

Prospective study of occupational asthma to laboratory animal allergens: Stability of airway responsiveness to methacholine challenge for one year

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The stability of airway hyperresponsiveness was studied in a group of 178 young adults working with laboratory animals. At the time of their entry into the study, 132 of 178 subjects (74%) had less than 20% response to the inhalation of 25 mg/ml methacholine, whereas 26 (15%) had a methacholine dose causing a 20% fall in forced expiratory volume in 1 second after fewer than 80 breath units. The distribution of methacholine responsiveness did not differ at 6 months and 1 year; 155 of 178 volunteers (90.4%) responded during the repeated challenges to doses within one dilution of their results at entry. One hundred forty-one subjects were consistently unreactive during the year, and 17 were consistently reactive. Approximately equal numbers gained and lost reactivity. Those with consistently positive responses to methacholine were more likely to have skin test reactivity and chest symptoms. The presence of consistent chest symptoms was loosely associated with consistent methacholine responsiveness; 55% of those with consistent hyperresponsive airways had symptoms, and 24% of those who consistently had symptoms had hyperresponsive airways. We concluded that the methacholine response is relatively stable during the course of a year in laboratory animal workers who remain at their jobs and that the presence of a positive skin test response to laboratory animals or of chest symptoms does not change the pattern of stable responsiveness. (J ALLERGY CLIN IMMUNOL 1995;95:707-15.)

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Laboratory animal allergy is an important industrial health problem affecting between 15% and 33% of laboratory workers.^{1,2} In the only prospective study of laboratory animal asthma (LAA)

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Abbreviations used

LAA:	Laboratory animal asthma
HRA:	Hyperresponsive airways
FEV ₁ :	Forced expiratory volume in 1 second
BU:	Breath unit (one breath of 1 mg/ml of solution)
PD ₂₀ FEV ₁ :	Methacholine dose causing a 20% decrease in FEV ₁ from control value

reported to date, Botham et al.³ showed that up to 37% of workers without symptoms who were exposed to animal allergens had symptoms within the first year. No studies have described the effects of either continuing exposure or stopping work. This is a very important issue because longitudinal

studies of workers exposed to chemical sensitizers⁴⁻⁶ have shown that symptoms and methacholine sensitivity tend to increase with continued exposure⁶ and to decrease in some but not all cases when exposure stops.^{4, 6}

As part of a prospective study of the epidemiology of laboratory animal allergy, we have assessed the variability in chest symptoms and responsiveness to methacholine among a group of laboratory workers, some of whom were sensitized to animal allergens. A hyperresponsive airway (HRA) was examined as an objective surrogate for asthma, and we had hypothesized that changes in HRA might precede frank LAA because longitudinal studies of patients with asthma found that many patients with HRA did not have symptoms⁷ and that HRA increased with allergen exposure⁸ and decreased with allergen avoidance.⁹

The present report focuses on one aspect of this question, airway hyperresponsiveness to methacholine, and examines changes in this measure and in chest symptoms during a year's exposure to laboratory animals.

METHODS

Ascertainment of subjects and study design

Volunteers were recruited from research personnel exposed to laboratory animals in their work at a medical research center. At entry into this study¹⁰ the median age of the 364 volunteers was 26 years. Forty-four percent were technicians, 23% were students, 23% were postdoctoral scientists or fellows, 6% were faculty, and 3% were support personnel without laboratory duties. In this prospective study of the incidence of allergic asthma, eligible persons denied ever having symptomatic asthma to laboratory animals, defined as three or more symptoms of wheezing, breathlessness, chest tightness, or productive cough associated with workplace exposure. However, they were not excluded if they had asthmatic symptoms related to other stimuli or if they had other allergic symptoms (i.e., rhinitis, conjunctivitis, rash) related to animal exposure. As a result, 46% reported allergic symptoms related to animal exposure, 30% had chest symptoms unrelated to work, and 8% had positive skin test reactions to laboratory animals. At enrollment in the study and again 6 months and 1 year later, subjects participated in pulmonary function tests, allergen skin tests, and personal interviews.

Consistency of testing sessions

Every effort was made to maintain consistency between the initial and follow-up testing sessions. Tests were performed at the same time of day, with standard test protocols. All bronchial challenge tests were carried out with the same spirometer and with methacholine chloride from the same supplier. The 1-year follow-up

sessions were scheduled within 2 months of the anniversary date of the initial visits, to minimize any bias from seasonal allergen exposure between initial and follow-up tests in the group as a whole.

