

Immunochemical measurement of airborne mouse allergens in a laboratory animal facility

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The major allergens from the mouse, including mouse serum albumin (MSA) and mouse urinary protein complex, are present in various concentrations in urine, serum, and pelts of mice. Subjects clinically sensitive to laboratory animals were screened by the RAST for specific IgE antibodies to mouse urine (MU), MSA, and mouse pelt extract (MPE). Twenty-four hour air samples from both a mouse-care room (2000 mice) and an immunology laboratory (five to 100 mice) were collected with a high-volume air sampler that retained particles greater than 0.3 μm with 95% efficiency. Eluates from the filter sheets were dialyzed, lyophilized, reconstituted in water, and used as fluid-phase inhibitors in the MU RAST and MPE RAST. MPE allergenic activity could be quantitated in filter eluates from both locations, but MU allergenic activity could be quantitated only in the mouse-care room. Airborne allergen content ranged from 1.8 to 825 ng/m³ and varied with both the number of mice and the degree of work activity in the rooms. The sampling and assay techniques described can be used to accurately quantitate amorphous airborne allergens. (J ALLERGY CLIN IMMUNOL 69: 522, 1982.)

Fifteen percent to 25% of laboratory scientists and technicians who work closely with laboratory animals develop hypersensitivity to those animals.¹⁻⁶ In fact, asthma due to animal exposure is now a prescribed industrial disease in Great Britain.⁴ Several laboratory mouse allergens have been described, including MSA^{7, 8} and MU.⁸⁻¹⁰ One of the allergenic components of the latter material has a molecular weight of 17,000 and may be identical to the mouse major urinary protein complex characterized earlier by Finlayson et al.¹¹

Lincoln et al.¹ attempted to quantify the mouse allergen in the workplace through three different techniques: morphologic identification of mouse dander in air samples obtained on Millipore filters, quantification of dust and extractable protein nitrogen in dust

collected with the air sampler, and double immunodiffusion in agar gel using rabbit antisera to mouse serum, dander, and urine. Although the results with the first two methods correlated positively with the number of animals in the room, these techniques were nonspecific in regards to allergen, and the double immunodiffusion was more qualitative than quantitative. Schumacher¹⁰ was able to show the presence of mouse allergen in the dust from a mouse-inhabited room by the RAST inhibition technique, but he was not able to quantitate the amount of allergen in the air.

In 1981 Agarwal et al.¹² reported a technique to immunochemically measure airborne allergens. Their technique involved collection of airborne particles on a filter sheet through which constant air flow was maintained. Particles greater than 0.3 μm in diameter were captured, eluted in suitable buffer, dialyzed, and lyophilized. These eluates were reconstituted and assayed for specific allergen activity by a RAST inhibition assay. With morphologically identifiable particulate allergens, i.e., ragweed pollen and *Alternaria* spores, a direct correlation was shown between the outdoor pollen and spore counts and the RAST inhibitory activity of eluates from filter sheets exposed to a known volume of air over a 24 hr period. The present study extended these observations by measuring

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Abbreviations used

RAST:	Radioallergosorbent test
MU:	Mouse urine
MSA:	Mouse serum albumin
MPE:	Mouse pelt extract
SRW:	Short ragweed

amorphous indoor airborne mouse allergens by the same technique.

MATERIALS AND METHODS

Antigen preparation

MU was collected from individual BALB/c males and BALB/cxATH males by manual expression of the bladder contents twice daily and was stored at -20°C until dialysis. The thawed MU was filtered through gauze and dialyzed at 4°C against 100 mM NH_4HCO_3 in Spectrapor-3 casing (mol. wt. cutoff approximately 3500). After 72 hr of dialysis the retentate was centrifuged at 20,000 rpm for 20 min. The resultant lipid layer was removed by aspiration, and the supernatant was decanted, lyophilized, and stored at 4°C . For testing, this dried material was reconstituted at 1 mg/ml (dry weight) in H_2O ; the protein content of this solution was 0.54 mg/ml by biuret analysis, with human serum albumin as a standard.

Pelts from six BALB/c mice were washed thoroughly in water, frozen in liquid nitrogen, lyophilized for 72 hr, and defatted with diethyl ether in a Soxhlet apparatus for 24 hr. These dry, defatted pelts were homogenized in a Virtis homogenizer and extracted in 240 ml of 100 mM phosphate-buffered saline overnight at 4°C with constant stirring. After centrifugation at 15,000 rpm for 30 min, the supernatant was dialyzed against 50 mM NH_4HCO_3 in Spectrapor-3 casing for 86 hr. The retentate was centrifuged at 15,000 rpm for 30 min, lyophilized, and stored at 4°C . For testing, the dried material was reconstituted at 1 mg/ml (dry weight) in H_2O ; by biuret analysis the protein content was 0.50 mg/ml.

