mic., Dm 6; and D.far., Df 6. Vertical axis, nanogram of allergen per gram of dust. Broken lines indicate detection limits.

ELISA. The 1:10 extracts were assayed in the three ELISAs for Dp 42, Df 6, and Dm 6, respectively.¹⁷ In short, 100 μ l of dust extracts and dilutions (1:10, 1:100, and 1:1000) were transferred to wells in polystyrene microtiter trays, previously coated with affinity-purified, monospecific, rabbit antibodies from separate immunizations with major allergens Dp 42, Df 6, and Dm 6. After overnight incubation and repeated washings, horse radish peroxidase-conjugated rabbit antibodies to allergens Dp 42, Df 6, and Dm 6, rendered species specific by Sepharose column immunoabsorption, were added. After a 1-hour incubation period and repeated washings, substrate (hydrogen peroxide/orthophenylenediamine) was added to each well, and reaction was carried out for 15 minutes in darkness. Reading of the absorption at 492 nm took place 5 minutes after addition of 1.0 mol/L of sulfuric acid. Standard response curves were constructed from reference whole mite culture-extract solutions of each species with major allergen concentrations in the range 1.2 to 600 ng/ml. On each tray, wells with concentrated solutions (64,000 to 90,000 ng/ml) of the two other species were run as specificity controls to ensure absence of cross-reactivity in the assay. Assay sensitivity was 7, 5, and 40 ng/ml for allergens Dp 42, Df 6, and Dm 6, respectively. Data obtained with these ELISAs

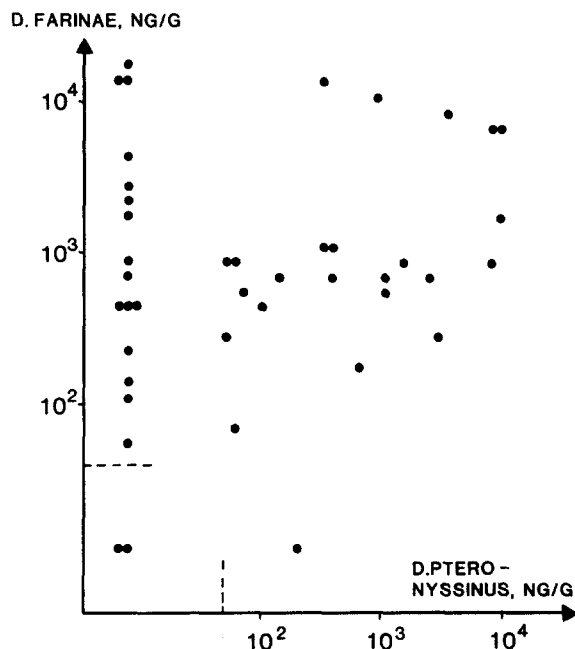


FIG. 2. Levels of *D. farinae* allergen plotted against level of *D. pteronyssinus* allergen. Figures on axes denote nanogram of allergen per gram of dust. Broken lines indicate detection limits.

are closely correlated with the number of mite specimens counted by microscopy, regarding both levels of allergen and species composition, and are not affected by the presence of human serum or epithelial proteins.¹⁷

Statistics

Correlations were tested by the Spearman rank correlation test or linear regression analysis as indicated.

RESULTS

Mite antigens

Of 42 dust samples investigated in ELISA, 40 contained *Dermatophagoides* mite antigen with values ranging from 50 (detection limit) to 16,000 ng/gm (median, 1250 ng/gm). *D. farinae* occurred most frequently, 39/42 positive samples (Fig. 1) with values from 50 to 15,000 ng/gm (median, 650 ng/gm). *D. pteronyssinus* antigen was recorded in 24 samples with values from 70 to 11,000 ng/gm (median, 75 ng/gm). None of the samples contained measurable quantities of *D. microceras* antigen. Levels of *D. farinae* and *D. pteronyssinus* did not correlate ($r_s = 0.21$; $p > 0.05$; $n = 42$). A considerable fraction (16/40) of the mite-positive samples contained detectable antigen of *D. farinae* only (Fig. 2). One sample was positive to *D. pteronyssinus* only, but three additional samples contained more than 10 times as much Dp 42 than Df 6.