Pulmonary function testing

Spirometry was performed according to American Thoracic Society guidelines,¹¹ with a rolling seal spirometer (model 822, Ohio Medical Products, Houston, Tex.). A technically acceptable spirogram met the following requirements: at least 6 seconds in duration, or 4 seconds if no change was observed in the slope of the tracing; no cough in the first second; no cough affecting accurate measurement of forced vital capacity; back-extrapolated volume less than 10% of measured forced vital capacity. Measurements were corrected for body temperature and ambient pressure. Three spiograms were inscribed, and the highest forced expiratory volume in 1 second (FEV₁) from an acceptable spirogram was recorded. Results were related to published norms.¹²

Methacholine challenges were performed according to a modification of the method of Chai et al.^{13, 14} Solutions of methacholine chloride (J. T. Baker Chemical Co., Phillipsburg, N.J.) were prepared monthly in phenolated buffered saline solution (0.04% KH₂PO₄, 0.11% Na₂HPO₄, 0.5% NaCl, 0.4% phenol; Greer Laboratories, Lenoir, N.C.) in concentrations of 1, 3, 10, and 25 mg/ml. For tests of persons known to be highly reactive to methacholine, 0.25 and 0.5 mg/ml concentrations were prepared by on-site dilution of the 1 mg/ml solution. Aerosols were generated by a DeVilbiss model 646 nebulizer attached to a dose-metering device, and five breaths were delivered for 0.6 seconds during slow and deep inspirations from functional residual capacity. Two minutes after the fifth breath of aerosol, two acceptable spiograms were obtained. The higher FEV₁ from either acceptable spirogram was recorded, and the percent change in FEV₁ with methacholine compared with that with saline solution alone was calculated. Increasing concentrations were administered until FEV₁ decreased 20% or more from the saline solution control values or until the 25 mg/ml solution was given.

The percent change in FEV₁ was plotted against the log of the cumulative methacholine dose up to a maximum of 197.5 breath units (BU). A methacholine BU is defined as the equivalent of one breath of a 1 mg/ml solution. The provocative dose that produced a 20% fall in FEV₁ (PD₂₀FEV₁) was calculated by linear interpolation between the two adjacent data points.

Interview and skin tests

Trained lay interviewers administered extensive standardized personal interviews including questions regarding symptoms of allergy and asthma. The symptoms of asthma recorded were intermittent episodes of wheezing or whistling of the chest; breathlessness (defined as episodes or attacks of difficulty breathing, shortness of breath, or inability to take a deep breath); chest tight-

TABLE I. Distribution of methacholine response ($PD_{20}FEV_1$) at entry and at 6 and 12 months

	Total	Not reactive*	$PD_{20}FEV_1$ (BU)					
			>160	160-81	80-41	40-21	20-11	<10
Entry								
n	178	132	8	12	9	9	3	5
%		74.2	4.5	6.7	5.1	5.1	1.7	2.8
6 mo								
n†	161	125	3	8	8	12	1	4
%		77.6	1.9	5.0	5.0	7.5	0.6	2.5
12 mo								
n	178	138	3	13	8	9	3	4
%		77.5	1.7	7.3	4.5	5.1	1.7	2.2

* FEV_1 decreased less than 20% from control value.

†Seventeen challenges not done or technically unacceptable.

ness (feeling of tightness or heaviness in the chest, possibly extending upward into the throat); and cough (3 consecutive weeks of productive cough with sputum or phlegm).

Immediate wheal-and-flare skin tests were performed by the prick puncture technique of Santilli et al.¹⁵ Materials included glycerinated extracts (1/20 wt/vol; Greer Laboratories) of house dust; *Alternaria*; *Aspergillus*; short ragweed and orchard grass pollen; rat, mouse, guinea pig, and rabbit epithelium; and histamine phosphate and glycerin controls. Duplicate tests were applied on the flexor surface of both forearms and read at 15 to 20 minutes. A positive test response was defined as at least one wheal 4 mm or greater in diameter, with a smaller wheal of at least 2 mm in diameter. All subjects denied taking oral or nasal medications containing antihistamines within 48 hours of testing.

Subjects included in analyses

For the purposes of this analysis data were included from those persons who were examined at entry and within 1 month of their scheduled visits at 6 months and 12 months. Of the 364 persons whose initial examination has already been described,¹⁰ 21 withdrew from the study during the year, 116 were seen before or after the 2-month "window" of their scheduled visits, and 24 did not have acceptable methacholine challenge data at one or more visits. Thus the group included in this analysis totaled 178 who had complete and timely studies.