MSA (fraction V; Sigma Chemical Co. St. Louis, Mo.) was dissolved in 200 mM NH_4HCO_3 and the fatty acids were removed by charcoal treatment according to the method of Chen.¹³ The albumin was partially purified by gel filtration through Sephadex G100 (Pharmacia, Piscataway, N. J.) on a 2.5 by 48 cm column equilibrated and eluted with 200 mM NH_4HCO_3 at 12 ml/hr.¹⁴ Two 277 nm absorbance peaks were obtained, one at the void volume and one coinciding with the elution volume of the bovine serum albumin marker. These two fractions were tested by immunoelectrophoresis against rabbit anti-whole mouse serum and rabbit anti-MSA (Cappel Laboratories, Cochranville, Pa.). Each fraction produced an identical banding pattern with each rabbit antisera, but only the second elution fraction was lyophilized, stored at 4°C , and used to prepare solid-phase RAST allergens and for skin testing. The

lyophilized material was reconstituted at 1 mg/ml (dry weight) in H_2O ; by biuret analysis the protein content was 0.45 mg/ml.

Skin testing

The lyophilized extracts of MU, MPE, and MSA were reconstituted (2 mg/ml) in 100 mM NaCl, sterilized by filtration through Millipore 0.22 μm filters, and diluted with glycerin to yield a 1 mg/ml solution in 50% glycerin. Prick skin tests were initially done with this solution, then intradermal skin tests were placed and scored according to the method of Norman.¹⁵ Tenfold dilutions were made using Sørensen's phosphate buffer with 0.5% human serum albumin. Nonallergic control subjects were intradermally skin tested with these materials to evaluate nonspecific irritant effects.

RAST

Cyanogen bromide activation of microcrystalline cellulose particles was performed according to the procedure of Adolphson et al.¹⁶ The MU and MPE described above were coupled separately to the activated microcrystalline cellulose with 50 mg of antigen/gm of cellulose; the MSA was coupled with 16 mg/gm of cellulose. These reagents were used to measure serum IgE antibodies to mouse allergens by the RAST procedure, as described by Adolphson et al.,¹⁶ and for RAST inhibition assays.

Patients

Subjects were recruited from an advertisement in the institution newsletter requesting individuals who had rhinitis, conjunctivitis, sneezing, or wheezing associated with laboratory animal contact. A questionnaire was completed by the 19 respondents, who also provided a serum sample for measurement of IgE antibody to various mouse allergen extracts. Sixteen of the 19 reported symptoms after contact with mice or rats. After IgE antibody results were available, six of these patients were selected for skin testing (Table I).

A plasmapheresis was obtained from these six subjects. After defibrination, the plasma obtained was pooled into three lots: an IgE anti-MU pool (patients 1, 2, 4, 5, and 6), an IgE anti-MPE pool (patients 1, 2, and 4), and an IgE anti-MSA pool (patients 2, 3, and 4). These pools were stored at -20°C until used.

Air sampling and allergen extraction

A high-volume air sampler (GMS 3210 Accu-Vol; General Metal Works, Inc., Cleves, Ohio) was used to collect airborne particles on a filter sheet (binderless glass fiber-sheets, 20 by 25 cm, grade GMS-810) through which a constant air flow was maintained. According to the manufacturer, the GMS 810 filter sheets retain particles greater than 0.3 μm with 95% efficiency. Ambient air was sampled continually over a 24 hr period in various areas of the Guggenheim research facility, and exposed filter sheets were eluted by a descending elution technique previously described by Agarwal et al.¹² The eluates were dialyzed

TABLE I. Symptoms, maxi-RAST results, and skin-test endpoints of subjects who donated sera for RAST inhibition studies