Positive RIE reaction to mite was obtained in

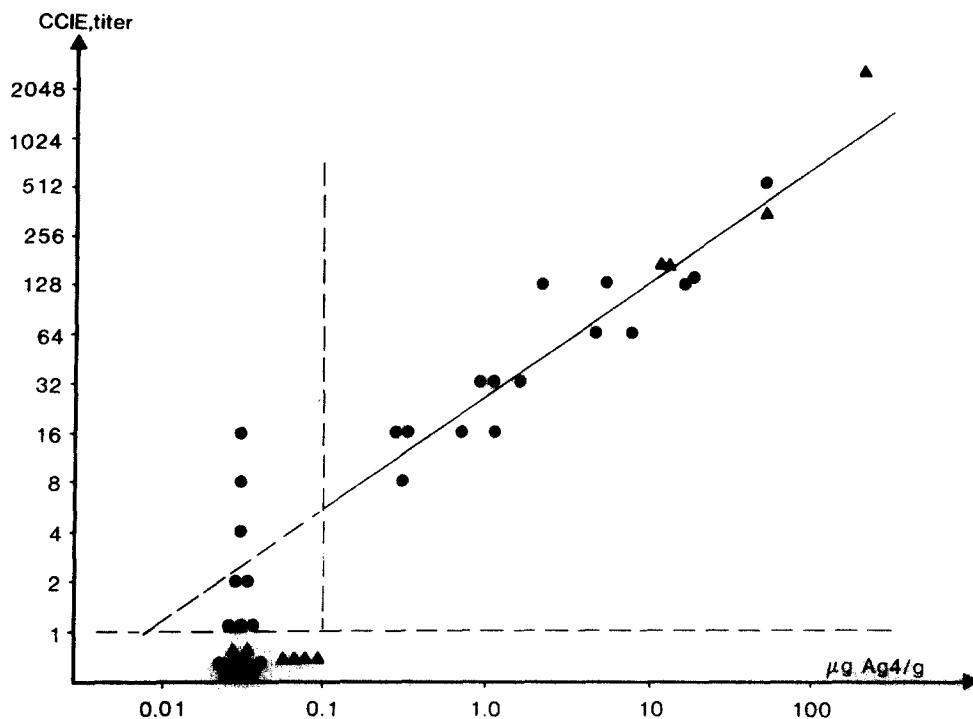


FIG. 3. CCIE titer for cat plotted against cat Ag 1 by RIE. CCIE performed with 2:1 wt/vol extracts (●). CCIE performed with 1:10 wt/vol extracts (▲). Broken lines indicate detection limits.

21/37 2:1 extracts and 2/5 of the 1:10 extracts. The frequent occurrence of double rockets and rockets with a morphology different from the standards (significantly weaker Coomassie staining) precluded a quantitation of antigen. Reactions were thus scored as positive/negative only.

Samples with positive RIE reaction elicited combined ELISA values in the range of 700 to 16,000 ng/gm ($n = 23$; median, 3300 ng/gm), whereas RIE-negative samples ranged from <50 to 700 ng/gm ($n = 16$; median, 275 ng/gm).

Animal-dander antigens

A positive RIE reaction for Cat Ag 1 was obtained in 19/36 2:1 extracts and 1/5 1:10 extracts, values ranging from 0.3 to 240 µg of Ag 1 per gram of dust. Positive CCIE reaction was found in 23/30 2:1 extracts and 4/10 1:10 extracts, titers ranging from 1 to 512. Fig. 3 illustrates the graphic plot of CCIE titer versus Ag 1 content. A linear correlation was found between the log CCIE titer and the log Ag 1 content (Fig. 3) for samples positive in both RIE and CCIE ($r = 0.95$; $p < 0.01$; $n = 19$). The slope of the regression line was 0.70 (SD 0.05), indicating a non-linear relationship between CCIE titer and Ag 1 content.