Subjects were between 19 and 48 years of age at the time of their initial test (median age, 27 years). Only 27 of 178 were current smokers. The frequency of skin test reactivity to common allergens among the 178 subjects (24% ragweed, 19% orchard grass, 6% *Alternaria*) closely approximates the frequency reported previously¹⁰ in these persons and in another large study in this geographic area.¹⁶

Analytic methods

Cross-sectional analysis of the frequency of HRA and its association with clinical, demographic, and occupa-

tional factors was performed with the SAS system.¹⁷ Odds ratios and confidence intervals of proportions were computed, and chi-square tests were performed.¹⁸

RESULTS

Frequency distributions of degree of responsiveness at each of three points in time

The distribution of methacholine responsiveness at entry, and at 6 and 12 months, is shown in Table I. The proportion of persons who did not have a $PD_{20}FEV_1$ was nearly identical at each test; that is, 132 of 178 (74%) had no $PD_{20}FEV_1$ at the first test compared with 125 of 161 (78%) 6 months later and 138 of 178 (78%) 1 year later. Moreover, the distribution of responsiveness as measured by the $PD_{20}FEV_1$ was nearly identical at the three tests. For example, 26 of 178 persons (15%) had responses in the asthmatic range ($PD_{20}FEV_1 \leq 80$ BU¹⁹) in the first test compared with 25/161 (16%) at the same dose level 6 months later and 24/178 (13%) 1 year after entry. However, only 17 of 178 persons (10%) had a $PD_{20}FEV_1$ of 80 BU or less at all three tests.

Intraindividual reproducibility

The consistency in the degree of responsiveness within persons between the three challenge tests is shown in Figs. 1 to 3. In each figure the individual challenge response at one time point is plotted against the result at the other time point. Although Fig. 1, illustrating data at entry versus data 12 months later, is discussed in greatest detail, Figs. 2 and 3 show that these test responses were also consistent when compared with the 6-month findings. Considering the presence or absence of a $PD_{20}FEV_1$ at any dose administered (through 197.5 BU), 118 of 178 persons did not have this

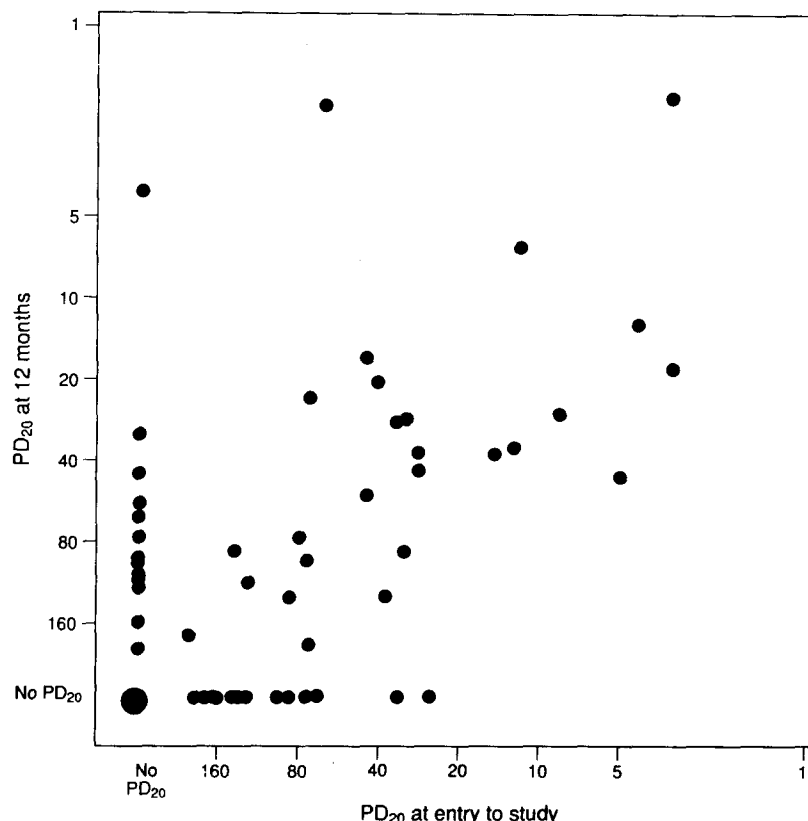


FIG. 1. Relationship in 178 participants between $PD_{20}FEV_1$ from methacholine challenges performed at entry to study and 12 months later. Large circle represents 118 persons who had less than 20% change in FEV_1 at either test.

degree of hyperreactivity either at entry to the study or 12 months later, whereas 25 of 178 had a $PD_{20}FEV_1$ at both tests and 35 had a $PD_{20}FEV_1$ at one test but not the other.