Subjects	Symptoms after exposure	RAST (% total counts bound)			Skin-test endpoint*		
		MSA	MU	MPE	MSA	MU	MPE
Sensitive							
1	R, O, U, W	0.76	5.18	1.04	10 ng/ml	1 ng/ml	10 ng/ml
2	R, S, U	3.1	7.06	1.76	10 ng/ml	1 ng/ml	10 ng/ml
3	R, O, S, U, W	6.8	2.27	1.41	10 ng/ml	100 ng/ml	10 ng/ml
4	R, O, S	4.29	22.80	2.98	100 ng/ml	10 ng/ml	100 ng/ml†
5	O, S, U	0.59	5.88	0.87	1 ng/ml	1 ng/ml	1 ng/ml
6	R, O, S, U	0.32	8.60	0.65	1 ng/ml	1 ng/ml	1 ng/ml
Control							
1	None	0.29	0.13	0.28	>100 µg/ml	>100 µg/ml	>100 µg/ml
2	None	ND	ND	ND	>100 µg/ml	>100 µg/ml	>100 µg/ml

R = rhinitis; O = ocular itching; S = sneezing; U = urticaria; W = wheezing; ND = not done.

*Lowest concentration of extract (dry weight/ml) that produced a 2+ intradermal skin test.

†Patient had taken 8 mg of chlorpheniramine 3 hr prior to skin testing.

using Spectrapor-3 casing with distilled water, lyophilized, reconstituted in 1 ml of distilled water, and frozen at -20°C .

Measurement of allergenic activity

The eluates obtained above were assayed for allergenic content by a mini- or maxi-RAST inhibition procedure¹⁶ using the solid-phase allergens and IgE antibody pools described above. Standard curves were obtained by assay of known amounts of MU, MPE, and MSA in the same assay.

Isoelectrofocusing

MU, MPE, MSA, and concentrated filter eluates were tested by thin-layer isoelectrofocusing in 5% polyacrylamide gel slabs by the method of Awdeh et al.¹⁷ with a 2% ampholyte concentration and pH gradient of 2.5 to 6. Gels were stained with Coomassie brilliant blue.

RESULTS

Air sampling

Immunochemical analysis. Preliminary studies in a mouse-care room housing 2000 mice showed that there was little day-to-day variation in allergenic activity eluted from the filter paper. Therefore filter eluates from 4 consecutive days of sampling in this room were pooled to provide sufficient material for further studies. Preliminary studies also revealed that these eluates were maximally inhibitory in the MPE RAST and the MU RAST; consequently studies with the solid-phase MSA were not continued.

To determine whether ambient levels of airborne mouse allergens were dependent on the number of mice or the amount of activity in various rooms, the sampler was operated in both the mouse-care room (2000 mice) and in an immunology laboratory, where an adjacent room housed a smaller number of mice

(<100). The sampler was operated in the laboratory for 2 consecutive days over a weekend, when the mice were separated from the laboratory by a closed door, and for 2 consecutive days during the week, when the adjoining door was open and mice were being killed in the laboratory.

Fig. 1 shows the MU RAST inhibitory activities of these eluates. The mouse-care room eluate contained an appreciable quantity of MU allergen, and the slope of this RAST inhibition curve is virtually identical to that of the MU standard. Minimal MU allergenic activity was found in the laboratory weekday filter eluate, but no MU allergenic activity could be shown in the laboratory weekend filter eluate. The MPE RAST inhibitory activities of the same eluates are shown in Fig. 2. Nearly identical findings were noted, except that some MPE allergenic activity could also be shown in the laboratory weekend filter eluate.

Allergen specificity was tested by adding SRW pollen extract (0.1 ml of 1:50 concentration) as a fluid-phase inhibitor to all three mouse RAST assays; no significant inhibition occurred in any of the three. Conversely, filter eluates from the mouse room were used as fluid-phase inhibitors in a SRW RAST with pooled sera from SRW pollen-sensitive individuals. None of these eluates demonstrated any inhibitory activity.

Isoelectrofocusing analysis. Fig. 3 shows the thin-layer isoelectrofocusing patterns produced by MU, MPE, MSA, and concentrated mouse room filter eluate. The filter eluate showed bands that appeared to be common to those produced by the MU, MPE, and MSA. Of note was the near identity of the MSA pattern and the MPE pattern.

