

21. Ayars GH, Altman LC, McManus MM, et al. Injurious effect of the eosinophil peroxidase-hydrogen peroxide-halide system and major basic protein on human nasal epithelium in vitro. *Am Rev Respir Dis* 1989;140:125-31.
22. Motojima S, Frigas E, Loegering DA, Gleich GJ. Toxicity of eosinophil cationic proteins for guinea pig tracheal epithelium in vitro. *Am Rev Respir Dis* 1989;139:801-5.
23. Gleich GJ, Flavahan NA, Fujisawa T, Vanhoutte PM. The eosinophil as a mediator of damage to respiratory epithelium: a model for bronchial hyperreactivity. *J ALLERGY CLIN IMMUNOL* 1988;81:776-81.
24. Wardlaw AJ, Dunnette S, Gleich GJ, Collins JV, Kay AB. Eosinophils and mast cells in bronchoalveolar lavage subjects with mild asthma: relationship to bronchial hyperreactivity. *Am Rev Respir Dis* 1988;137:62-9.
25. Brofman JD, White SR, Blake JS, Munoz NM, Gleich GJ, Leff AR. Epithelial augmentation of trachealis contraction caused by MBP of eosinophils. *J Appl Physiol* 1989;66:1867-73.
26. Barnes PJ, Cuss FMC, Palmer JBD. The effect of airway epithelium on smooth muscle contractility in bovine trachea. *Br J Pharmacol* 1985;86:685-91.
27. Flavahan NA, Aarhus LL, Rimele TJ, Vanhoutte PM. Respiratory epithelium inhibits bronchial smooth muscle tone. *J Appl Physiol* 1985;58:834-8.
28. Sasaki T, Shimura S, Okayama H, Sasaki H, Takishima T. Regulatory effect of airway epithelium on secretion of isolated submucosal gland from feline trachea [Abstract]. *Am Rev Respir Dis* 1986;133:A295.
29. White SR, Ohno S, Blake JS, Munoz NM, Gleich GJ, Leff AR. Spontaneous contraction and mechanism of epithelial activation in augmented contractility of tracheal smooth muscle elicited by eosinophilic major basic protein (MBP) in guinea pigs in vivo [Abstract]. *Am Rev Respir Dis* 1989;139:A84.
30. Barnes PJ. Asthma as an axon reflex. *Lancet* 1986;1:242-5.

Occupational challenge studies with laboratory workers allergic to rats

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To relate airborne Rat N I concentrations to airway responses in allergic laboratory workers, we collected allergen on personal monitors while workers and control subjects were exposed in a rat vivarium for 1 hour. Allergen concentrations ranged from <1.5 to 310 ng/m³ and were significantly (p < 0.001) higher during cage cleaning (19 to 310 ng/m³) than during quiet activity (<1.5 to 9.7 ng/m³). Among 12 rat-allergic volunteers, all had a nasal response during the exposure with an increase in nasal lavage histamine concentrations or TAME-esterase activity, but only five volunteers had a lower respiratory tract response (fall in FEV₁ >10%). Two of five allergic control subjects had a small change in nasal TAME-esterase activity, and none had a 10% fall in FEV₁. A dose response was demonstrated between the allergen concentrations and the intensity of the nasal allergic response. This occupational challenge procedure thus was able to measure the nasal and lower respiratory tract response to airborne allergen exposure in a work environment. It can be used to define further the determinants of this response. (J ALLERGY CLIN IMMUNOL 1990;86:63-72.)

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Supported by National Institutes of Health Grants HL30532, AI21073, and the Hospital for Consumptives of Maryland (Eudowood fund).

Received for publication Nov. 13, 1989.

Revised Feb. 26, 1990.

Accepted for publication March 12, 1990.

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Abbreviations used

OEC: Occupational exposure challenge

PEFR: Peak expiratory flow rate

Allergy to laboratory animals is an important occupational illness that affects 15% to 30% of laboratory workers.¹⁻⁴ As part of an ongoing project to examine risk factors for laboratory-animal allergy, we reported earlier that the airborne concentration of a

representative allergen (*Rat N I*) varied widely in workers' personal breathing zones (air within 1 foot radius of the head), depending on the tasks they performed.⁵ For instance, while workers were cleaning cages or manipulating conscious animals, concentrations as high as 69 ng/m³ were measured, whereas the median concentration was 3 ng/m³ when unconscious animals were handled or when no activity was taking place in a rat vivarium. These differences implied that certain laboratory tasks presented a greater risk for the sensitized worker. Based on data from persons allergic to cats⁶ that demonstrated that the airway response to animal allergens had a steep dose response, an increase in airborne allergen of tenfold to hundredfold should be enough to worsen symptoms significantly. To test this relationship in sensitized laboratory workers, we measured airborne rat allergen *Rat N I* concentrations in the personal breathing zones of sensitized workers while, at the same time, we were monitoring their nasal and lower respiratory tract responses. The changes encountered are the subject of the present article.

METHODS

Volunteers

Seventeen atopic young adults were recruited from The Johns Hopkins Medical Institutions and volunteered for the study (Table I). The protocol was approved by The Johns Hopkins Joint Committee on Clinical Investigation, and all volunteers gave their informed consent. Fifteen volunteers complained of airway symptoms when they were exposed to laboratory rats, and 12 also had positive skin tests to rat-pelt antigen. These 12 volunteers were designated rat-allergic volunteers. Five atopic volunteers with negative skin tests to rat-allergen extract were designated atopic control subjects. Eleven of the rat-allergic volunteers and all but one of the atopic control subjects had positive skin tests to other inhalant allergens. Serum IgE antibody to rat allergen ranged from <1 to 2037 U/ml (median, 85 U/ml) in the volunteers and from <1 to 63 U/ml (median, <1 U/ml) in the control subjects. One volunteer had chronic asthma requiring medications, including beclomethasone, theophylline, and albuterol. Two volunteers had methacholine inhalation-challenge results in the severe range, and six other volunteers were in the moderate range,⁷ but baseline values of FEV₁ and PEFR were normal.⁸

Skin tests

Skin testing was performed by a prick puncture method⁹ with glycerinated full-strength extracts of *Dermatophagoides farinae*, *Alternaria*, *Helminthosporium*, *Aspergillus*, timothy grass, short ragweed, white oak, cat, dog, guinea pig, rabbit, mouse, and rat pelt (Hollister-Stier Laboratories, Spokane, Wash.).