Of the 35 persons who had a $PD_{20}FEV_1$ on one test but not at the other, approximately equal numbers gained or lost responsiveness. Fourteen volunteers were unresponsive at entry but had $PD_{20}FEV_1$ values ranging from 4.1 to 198 BU (median, 92.5 BU) at 12 months. Twenty volunteers had a $PD_{20}FEV_1$ ranging from 24 to 195 BU (median, 134 BU) at entry but not at 12 months. Those 12 volunteers with more severe HRA ($PD_{20}FEV_1 \leq 80$ BU) at entry tended to be more consistent, and 18 responded in this range a year later.

Risk factors for methacholine responsiveness

To analyze for risk factors, we designated a $PD_{20}FEV_1$ of 80 BU or less a positive response (+) and any result greater than 80 BU a negative response (-). Next we categorized the results as all positive (+/+), all negative (-/-), or variable,

either with one positive (+/-) or one negative (-/+) test result. As seen in Table II, 161 persons had data for all three visits whereas 17 had no data at 6 months. One hundred forty-one had negative test results at all visits, and 17 had positive test results at all visits. The responsiveness of the remaining 19 was inconsistent in that results of one of the three tests differed from the other two tests.

The first association examined was specific allergy as indicated by the presence or absence of a positive skin test result at the initial evaluation. Results are shown in Table III. Of the 178 volunteers, 173 had interpretable skin test results. It can be seen that persons with a positive skin test response to pollen allergens were more likely to have either consistent or transient HRA ($\chi^2 13.3, p = 0.004$). On the other hand, a positive skin test response to one of the four laboratory animal allergens or to household allergens was not significantly related to either consistent or transient HRA. Thus IgE-mediated sensitivity to animal allergens was not associated with either the presence of HRA or with the development of HRA despite ongoing workplace exposure to animal allergens.

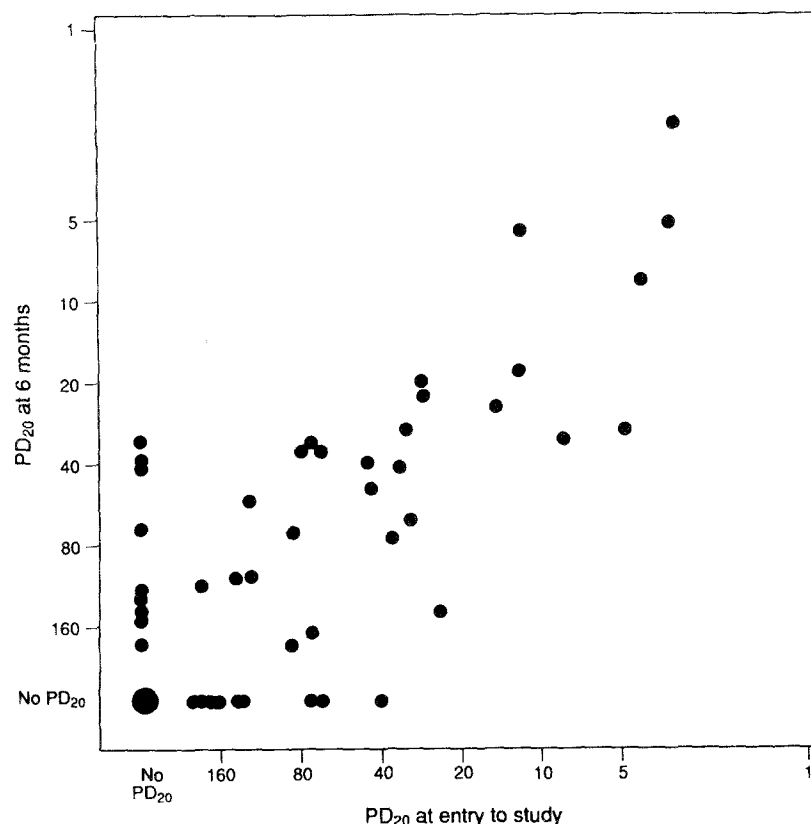


FIG. 2. Relationship in 161 participants between $PD_{20}FEV_1$ at entry and at 6 months. Large circle represents 127 persons.