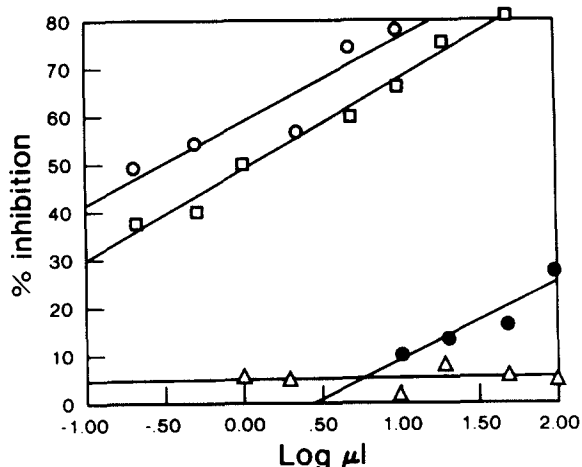


FIG. 1. MU RAST inhibition using MU standard (1 mg dry weight/ml) ($\circ$) and filter eluates from mouse-care room ($\square$), weekend day in immunology laboratory (Δ), and working day in same laboratory ($\bullet$). Values shown are means of duplicate determinations made by the mini-RAST procedure.

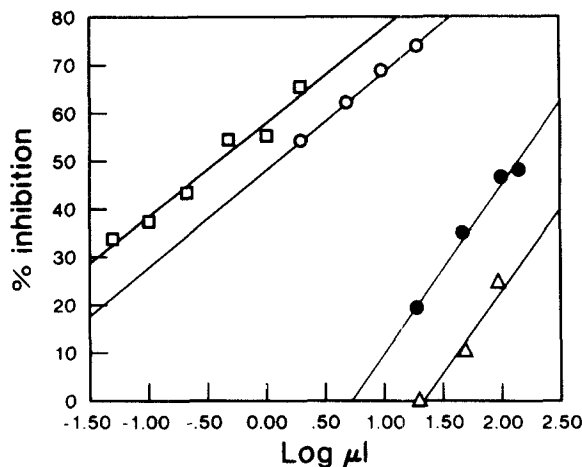


FIG. 2. MPE RAST inhibition using MPE standard (1 mg dry weight/ml) ($\circ$) and filter eluates from mouse-care room ($\square$), weekend day in immunology laboratory (Δ), and working day in the same laboratory ($\bullet$). Values shown are means of duplicate determinations using the maxi-RAST procedure.

DISCUSSION

By RAST inhibition studies, Schumacher¹⁰ was able to detect MU and MPE allergens in dust samples and air-conditioning filters from a mouse care room. Although these studies were quantitative in that the amount of allergenic activity could be calculated per gram of dust, the studies did not measure the quantity of airborne allergen. The technique reported by Agarwal et al.¹² provides this quantitation, measuring known volumes of air.

In our study we applied this technique to a heavy allergen exposure area (mouse-care room) and a light exposure area (immunology laboratory). By measurement of the protein content of mouse allergen standards, comparison of the quantities of eluates or mouse allergen standards required for 50% RAST inhibition, and assumption of 100% recovery in elution of allergen from filter sheets, we estimated that the mouse-care room air contained approximately 825 ng/m³ MPE protein and 59 ng/m³ urine protein. Using similar computations we estimated that the amount of MPE protein in the air on a weekday in the immunology laboratory, when five mice were in the room, was 1.8 ng/m³, or less than 1% of the airborne MPE in the mouse-care room.

Lincoln et al.¹ suggested the use of top covers for animal cages to decrease the dispersal of dust. The animal cages used in the rooms surveyed in this study are covered, but the wood shavings are dumped into garbage cans when these cages are cleaned, releasing much dust into the ambient air. The covers are also removed when mice are retrieved for study, and dust

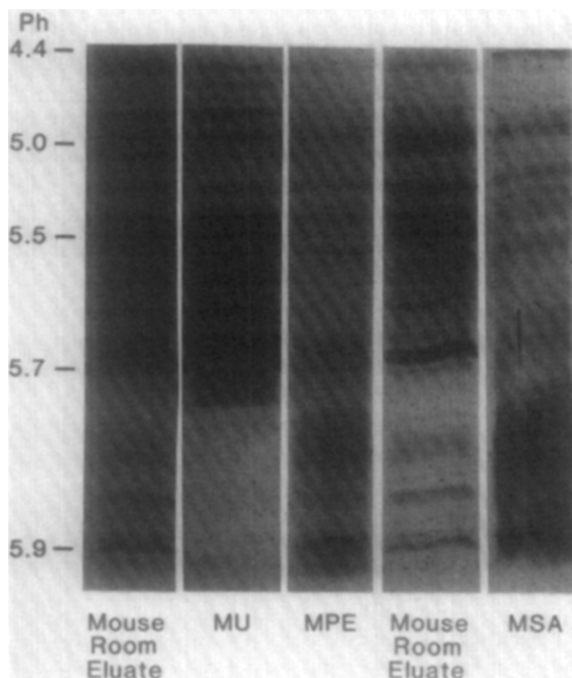


FIG. 3. Thin-layer polyacrylamide gel isoelectrofocusing (pH 3 to 6) of MU, MPE, MSA, and concentrated mouse room filter eluate; anode is at the top. Samples were applied at cathode side of gel.

is again raised at such times. It would be of interest to modify the procedures and materials used in these care facilities and evaluate the efficacy of such changes by quantitatively measuring the amorphous airborne allergen with the method presented. It would also be interesting to expose mouse-sensitive subjects

to different mouse-care rooms while sampling the ambient air, permitting the correlation of allergen levels with the clinical symptoms produced.