IgE antibody to rat allergen

Serum IgE antirat antibody was measured with an ELISA antibody. Polystyrene microtiter plates (Nunc GmbH, Roskilde, Denmark) were coated with a dilution of rat-allergen extract of hair and urine containing 0.05 µg *Rat N I* allergen provided by Hollister-Stier Laboratories. Test serum or dilutions (1:5 to 1:2560) of a reference serum from a single highly sensitized donor were incubated in the wells overnight. The plates were then washed, incubated with alkaline phosphate-conjugated anti-IgE (Kirkegaard & Perry Laboratories, Gaithersburg, Md.), and developed with p-nitrophenyl phosphate. Reference serum dilutions of 1:640 or less contained detectable antibody activity. The reference serum was assigned a nominal potency of 1000 arbitrary units per milliliter.

Methacholine challenge

Methacholine challenges were performed according to the method of Chai et al.¹⁰ Methacholine (Provacholine; Hoffman La Roche, Nutley, N.J.) was nebulized from a Devilbiss 646 nebulizer (Devilbiss Co., Somerset, Pa.) activated by a nebulization dosimeter. Spirometry was performed on a Stead-Wells instrument with automated derivation of FVC, FEV₁, FEV₃, PEFR, and ratio of expiratory flow to inspiratory flow at 50% of FVC (Eagle II, Warren E. Collins, Inc., Braintree, Mass.), and results were compared to published norms.⁸ Baseline FEV₁ was measured in triplicate and had to be within 80% of predicted norms to proceed. After five inhalations of buffered saline, FEV₁ had to fall within 10% of baseline. Five inhalations of increasing doses of methacholine (0.1, 1.0, 3, 10, and 25 mg/ml) were administered, and the test was stopped when FEV₁ had fallen 20% from control values. Results were expressed as the extrapolated dose causing a 20% fall in FEV₁.

Occupational challenge

OEC consisted of standing in an animal holding room for 1 hour while airborne *Rat n I* concentrations, symptoms, nasal lavage, and spirometry were measured.

Before the OEC certain medications that might modify the challenge were withheld. These included antihistamines (2 days), theophylline, and oral β-adrenergic agonists (12 hours), inhaled β-adrenergic agonists (4 hours), corticosteroids (4 hours), and cromolyn (12 hours).

The challenges were conducted in one of three rat vivaria. In each vivarium, floors were swept and hosed daily, and the cage litter was changed biweekly. Ventilation was provided through a filtered single-pass system that delivered 19.7 to 21.9 room volume changes in 1 hour (540 to 560 cu ft/min). In one vivarium, 35 to 62 rats were housed in cages with coarse wire mesh floors and shallow litter pans underneath. Cage cleaning consisted of emptying the soiled litter from these trays into a large open container and replenishing the litter. In the other two vivaria, 206 to 308 rats were housed in plastic cages with litter on the bottom; here, cage cleaning consisted of transferring the rats to clean

TABLE I. Occupational challenge volunteers: Background and history of rhinitis and asthma*

Sex	Age	Skin tests		Serum IgE antirat (U/ml)	PD ₂₀ FEV ₁	FEV ₁ (% pred)	PEFR (% pred)	Symptoms to allergens		
		Rat (mm)	Other pos					Rat	Other	
Rat-allergic volunteers										
1	F	40	2	5	301	110	98	104	AS, AR	AS, AR
2	M	31	7	5	122	112	108	102	AR	AR
3	M	36	10	6	101	24	89	74	AS, AR	AS, AR
4	F	33	9	1	274	115	97	115	AS, AR	
5	M	35	3	1	19	>195	92	119	AR	AR
6	F	25	3	5	<1	185	98	108	AR	AR
7	F	26	3	6	69	91	102	90	AS, AR	AS, AR
8	F	27	6	2	53	73	107	106	AS, AR	AR
9	F	27	10	0	49	11	112	99	AR	AR
10	M	31	13	6	2037	89	98	106	AS	
11	F	27	17	1	69	52	102	123	AR	AS, AR
12	M	23	1	4	272	2.5	94	102	AS, AR	AS, AR
Atopic control subjects										
1	F	27	1	1	63	>195	117	102	AR	AR
2	F	23	0	1	<1	39	83	109	AR	AR
3	F	30	0	1	<1	28	118	111		AR
4	F	27	0	2	ND	>195	108	121		AR
5	F	43	0	4	ND	>195	104	106	AR	AR

Pos, Positive; AS, asthma; AR, allergic rhinitis; ND, not done.

*History of typical symptoms of asthma or allergic rhinitis for which medications are administered.

cages with fresh litter and then emptying the soiled litter into a large open container. During a challenge, either the volunteer cleaned the cages or animal-care personnel cleaned them with the volunteer standing within 3 feet of the litter receptacle. Low allergen-dose challenges were conducted in the same vivarium without cage cleaning.

Before the challenge, baseline nasal lavages and spirometry were obtained in a hallway adjacent to the rat vivarium. On preliminary testing, this location had detectable airborne allergen only twice (0.18 ± 0.04 ng/m³) in 16 collections. Every 15 minutes during the challenge, the volunteer returned to the hallway for approximately 2 minutes to repeat the nasal lavages and spirometry.

Symptoms (nasal and lung) were recorded every 2 to 5 minutes during the challenge. Nasal congestion and chest tightness were scored from 0 to 3, and the scores averaged during 15 minutes. The total number of sneezes and coughs were counted.