Positive CCIE reaction to dog antigens were re-

corded in 20/32 2:1 extracts and 5/10 1:10 extracts, titers ranging from 1 to 1024.

A weak correlation was found between CCIE titer values for dog and cat ($r_s = 0.36$; $p < 0.05$; $n = 40$).

The distribution of titers is presented in Table I.

Positive RIE reaction to horse antigen (>1.5 µg of Ag 11 per gram) was observed in two dusts (3 and 7 µg/gm, respectively). One sample eliciting a precipitate with deviant morphology may have contained degraded antigen.

Concentrations of HSA measured by RIE ranged from 15 to 640 µg/gm of dust (median, 100 µg/gm). No correlation existed between levels of HSA and combined mite allergen by ELISA ($r_s = 0.07$; $p > 0.05$) or between HSA and CCIE titer to cat ($r_s = 0.16$; $p > 0.05$) or dog ($r_s = 0.03$, $p > 0.05$).

DISCUSSION

We have studied the prevalence of common indoor allergens (house dust mites and animal danders) in a cross section of homes in a metropolitan area and used the recorded data for investigation of some quantitative aspects of the immunochemical techniques used.

House dust-mite (*Dermatophagoides* sp.) allergens were detected by ELISA in 95% of the samples. The frequency of occurrence was higher for *D. farinae*

TABLE I. Distribution of titers to cat and dog antigens in 2:1 wt/vol extracts

	Cat CCIE						Total
	<1	1-2	4-8	16-32	64-128	>128	
Dog CCIE							
<1	4	1	1	6	0	0	12
1-2	1	1	0	2	0	0	4
4-8	1	2	0	0	1	1	5
16-32	1	1	0	0	3	1	6
64-128	0	0	0	0	2	1	3
>128	0	0	2	0	0	0	2
Total	7	5	3	8	6	3	32

(92%) than for *D. pteronyssinus* (57%), which seldomly occurred alone (Figs. 1 and 2). A very similar species composition was found by microscopic examinations in Ohio,¹⁸ whereas in Texas,¹⁹ a complete dominance of *D. farinae*, and in Georgia,²⁰ a relative dominance of *D. pteronyssinus* was found. In California²¹ both species appear frequently, with *D. pteronyssinus* dominating in the coastal areas.

Longitudinal studies of Danish dusts have demonstrated a striking constancy of the species composition in individual habitations from year to year.* Detailed studies of the dynamics of mite populations in mattresses revealed no essential seasonal difference between *D. pteronyssinus* and *D. farinae*.²² Thus, the mite-species composition appears to be a specific characteristic of a given geographic area.

The amount of antigen corresponding to one mite detected by microscopy is 40 to 70 ng of Dp 42/Df 6 as an average for all life stages.¹⁷ Because only 50% of the mattress dusts sampled in the present study contained mite allergen >1200 ng/gm (approximately 1 mite per 0.05 gr of dust), the levels of mite infestation appear lower than in the Ohio study¹⁸ in which at least one mite per 0.05 gr of dust was found in 25/26 homes.

Several factors, like actual site of the home sampled, duration of sampling, equipment, and season of the year will affect the allergen levels recorded. Also, in population studies, the selection of homes (mite-allergic/healthy persons) may exert influence.²³ In a study of mattress dusts collected in Copenhagen, Denmark, during the month of October, antigen levels up to 300,000 ng/gm were measured by ELISA with 20/22 samples >1200 ng/gm.¹⁷ Similar figures were obtained in the United Kingdom by RIA for Ag P₁ (equal to DP 42) in mattresses and blankets.²⁴ In the

present study, the mattress dusts originated from a normal population, and collection took place at a season unfavorable to mites.¹⁸ Thus, the observed degree of infestation is probably a minimal value. It is, however, also possible that different cultural habits in Europe and the United States, such as the type of mattress covering used, may influence the degree of mattress colonization by mites. Such factors were suggested by Arlian et al.¹⁸ as an explanation for the low mattress levels of mites compared to floor and furniture mite levels observed in Ohio.