By thin-layer isoelectric focusing experiments we showed that the air sampler filter eluates produced protein bands that appeared common to all three mouse allergen preparations. This finding corroborates most of the results from the RAST inhibition experiments. Although the isoelectrofocusing patterns of MSA and MPE were similar, MSA was not well detected by MSA RAST inhibition. This discrepancy may have been because the MSA used for solid-phase coupling was partially purified by gel filtration, whereas the MSA used for isoelectrofocusing was MSA fraction V as supplied by Sigma Chemical Co.

Even though the same mouse allergens were used for both skin tests and RAST, it is interesting to note that the skin-test endpoint titration of the subjects did not correlate significantly with the RAST determinations. Larger numbers of patients would have to be studied to explore this relationship.

We conclude that airborne mouse allergens are present in various quantities in direct relationship to the number of mice present and that the immunochemical air sampling technique described by Agarwal et al.¹² can be used to measure such amorphous airborne allergens.

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REFERENCES

1. Lincoln TA, Bolton NE, Garrett AS Jr: Occupational allergy to animal dander and sera. *J Occup Med* **16**:465, 1974.
2. Lutsky II, Neuman I: Laboratory animal dander allergy. I. An occupational disease. *Ann Allergy* **35**:201, 1975.
3. Taylor G, Davies GE, Altounyan REC, Morrow Brown H, Frankland AW, Morrison Smith J, Winch R: Allergic reactions to laboratory animals. *Nature* **260**:280, 1976.
4. Cockcroft A, McCarthy P, Edwards J, Andersson N: Allergy in laboratory animal workers. *Lancet* **1**:827, 1981.
5. Slovak AJM, Hill RN: Laboratory animal allergy: a clinical survey of an exposed population. *Br J Ind Med* **38**:38, 1981.
6. Rajka G: Ten cases of occupational hypersensitivity to laboratory animals. *Acta Allergol* **16**:168, 1961.
7. Levy DA: Allergenic activity of proteins from mice. *Int Arch Allergy Appl Immunol* **49**:219, 1975.
8. Siraganian RP, Sandberg AL: Characterization of mouse allergens. *J ALLERGY CLIN IMMUNOL* **63**:435, 1979.
9. Newman Taylor A, Longbottom JL, Pepys J: Respiratory allergy to urine proteins of rats and mice. *Lancet* **2**:847, 1977.
10. Schumacher MJ: Characterization of allergens from urine and pelts of laboratory mice. *Mol Immunol* **17**:1087, 1980.
11. Finlayson JS, Asofsky R, Potter M, Runner CC: Major urinary protein complex of normal mice: origin. *Science* **149**:981, 1965.
12. Agarwal MK, Yunginger JW, Swanson MC, Reed CE: An immunochemical method to measure atmospheric allergens. *J ALLERGY CLIN IMMUNOL* **68**:194, 1981.
13. Chen RF: Removal of fatty acids from serum albumin by charcoal treatment. *J Biol Chem* **242**:173, 1967.
14. Wahn U, Peters T Jr, Siraganian RP: Studies of the allergenic significance and structure of rat serum albumin. *J Immunol* **125**:2544, 1980.
15. Norman PS: Skin testing, in Rose NR, Friedman H, editors: *Manual of clinical immunology*, ed. 2., Washington, D. C., 1980, American Society for Microbiology, chapter 106, p. 789.
16. Adolphson CR, Yunginger JW, Gleich GJ: Standardization of allergens, in Rose NR, Friedman H, editors: *Manual of clinical immunology*, ed. 2. Washington, D. C., 1980, American Society for Microbiology, chapter 105, p. 778.
17. Awdeh ZL, Williamson AR, Askonas BA: Isoelectric focusing in polyacrylamide gels and its application to immunoglobulins. *Nature* **219**:66, 1968.