Nasal lavages were collected according to the technique of Naclerio et al.¹¹ Three baseline lavages were performed in quick succession. Five milliliters of normal saline was instilled into each nostril while the volunteer sat with the head tilted slightly backward and closed the posterior nasopharynx with the soft palate. After 10 seconds, the head was tilted forward and the mixture of saline and mucus was

expelled into a plastic basin. The fourth baseline and all subsequent lavage were accomplished with 2.5 ml of normal saline, and the saline-mucus was collected in the basin and was transferred to a 15 ml polystyrene tube. Generally, 80% or more of the lavage is returned. The tube was shaken vigorously to break the mucus gel and centrifuged at 1500 g for 15 minutes. Histamine was analyzed on acidified aliquots of the supernatant according to the automated procedure of Siraganian,¹² which, in our laboratory, has a sensitivity of 0.5 ng/ml. In another aliquot, enzymes having arginine esterase activity were assayed according to the method of Imanari et al.,¹³ based on the liberation of tritiated methanol from the synthetic substrate TAME. Although a protease with similar activity is produced by mast cells,¹⁴ serum and glandular kallikreins may contribute to TAME and also be measured by the assay.

Spirometry was measured on a Stead-Wells instrument (Eagle II, Warren E. Collins, Inc.) and compared to published norms.⁸ The challenge was not conducted if FEV₁ was <70% of predicted before exposure to rat allergen.

Airborne concentrations of Rat n I in the volunteer's personal breathing zone were obtained as previously reported⁵ with a battery-powered sampling pump (model P-2500, DuPont Co., Wilmington, Del.) calibrated at a flow rate of approximately 4.2 L/min. The pump was worn at

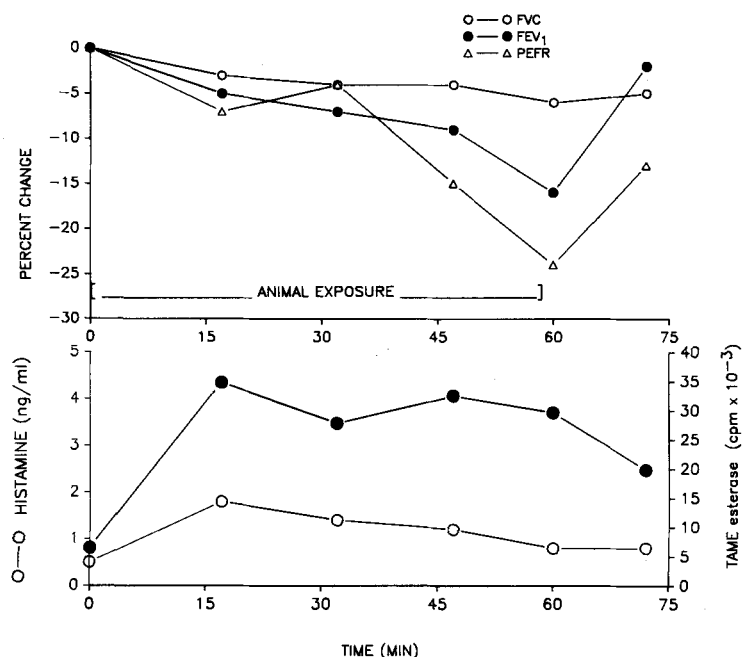


FIG. 1. A representative airway response to an occupational exposure challenge in a single volunteer. Pulmonary function test results are plotted in the *upper panel* as a percent change from baseline. Mediator concentrations in nasal lavage fluids are plotted in the *lower panel*; histamine (○) in nanograms per milliliter or TAME-esterase activity (●) in counts per minute × 10⁻³.

the waist and attached by a length of plastic tubing to a plastic cassette worn on the collar containing a 37 mm Gelman-type AE glass fiber filter (Millipore Corp., Bedford, Mass.) with a thick paper support pad. The filter is 99.9% efficient in collecting spherical particles >0.3 μ m in diameter. The sampling pump flow rate was calibrated before each OEC with a bubble meter as a primary standard. At the end of the 1-hour OEC, the cassettes were stored sealed at -20° C. Antigen was extracted from the filters by removing the supportive paper pad, filling the cassettes with 3 ml of buffer, 25 mmol/L of piperazine-*N,N*-bis-(2-ethanesulfonic acid), and 110 mmol/L of NaCl, 5 mmol/L of KCl, pH 7.4, containing 0.3 % human serum albumin, and reassembling them. After overnight shaking at 4° C, the fluid was collected, centrifuged at 3000 g to remove debris, and stored at -20° C, for antigen analysis.

Rat n I concentration was determined by an inhibition radioimmunoassay.⁵ The filter extract was mixed with diluted pooled immune human serum and radiolabeled *Rat n I* B (kindly supplied by Dr. Joan Longbottom). After a 4-hour incubation at 23° C, goat antihuman IgG was added to precipitate the complexes, and the bound ¹²⁵I was quantitated. The assay was capable of detecting 0.4 ng per filter. The airborne allergen concentration was expressed in nanograms per cubic millimeter (allergen mass divided by [flow rate × sampling time] × 1000 L/m³). At a flow rate of 4.2 L/min, the collection system could detect average allergen concentrations >1.5 ng/m³.

Statistical analysis

Since most of the data were not normally distributed, results were analyzed with nonparametric techniques, usually Wilcoxon sign-rank test of Spearman's correlation coefficient. Exceptions, where the data were paired, analyzed categorically or where the number of replicates precluded the use of nonparametric tests, are indicated.

RESULTS

A typical response to the OEC by a rat-allergic volunteer is illustrated in Fig. 1, and spirometry changes are illustrated in the *upper panel*. Beginning at a baseline value of 112% predicted, FEV₁ gradually decreased by 16% during the hour; PEFR began at 99% of predicted and decreased by 24% by 1 hour. Both values returned toward normal after the volunteer left the room and received treatment with metaproterenol. The volunteer experienced no chest tightness but coughed eight times beginning 15 minutes after exposure.