Although mattress dust in Baltimore had somewhat lower concentrations of mite allergen than mattresses in some European countries, the frequency with which both *D. pteronyssinus* and *D. farinae* were found indicates that these are significant allergens in the Baltimore area and that both allergens (not just *D. farinae* alone) need to be considered in patients with respiratory symptoms consistent with dust allergy.

The combined ELISA values correlated well with results obtained with the gel-precipitation technique, RIE. The sensitivity of ELISA was more than tenfold higher (in terms of nanograms of antigen per grams of dust), despite the fact that most extracts analyzed in RIE were concentrated 20 times for freeze-drying. Furthermore, ELISA elicited valuable information on the species distribution.

House dust mites are the source of multiple allergens, and the significance of the various allergens varies both between and within patients.^{25, 26} The techniques used in the present study make use of the best investigated and, on a population basis, most potent allergens, Dp 42 (equal to P₁) and analogues, as markers for levels of mite infestation. In at least two independent studies,^{17, 24} excellent correlations were obtained between levels of counted mite specimens in mattress dusts and quantitations of well-defined single components. It is, however, not known whether the

*Mosbech H, Schwartz B, Lind P. In preparation.

various other allergens may react in different ways to factors like breakdown, accumulation in the dust, and dispersion to other home locations. This aspect clearly deserves further study.

The frequency of CCIE detection of cat and dog antigens (77% and 63%, respectively, in the 2:1 extracts) was higher than the frequencies previously observed²⁷ for room dusts in Denmark (46% and 37%, respectively), which were, however, investigated as 1:2 wt/vol extracts and thus fourfold less concentrated. Bed and mattress would not be expected to be the optimal collection site for detection of animal-dander antigen. The figures presented here, however, appear to indicate an efficient dispersion of the antigen-bearing particles around the habitation.

A strong correlation existed between CCIE titer to cat and RIE quantitation of the major allergen, Ag 1, of Ohman et al.¹¹ (Fig. 3). CCIE was apparently able to detect about 10 times lower amounts of cat antigen than RIE. Regression analysis, however, demonstrated a nonlinear relationship, the log-log slope being significantly different from unity. Thus, CCIE should be considered a semiquantitative determination, and titers should not be translated into weight amounts of major allergen.

Up to fortyfold differences were found in the concentration of HSA per gram of dust with 640 µg/gm as the maximum. The content of HSA in pooled human dander (from electric shavers) is approximately 100 µg/gm as determined by RIE, but large variations between human individuals are found.* The data from the present study indicate that human skin scales contribute a dominating fraction of mattress dust weight. However, it is worth noting that in extreme cases, we observed mattress dust levels of major cat allergen as high as 240 µg/gm, a figure corresponding to the maximal 300 µg/gm of mite-excrement allergen (Dp 42 and analogues) observed in mattress dusts, collected in the most favorable season.¹⁷

The mattress dusts collected in the present study originated from a normal (nonallergic) population; therefore, it was not possible to correlate the findings with thresholds for allergic symptoms and provide clinically significant limits for allergen levels in dust. Accumulation of data like the data presented here obtained from allergic patients' homes and preferably compared to symptom scores will help define such values.

We gratefully acknowledge the skillful technical assistance of Gitte Nordskov Hansen and Anne Ravn Mortensen.

*Lind P. Unpublished observations.