A relationship between HRA and chest symptoms was also sought. This relationship was expected to be circular in that those with HRA should have more symptoms⁷ and those with symptomatic allergen exposures would be expected to have increased HRA as a result.⁸ This relationship is shown in Table IV. The consistency of reported symptoms has been approached in the same way as HRA (see Table II). Both have been categorized as consistently absent ($-/-$), present at only one visit ($-/+$), present at all but one visit ($+/-$), or present at all visits ($+/+$). It can be seen that reported symptoms and HRA are related but not strikingly so. In general, those participants who never reported chest symptoms were more likely to have consistently negative methacholine inhalation challenges and those consistently reporting symptoms were more likely to have consistent HRA; these differences were statistically significant ($\chi^2 = 30.8$, $p < 0.001$). The relationship is clearer if Table IV is simplified by combining certain data. For example, 100 persons never had symptoms and an additional 37 reported symptoms at only one visit; 12 of these 137 persons (8%) had consistent

HRA, compared with 10 of 41 persons (24%) who consistently had symptoms. With the same approach it is possible to examine the predictive value of a consistently positive methacholine challenge test. Thus 17 persons had HRA at all tests and an additional five had HRA at all but one test; 10 of these 22 persons (46%) consistently reported chest symptoms, compared with 31 of 156 (20%) in whom HRA was demonstrated at one visit or less. Therefore the sensitivity of the methacholine challenge is 24% and the specificity is 91%. In this population, where the prevalence of symptoms is 23%, the positive predictive value of a positive methacholine challenge is 46%.

DISCUSSION

These data suggest that during occupational exposure to laboratory animals neither hyperresponsiveness to methacholine (HRA) nor chest symptoms changed markedly during this 1-year observation. Only 35 of 178 persons had a $PD_{20}FEV_1$ at one methacholine test but not at another, and there was no tendency to shift more in one direction than in the other. Symptoms were

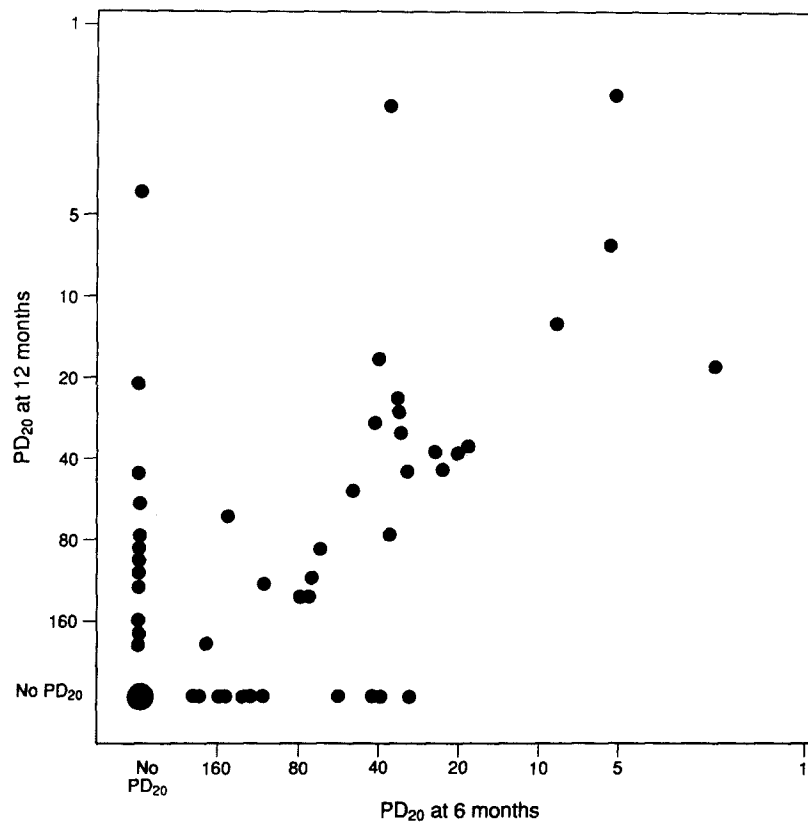


FIG. 3. Relationship in 161 participants between $PD_{20}FEV_1$ at 6 and 12 months after entry. Large circle represents 129 persons.