Mediator concentrations in the nasal lavage fluid are illustrated in Fig. 1, *lower panel*. Histamine rose from 0.5 ng/ml before exposure to 1.8 ng/ml after 15 minutes of exposure and then gradually declined as exposure continued. TAME-esterase activity increased more strikingly, from 6.4 cpm × 10⁻³ before exposure to 34.8 cpm × 10⁻³ after 15 minutes of

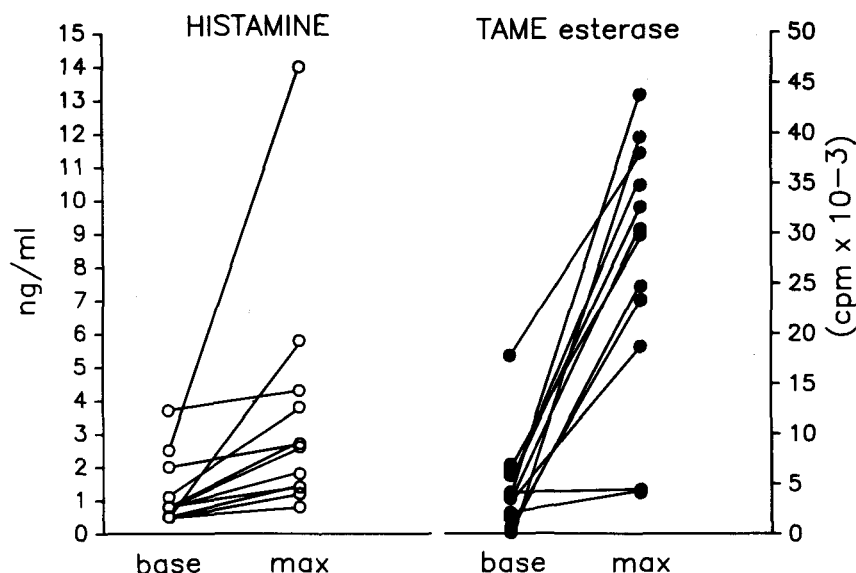


FIG. 2. Mediator concentrations in nasal lavage fluids in rat-allergic volunteers. Baseline concentrations were measured in fluids before exposure; values after exposure are the highest values measured in lavages during the challenge.

exposure; this activity was maintained for the remainder of the hour but declined when exposure was stopped. The volunteer did not sneeze but experienced nasal congestion and coryza that was rated 1.4 of 3 during each 15-minute period. The airborne concentration of *Rat N I* allergen in the room was 36.1 ng/m³.

Upper respiratory tract changes

Mediator concentrations in nasal lavages increased in all 12 rat-allergic volunteers, as illustrated in Fig. 2. Histamine concentrations increased from baseline values of <0.5 to 3.7 ng/ml (median, 0.5) to maximal concentrations during the challenge ranging from 0.8 to 14.0 ng/ml (median, 2.7). TAME-esterase activity also rose consistently during the challenge. As observed in Fig. 3, the net change in histamine ranged from 0.3 to 11.5 ng/ml (median, 1.1 ng/ml) with nine of 12 individuals having at least a threefold change, which is considered the minimal positive response.¹⁵ The net change in TAME-esterase activity ranged from 2.0 to 39.7 cpm $\times 10^{-3}$ (median, 23.5 cpm $\times 10^{-3}$) with nine of 12 individuals having at least a threefold change. The five atopic control subjects who were not allergic to rats had significantly smaller changes in both histamine and TAME-esterase ($p < 0.01$). Two control subjects had at least a threefold change in TAME-esterase activity, but none had a significant change in histamine.

Symptoms were more severe in allergic volunteers. Nine of the 12 volunteers sneezed, whereas one atopic control subject sneezed ($\chi^2 = 8.0$; $p < 0.05$); the frequency of sneezing was much higher in the allergic

volunteers (0 to 11/hr; median, 1.5) than in the atopic control subjects (0 to 1/hr; $p < 0.01$). Nasal congestion scores were also higher in the allergic volunteers. Ten of 12 volunteers complained of nasal congestion or coryza, and their mean symptom scores were 1 of a possible 3 compared to a mean of 0.2 in the atopic control subjects ($p < 0.05$).

In aqueous nasal challenges, sneezing correlated significantly with mediator changes in nasal lavages.¹¹ As illustrated in Fig. 4, in a total of 16 OECs performed by the 12 allergic volunteers, the maximum change in TAME-esterase activity also correlated with symptoms. The correlation coefficient with the mean symptom score was significant ($r_s = 0.61$; $p < 0.05$), but the number of sneezes was not ($r_s = 0.40$). However, the relationship was not linear, and there appeared to be a threshold of TAME-esterase activity at 15 cpm $\times 10^{-3}$, which was associated with symptoms. Five volunteers had changes smaller than that, and three of the five had significant mediator changes without symptoms. Above this level, symptom scores were significantly higher (median, 1.0 versus 0; $p < 0.05$), although the number of sneezes (median, 2 versus 0; $p > 0.05$) were not significantly higher. A similar pattern was observed when histamine changes were >0.5 ng/ml (data not presented), with significantly higher symptom scores ($p < 0.05$) but not a higher number of sneezes ($p > 0.05$).