REFERENCES

1. Dirksen A. Clinical vs. paraclinical data in allergy. *Dan Med Bull* 1982;29(suppl 2):5-72.
2. Vanto T, Koivikko A. Dog hypersensitivity in asthmatic children. *Acta Paediatr Scand* 1983;72:571-5.
3. Blythe ME. Some aspects of the ecological study of the house dust mites. *Br J Dis Chest* 1976;70:3-31.
4. Bronswijk JEMH. Hausstaubmilben, vorkommen, und bedeutung. *Allergologie* 1978;1:55-60.
5. Carswell F, Robinson DW, Oliver J, Clark J, Robinson P, Wadsworth J. House dust mites in Bristol. *Clin Allergy* 1982;12:533-45.
6. Korsgaard J. Preventive measures in mite asthma—a controlled trial. *Allergy* 1983;38:93-102.
7. Sarsfield JK, Gowland G, Toy R, Norman ALE. Mite-sensitive asthma of childhood: trial of avoidance measures. *Arch Dis Child* 1974;49:716-21.
8. Chapman MD, Platts-Mills TAE. Purification and characterization of the major allergen from *Dermatophagoides pteronyssinus*—antigen P₁. *J Immunol* 1980;125:587-92.
9. Lind P. Purification and partial characterization of two major allergens from the house dust mite, *Dermatophagoides pteronyssinus*. *J ALLERGY CLIN IMMUNOL* 1985;76:753-61.
10. Lind P. Demonstration of close physicochemical similarity and partial immunochemical identity between the major allergen, Dp 42, of the house dust mite, *D. pteronyssinus*, and corresponding antigens of *D. farinae* (Df 6) and *D. microceras* (Dm 6). *Int Arch Allergy Appl Immunol* 1986;79:60-5.
11. Ohman JL Jr, Lowell FC, Bloch KJ. Allergens of mammalian origin. III. Properties of a major feline allergen. *J Immunol* 1974;113:1668-77.
12. Løwenstein H, Lind P, Weeke B. Identification and clinical significance of allergenic molecules of cat origin: a part of the DAS 76 study. *Allergy* 1985;40:430-41.
13. Løwenstein H. Allergene von Katze, Hund, Rind und Pferd. *Allergologie* 1981;4:265-9.
14. Løwenstein H, Markussen B, Weeke B. Isolation and partial characterization of three major allergens of horse hair and dandruff. *Int Arch Allergy Appl Immunol* 1976;51:48-67.
15. Løwenstein H. Quantitative immunoelectrophoretic methods as a tool for the analysis and isolation of allergens. *Prog Allergy* 1978;25:1-62.
16. Lind P, Korsgaard J, Løwenstein H. Detection and quantitation of *Dermatophagoides* antigens in house dust by immunochemical techniques. *Allergy* 1979;34:319-26.
17. Lind P. Enzyme-linked immunosorbent assay (ELISA) for determination of major excrement allergens of the house dust mite species *D. pteronyssinus*, *D. farinae*, and *D. microceras*. *Allergy* 1986;41:442-51.
18. Arlian LG, Bernstein IL, Gallagher JS. The prevalence of house dust mites, *Dermatophagoides* spp, and associated environmental conditions in homes in Ohio. *J ALLERGY CLIN IMMUNOL* 1982;69:527-32.
19. Hall CC, McMahon B, Sams JT. Collecting and rearing *Dermatophagoides farinae* Hughes, a mite from house dust. *Ann Allergy* 1971;29:81-5.
20. Smith TF, Kelly LB, Heymann PW, Wilkins SR, Platts-Mills TAE. Natural exposure and serum antibodies to house dust mite of mite-allergic children with asthma in Atlanta. *J ALLERGY CLIN IMMUNOL* 1985;76:782-8.
21. Mulla MS, Harkrider JR, Galant SP, Amin L. Some house dust mite control measures and abundance of *Dermatophagoides* mites in Southern California (Acarina: Pyroglyphidae). *J Med Entomol* 1975;12:5-9.