Lower respiratory tract changes

The pulmonary function test changes in all 12 volunteers are compared to changes in atopic control

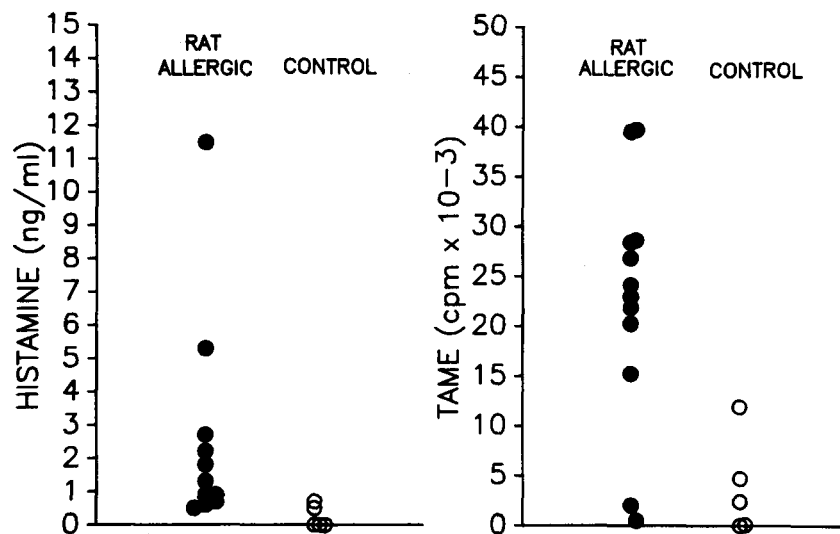


FIG. 3. Net change in nasal mediator concentrations calculated by subtracting concentrations in baseline lavage fluids from the maximum concentrations observed during the challenge. Closed circles (●) represent values in rat-allergic volunteers; open circles (○) represent values in atopic control subjects.

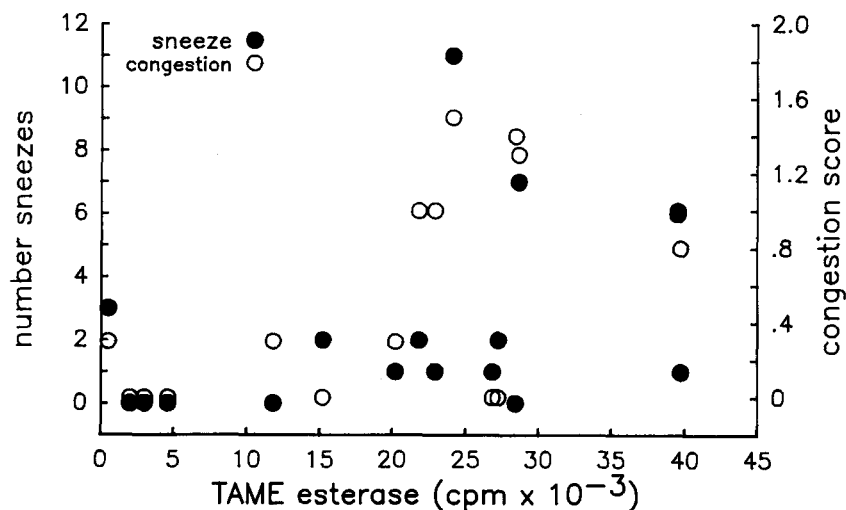


FIG. 4. Relationship of maximum TAME-esterase changes during individual OECs to the total number of sneezes and mean nasal congestion scores recorded during the challenge. Each pair of points (sneezes, ●; congestion scores, ○) represents symptoms during one of 16 challenges by 12 allergic volunteers.

subjects in Fig. 5. The changes were small even in the rat-allergic volunteers. The maximum fall in FEV_1 ranged from 1% to 29% (median, 8%). In five volunteers, FEV_1 fell at least 10% but fell >20% in only one volunteer. None of the five atopic control subjects had an FEV_1 change >10%, and the median change (3%) was significantly less than that of the allergic volunteers ($p < 0.05$). The change in PEFR was somewhat higher than in FEV_1 , but the fall in the rat-allergic volunteers (1% to 33%; median, 14%) and in the control subjects (4% to 22%; median, 8%) was

not significantly different ($p > 0.1$). Other pulmonary function tests differed as well, but only FEV_3 changed significantly more in volunteers than in control subjects. Thus, despite upper respiratory tract changes that were consistent and sometimes striking, the lower respiratory tract response to allergen exposure was modest, and only one person developed the degree of obstruction usually observed with aerosol bronchoprovocation tests.^{10, 16}

The time course of the pulmonary function test changes was quite different from the nasal response,

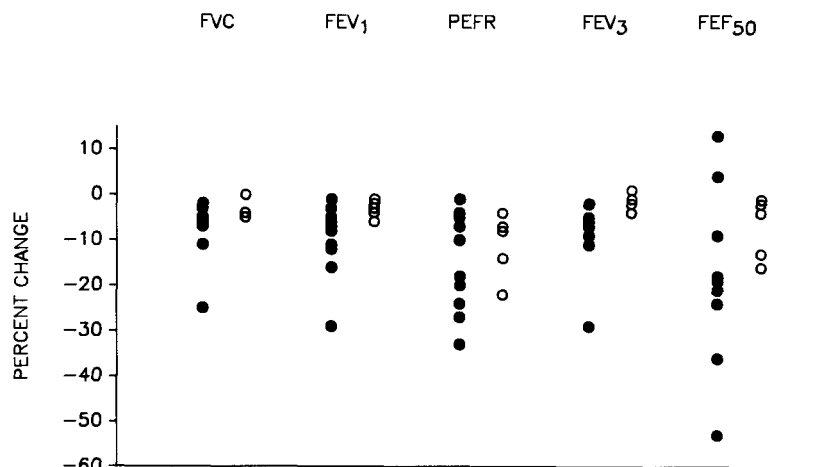


FIG. 5. Pulmonary function test changes in rat-allergic volunteers. Each *point* represents the maximum percentage change from baseline. *Closed circles* (●) represent rat-allergic volunteers; *open circles* (○) represent atopic control subjects.