22. Dusbabek F. Dynamics and structure of mixed populations of *Dermatophagoides farinae* and *D. pteronyssinus*. *Rec Adv Acarology* 1979;2:173-7.
23. Korsgaard J. Mite asthma and residency: a case-control study on the impact of exposure to house dust mites in dwellings. *Am Rev Respir Dis* 1983;128:231-5.
24. Tovey ER, Chapman MD, Wells CW, Platts-Mills TAE. The distribution of dust mite allergen in the houses of patients with asthma. *Am Rev Respir Dis* 1981;124:630-5.
25. Lind P, Løwenstein H. Identification of allergens in *Dermatophagoides pteronyssinus* mite body extract by crossed radioimmuno-electrophoresis with two different antibody pools. *Scand J Immunol* 1983;17:263-73.
26. Lind P, Weeke B, Løwenstein H. A reference allergen preparation of the house dust mite *D. pteronyssinus*, produced from whole mite culture—a part of the DAS 76 study. Comparison with allergen preparations from other raw materials. *Allergy* 1984;39:259-74.
27. Schwartz B, Lind P. Immunochemical methods for investigations of allergic patients' environment: results of elimination treatment of the allergen source [Abstract]. *J ALLERGY CLIN IMMUNOL* 1984;73:156.

Characterization of mononuclear cell-derived histamine release enhancing factor

Robert E. Younger, M.D., Henry G. Herrod, M.D.,
Phillip L. Lieberman, M.D., William R. Valenski, B.S., Robert L. Trouy, M.S.,
and Lloyd V. Crawford, M.D. *Memphis and Chattanooga, Tenn.*

Histamine release enhancing factor (HREF) is a product of phytohemagglutinin-stimulated mononuclear cells that substantially augments in vitro IgE-mediated basophil histamine release. The factor is stable at 56° C and has a molecular weight in the 10,000 to 30,000 dalton range. The magnitude of HREF activity produced is dependent on the concentration of mononuclear cells cultured and the final concentration of HREF during basophil challenge. The HREF phenomenon could not be attributed to phytohemagglutinin, α - or γ -interferon, arachidonic acid metabolites, or interleukin-1 or 2. HREF appears to be a unique cytokine of potential importance in the immunology of inflammatory and atopic processes. (J ALLERGY CLIN IMMUNOL 1987;80:547-57.)

The ability of mast cells and basophils to synthesize and release chemical mediators is an integral component of the host inflammatory response. Major advances have been made in our understanding of degranulation mechanisms that operate in these cells and of agents, both endogenous and pharmacologic, that influence or trigger degranulation. The importance of mononuclear cells, particularly T-lymphocytes, in the initiation and modulation of various immune responses is well established,¹ but only recently have

Abbreviations used

HREF:	Histamine release enhancing factor
DHRF:	Direct histamine-releasing factor
IH:	Intrinsic histamine
PBMC:	Peripheral blood mononuclear cells
CM:	Conditioned media
HR:	Histamine release
PIPES:	Piperazine- <i>N</i> , <i>N'</i> -bis(2-ethanesulfonic acid)
RIA:	Radioimmunoassay
MW:	Molecular weight
IL-1, IL-2:	Interleukin-1 and 2
PHA:	Phytohemagglutinin
LT:	Lymphocyte transformation

From the Section of Allergy-Immunology, Department of Pediatrics, University of Tennessee Center for the Health Sciences, LeBonheur Children's Medical Center, and the Department of Medicine, Baptist Memorial Hospital, Memphis, Tenn.

Received for publication Aug. 5, 1985.

Accepted for publication March 24, 1987.

Reprint requests: Robert E. Younger, M.D., University of Tennessee Center for the Health Sciences, Chattanooga Unit, 960 East Third St., Suite 306, Chattanooga, TN 37403.

there been studies describing mononuclear cell products that may directly induce HR from basophils^{2,4} or augment IgE-mediated degranulation.⁵ Among the factors described that may enhance mediator release