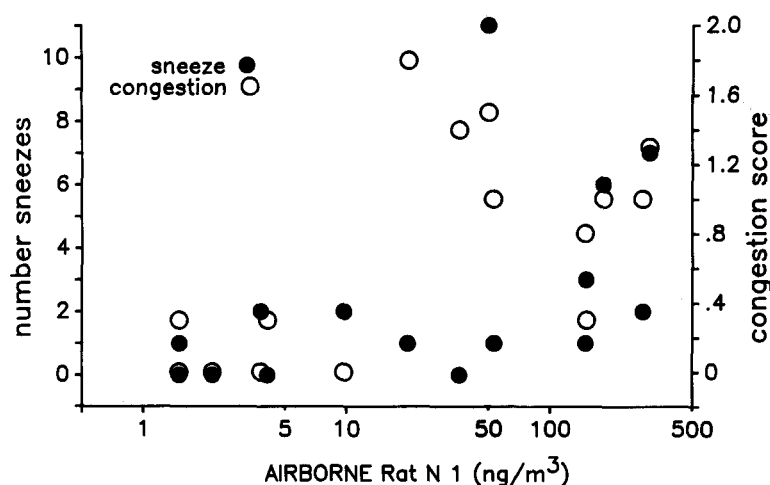


FIG. 6. Relationship of airborne allergen concentrations to the total number of sneezes and the mean nasal congestion score during the challenge.

as typified by the example illustrated in Fig. 1. Maximal pulmonary function changes were observed after 45 minutes in all five volunteers who had asthmatic responses and did not occur until 60 minutes in four of five volunteers. By contrast, the nasal mediator response tended to be highest at 15 minutes and then to plateau. For instance, TAME-esterase activity was $89\% \pm 8\%$ of maximum at 15 minutes, $82\% \pm 8\%$ at 30 minutes, $75\% \pm 7\%$ at 45 minutes, and $70\% \pm 11\%$ at 60 minutes.

Despite their modest changes in spirometry, most of the allergic volunteers had chest symptoms. Nine of the volunteers coughed (median, six coughs per hour), whereas only two of five atopic control subjects coughed (median, one cough per hour); both the incidence and rate of coughing were significantly higher

in the volunteers ($p < 0.05$). Six volunteers complained of chest tightness when they were compared to one control subject. Only one volunteer had no chest symptoms.

Allergen concentrations

Allergen concentrations in the vivaria varied during the OECs from <1.5 to 310 ng/m^3 but fell into predictable ranges. Cage-cleaning activity had been demonstrated previously to generate high airborne allergen concentration⁵; in the 12 challenges in which cage cleaning was performed, concentrations ranged from 19 to 310 ng/m^3 (median, 85). In the nine challenges without cleaning activity, the allergen concentration ranged from <1.5 to 9.7 ng/m^3 (median, <1.5).

TABLE II. Spirometry and nasal mediator changes with and without cage cleaning: Allergen dose-response relationship

Vol	Cage cleaning				Quiet sitting			
	Rat NI (ng/m ³)	FEV ₁ (%) change	Histamine change (ng/ml)	TAME change (cpm × 10 ⁻³)	Rat NI (ng/m ³)	FEV ₁ (%) change	Histamine change (ng/ml)	TAME change (cpm × 10 ⁻³)
1	53	-12	0.6	22.9	4.1	-3	1.3	11.8
5	287	-1	5.5	21.8	2.2	-1	0.8	3.0
3	20	-29	11.5	26.8	<1.5*	-47	0.2	6.0
4	149	-11	0.7	39.7	9.7	-3	0.2	27.2
Median	101	-11.5	3.1†	33.2	3.1†	-3	0.5	8.9†

Vol, Volunteer.

*Assay detection limit is 1.5 ng/m³.†*p* < 0.05 compared to cage cleaning; Student's paired *t* test.

Determinants of airway response

When the determinants of the airway response were examined, it was assumed that nasal mediator changes would be related to individual sensitivity to rat allergen and to the airborne allergen exposure. For instance, the allergic volunteers in Figs. 2 and 3 had significantly higher responses than atopic control subjects. In addition, in the 12 rat-allergic volunteers, the skin test wheal diameter correlated weakly with TAME-esterase changes by regression analysis ($r_s = 0.59$; $p < 0.05$) but not with histamine changes ($r_s = 0.16$). Serum IgE antibody to rat allergen did not correlate with mediator changes ($r_s = 0.17$).

Airborne allergen concentrations also appeared to determine airway response. For example, the 12 rat-allergic volunteers underwent nine challenges with cleaning activity and seven challenges with quiet sitting. As illustrated in Fig. 6, both sneezing ($r_s = 0.69$; $p < 0.01$) and nasal congestion scores ($r_s = 0.65$; $p < 0.01$) correlated with airborne allergen concentrations during the OEC. There appeared to be a symptomatic threshold at 5 to 10 ng/m³, below which symptoms are less severe in terms of nasal congestion scores (median, 0 versus 1.0; $p < 0.01$) but not of sneezing (median, 0 versus 2; $p < 0.05$). Airborne allergen concentrations also correlated with increased TAME-esterase activity ($r_s = 0.53$; $p < 0.05$; data not presented). Comparing these data with data in Fig. 4, it should be pointed out that of the six individuals exposed to allergen concentrations <5 ng/m³, five had significant increases in mediators during the hour, all associated with mild symptoms; two had no symptoms, and a third individual had two sneezes during the hour.

Of the 16 OECs described, four were repeat chal-

lenges in which volunteers who had previously been exposed to high allergen concentrations sat in the rat vivarium without cage cleaning. As presented in Table II, higher levels of both nasal mediators were observed during high antigen exposure. In the case of TAME-esterase, this difference, 33.2 versus 11.7 cpm × 10⁻³, was significant ($p < 0.05$, paired *t* test), but histamine changes were not significant. The lower respiratory tract response was less striking in the challenge with quiet sitting but not significantly so; FEV₁ fell 3% compared to 11.5% ($p < 0.2$, paired *t* test). Thus, the challenge exhibits a clear dependence on airborne allergen dose, although nasal lavage mediator changes could be observed at very low concentrations.

DISCUSSION

These studies have presented a method to examine allergic individuals' nasal and pulmonary response to environmental allergens. Allergic laboratory workers usually complain of rhinoconjunctivitis.^{1, 3, 4} Only 35% of symptomatic workers report asthmatic symptoms, and asthma almost never occurs without accompanying rhinitis. This pattern of illness is similar to illness observed in the general allergic population in which allergic rhinitis is two to three times more common than asthma.^{18, 19} Our current understanding of the pathophysiology of these diseases, especially of the role of mast cell-dependent inflammation, has been benefitted tremendously by experiments with allergen provocation techniques.^{11, 16, 19-21} However, the relevance of the results of these experiments to a chronic airway disease state can be questioned because the procedures are brief, large allergen doses are required, and in the case of bronchoprovocation, obstruction may be induced in anyone with IgE antibody, regard-

less of their history of asthma^{16, 19, 21} or airway hyperresponsiveness.^{16, 21} Environmental challenges, including the OEC described here, provide an important link between aqueous allergen challenges and the clinical disease state.

Earlier studies of environmental challenges demonstrated that it was possible to induce acute asthmatic responses to particulate environmental allergens.^{22, 23} More recently, Van Metre et al.⁶ developed methods to quantitate airborne particulate allergens during exposure and demonstrated that a dose-response relationship could be established that correlated well with aqueous bronchoprovocation challenges in the same patients. The OEC reported here carries the method a step farther by monitoring allergen concentrations in the workers' personal breathing zone⁵ and also by providing an objective measure of the nasal inflammatory response.

One of the major advantages of OECs is that airborne particles reach the lung through the nasal airway, which allows the nose both to modify the lung dose and to participate in the allergic airway response. Virtually all particles $>10\ \mu\text{m}$ in diameter are removed from the inspirate by the nose.¹⁵ Smaller particles are removed more efficiently as nasal resistance increases,²⁴ therefore, this "scrubbing" efficiency would be expected to increase as rhinitis is induced by allergen exposure. In contrast, obstruction may cause a transition to oronasal breathing²⁵ that would decrease scrubbing efficiency²⁶ and increase the lung dose. The ability to measure nasal inflammation, airflow obstruction, and airborne allergen concentrations simultaneously during the OEC promises to allow this complex protective mechanism to be examined experimentally.

The nasal response to the OEC was observed at low airborne rat-allergen concentrations and demonstrated a dose-response relationship. Mediator concentrations in the first lavage fluids after exposure were as high as concentrations reported with the highest allergen doses administered in aqueous nasal challenges, and concentrations remained high throughout the exposure. Naclerio et al.¹¹ administered 0.1 ml of ragweed-pollen extract intranasally in tenfold-increasing doses. With the extracts that they used, the highest allergen dose contained approximately 3 to 400 ng of antigen E. If the deposited nasal dose in the present experiments is calculated from the airborne *Rat N I* concentrations that we measured during cage cleaning (19 to 310 ng/m³), an estimated tidal volume of 7 L/min,²⁷ and a nasal antigen deposition fraction of 50%,²³ then the nasal dose during the 15 minutes before each lavage would have been in the range of 1 to 16 ng of *Rat N I*. Thus, the nasal lavage mediator

concentrations in the OEC were comparable to concentrations observed with aqueous challenges with tenfold to hundredfold higher allergen concentrations. The sensitivity of the nasal response is also emphasized by measurable mediator changes that we found during exposure to nasal doses $<0.1\ \text{ng}$, as calculated from the concentrations measured during the OEC performed without cage cleaning. It is not clear why the threshold dose for mediator release is so much lower in the OEC than in the aqueous challenge. We could speculate that allergen was carried on particulate vectors that became trapped on mucosal surfaces and maintained high local concentrations or that the continuous allergen exposure in the OEC might present a more efficient stimulus to mucosal mast cells, but at the present time, there is no data to support either possibility.

Another surprising finding in these studies is that mediators appeared in nasal lavages without associated symptoms. As illustrated in Fig. 4, symptoms tended to occur when increased mediator concentrations were highest, that is, when TAME-esterase activity increased $>15\ \text{cpm} \times 10^{-3}$ or histamine increased $>0.5\ \text{ng/ml}$. This suggests that there may be a threshold for symptoms in acute allergic disease, below which subclinical inflammation may occur. The fact that only one of the volunteers in this study developed lower airway obstruction sufficient to cause a 20% fall in FEV₁ (and only four volunteers to develop a 10% fall), despite striking upper airway mediator release and despite the large proportion (30% to 50%) of airborne animal allergen that is found in particles capable of lower airway deposition,^{6, 28-31} may be another example of subclinical airway inflammation. The present study can only suggest that subclinical inflammation occurs, but the implications for the natural history of chronic allergic airway disease requires that it be the subject of future studies.

The airborne allergen concentrations reported here were similar to concentrations detected in workplace environments in our earlier study with the same personal breathing zone monitors⁵ and confirms their pathologic significance. In those studies, *Rat n I* concentrations up to 70 ng/m³ were found when unanesthetized animals were handled or when cages were cleaned; these concentrations were significantly higher than concentrations found during surgery or when animals were killed (up to 13 ng/m³). Thus, the present studies confirm the practicality of personal monitoring devices for detecting individual allergen exposure in the workplace. These studies also demonstrate a steep dose-response relationship with nasal mediators, with significantly increased nasal mediator changes on exposure to higher doses. This relationship

was not observed for asthmatic changes, but Van Metre et al.⁶ did find a similar relationship in cat-allergic subjects with asthma. Taken together, these studies suggest that task assignment in a laboratory may be an important preventive strategy in laboratory animal asthma. However, because mediator release can be observed at extremely low airborne allergen doses, any exposure appears to carry a risk for the sensitized worker.

We gratefully acknowledge the technical help of Ms. Marie Martin and Kim Mudd, the advice of Dr. Carol Newill, and the secretarial help of Ms. Donna Dieterich.

REFERENCES

- Cockcroft A, Edwards J, McCarthy P, Anderson N. Allergy in laboratory animal workers. *Lancet* 1981;1:827-30.
- Schumaker MJ, Tait BAD, Holmes MC. Allergy to murine antigens in a biological research institute. *J ALLERGY CLIN IMMUNOL* 1981;68:310-8.
- Beeson MF, Dewdney JM, Edwards RG, Lee KD, Orr RG. Prevalence and diagnosis of laboratory animal allergy. *Clin Allergy* 1983;13:433-42.
- Gross NJ. Allergy to laboratory animals: epidemiologic, clinical, and physiologic aspects and a trial of cromolyn in its management. *J ALLERGY CLIN IMMUNOL* 1980;66:158-65.
- Eggleston PA, Newill CA, Ansari AA, et al. Task-related variation in airborne concentration of laboratory animal allergens: studies with *Rat n* I. *J ALLERGY CLIN IMMUNOL* 1989;84:347-52.
- Van Metre TE, Marsh DG, Adkinson NF Jr, et al. Dose of cat (*Felis Domesticus*) allergen I (*Fel d* I) that induces asthma. *J ALLERGY CLIN IMMUNOL* 1986;78:62-75.
- Woolcock A. Expression of results of airway hyperresponsiveness. In: Hargreave FE, Woolcock AJ, eds. *Airway responsiveness: measurements and interpretation*. Ontario: Astra Pharmaceuticals Canada, Ltd, 1985:80-5.
- Morris JF, Koski A, Johnson LC. Spirometric standards for healthy nonsmoking adults. *Am Rev Respir Dis* 1971;103:57-67.
- Freidhoff LR, Marsh DG, Meyers DA, Hussain R. The structuring of an allergy index based on IgE-mediated skin sensitivity to common environmental allergens. *J ALLERGY CLIN IMMUNOL* 1983;72:274-87.
- Chai H, Farr RS, Froelich LA, et al. Standardization of bronchial inhalation challenge procedures. *J ALLERGY CLIN IMMUNOL* 1975;56:323-8.
- Naclerio RM, Proud D, Togias AG, et al. Inflammation mediators in late antigen-induced rhinitis. *N Engl J Med* 1985;313:65-70.
- Siraganian R. An automated continuous flow system for the extraction and fluorometric analysis of histamine. *Anal Biochem* 1974;57:283-94.
- Imanari T, Kaizu T, Yoshida H, Yates H, Pierce JV, Pisano JJ. Radiochemical assays for human urinary, salivary, and plasma kallikreins. In: Pisano JJ, Austen KF, eds. *Chemistry and biology of the kallikrein-kinin system in health and disease*. Washington, D.C.: DHEW Publication No. 76-791, 1976: 205-213.
- Baumgarten CR, Nichols RC, Naclerio RM, Lichtenstein LM, Norman PS, Proud D. Plasma kallikrein during experimentally induced allergic rhinitis: role of kinin formation and contribution to TAME-esterase activity in nasal secretions. *J Immunol* 1986;137:977-82.
- Stuart B. Deposition and clearance of inhaled particles. *Environ Health Perspect* 1984;55:369-90.
- Bruce CA, Rosenthal RR, Lichtenstein LM, Norman PS. Quantitative inhalation bronchial challenge in ragweed hay fever patients: a comparison with ragweed-allergic asthmatics. *J ALLERGY CLIN IMMUNOL* 1975;56:331-7.
- Van Arsdel PP Jr, Motulsky AG. Frequency and heritability of asthma and allergic rhinitis in college students. *Acta Genetica* 1959;9:101-14.
- Broder I. Epidemiology of asthma and allergic rhinitis in a total community, Tecumseh, Michigan. *J ALLERGY CLIN IMMUNOL* 1974;53:127-38.
- Cockcroft DW, Ruffin RE, Frith PA, et al. Determinants of allergen-induced asthma: dose of allergen, circulating IgE antibody concentration and bronchial responsiveness to inhaled histamine. *Am Rev Respir Dis* 1979;120:1053-8.
- Cockcroft DW, Ruffin RE, Dolovich J, Hargreave FE. Allergen-induced increase in nonallergic bronchial reactivity. *Clin Allergy* 1977;7:503-7.
- Townley RG, Dennis M, Itkin IH. Comparative action of acetyl-beta-methylcholine, histamine, and pollen antigens in subjects with hay fever and patients with bronchial asthma. *J ALLERGY* 1965;36:121-37.
- Cartier A, Malo J-L, Forest F, et al. Occupational asthma in snow crab-processing workers. *J ALLERGY CLIN IMMUNOL* 1984;74:261-9.
- Pepys J, Hutchcroft BJ. Bronchial provocation tests in etiologic diagnosis and analysis of asthma. *Am Rev Respir Dis* 1975;112:829-59.
- Hounam R, Black A, Walsh M. The deposition of aerosol particles in the nasopharyngeal region of the human respiratory tract. In: *Inhaled particles III*. Walton WH, ed. Old Working, UK: Gresham Press, 1971:71-80.
- Niinimaa V, Cole P, Mintz S, Shephard R. The switching point from nasal to oronasal breathing. *Respir Physiol* 1980;42:61-71.
- Bowes S, Swift D. Deposition of inhaled particles in the oral airway during oronasal breathing. *Aerosol Sci Technol* 1989; 11:157-67.
- Saltin B, Astrand P-O. Maximal oxygen uptake in athletes. *J Appl Physiol* 1967;23:353-61.
- Findlay SR, Stotsky E, Leiterman K, Khemady Z, Ohman JL Jr. Allergens detected in association with airborne particles capable of penetrating into the peripheral lung. *Am Rev Respir Dis* 1983;128:1008-12.
- Platts-Mills TAE, Heymann PW, Longbottom JL, Wilkins SR. Airborne allergens associated with asthma: particle sizes carrying dust mite and rat allergens measured with a cascade impactor. *J ALLERGY CLIN IMMUNOL* 1986;77:850-7.
- Price JA, Longbottom JL. ELISA method of measurement of airborne levels of major laboratory animal allergens. *Clin Allergy* 1988;18:95-107.
- Corn M, Koegel A, Hall T, Scott A, Newill C, Evans R. Characteristics of airborne particles associated with animal allergy in laboratory workers. *Ann Occup Hyg* 1988;32(suppl): 435-6.