TABLE II. Consistency of HRA within 120-month follow-up

At entry, and at 6 and 12 mo (<i>n</i> = 161)		At entry and 12 mo later (<i>n</i> = 17)	
HRA status*	Frequency (%)	HRA status*	Frequency (%)
-00/-06/-12	126 (78.3)	-00/-12	15 (88.2)
-00/-06/-12	5 (3.1)	+00/-12	1 (5.9)
-00/+06/-12	5 (3.1)	+00/+12	1 (5.9)
+00/-06/-12	4 (2.5)		
+00/+06/-12	3 (1.9)		
+00/-06/+12	1 (0.6)		
-00/+06/+12	1 (0.6)		
+00/+06/+12	16 (9.9)		

*00/06/12 represents evaluation at entry, and at 6 and 12 months. -, $PD_{20}FEV_1$ greater than 80 BU or FEV_1 decrease less than 20%; +, $PD_{20}FEV_1$ 80 BU or less.

also consistent but tended to be reported by more workers. The presence of a positive skin test reaction to laboratory animals or of chest symptoms did not change the pattern of stable airway responsiveness.

This is the first description of the natural history of occupational asthma caused by an IgE-mediated mechanism among workers who continue their exposure to the allergen. Few reports exist of

occupational asthma from any cause among persons who remain in the same workplace. Chan-Yeung et al.⁶ demonstrated that spirometry and HRA worsens with continued exposure to western red cedar and that asthma may continue at the same severity even when exposure stops. Asthma occurring among snow crab workers, like LAA, is IgE-mediated, and snow crab workers with asthma had only partial resolution after stopping work,²⁰

TABLE III. Relationship of HRA to skin test response at entry evaluation

HRA pattern	No. (%) of subjects with positive skin test reactions at initial evaluation			
	Lab animal	Household†	Pollen	Total No. (%)*
Consistently negative	8 (62)	24 (65)	32 (65)	137 (79)
1 test positive	2 (15)	4 (11)	5 (10)	14 (8)
1 test negative	1 (8)	2 (5)	1 (2)	5 (3)
Consistently positive	2 (15)	7 (19)	11 (23)	17 (10)
Total	13	37	49	173
Chi-square test (<i>p</i>)	0.385	0.084	0.004	

*Some persons had positive skin tests to more than one allergen.

†Includes dust, mold, cat, and dog allergens.

TABLE IV. Association between consistency of symptoms and consistency of HRA

HRA pattern	No. (%) of subjects with symptoms* at entry and at 6 and 12 mo			
	Never	Once	Twice	All visits
Consistently negative (<i>n</i> = 141)	85 (60)	31 (22)	21 (15)	4 (3)
1 test positive (<i>n</i> = 15)	6 (40)	3 (20)	5 (33)	1 (7)
1 test negative (<i>n</i> = 5)	2 (40)	0 (0)	3 (60)	0 (0)
Consistently positive (<i>n</i> = 17)	7 (41)	3 (18)	2 (12)	5 (29)
Total (<i>n</i> = 178)	100	37	31	10
Chi-square test (<i>p</i>)	0.005	0.67	0.07	<0.001

*Any asthma symptom (wheezing, breathlessness, chest tightness, and productive cough) since the last visit, except at entry, when symptoms were assessed 6 months previously.

suggesting that IgE-mediated asthma may also carry a risk of chronic asthma with continued symptoms and methacholine hyperresponsiveness. Although our data do not address this question directly, they suggest that continued exposure to allergen does not induce progression of symptoms or HRA during a year's observation.

This article derives from a study of the epidemiology of LAA. When the study began in 1981 we defined asthma by both symptoms and airway responsiveness to methacholine, and hypothesized that bronchial hyperresponsiveness would appear first, followed by symptoms. When the data were examined we found that 36% of subjects had chest symptoms on entry, although they did not meet the study criterion of having all three symptoms, and that 24% already had HRA.¹⁰ The present report demonstrated that neither chest symptoms nor HRA changed in a consistent direction during the first year of observation (i.e., there was no net increase in asthma in this group of workers who were exposed to a potent allergen in their work).

This report shows that responsiveness to methacholine was highly reproducible in 1 year among a sample of young adults who were routinely exposed to laboratory animal allergens in their work. When the grouped data from the initial test with

the test performed 1 year later were compared, not only was the proportion of persons who did not respond to the highest dose of methacholine nearly identical at the beginning of the study and after 6 and 12 months (74%, 78%, and 78% respectively), but the frequency distributions of persons with each level of PD₂₀FEV₁ was also similar. The degree of responsiveness to methacholine was seen to be highly reproducible within subjects as well. Only 23 of 178 persons shifted PD₂₀FEV₁ by more than one dose level in 1 year, and there was no tendency to shift more in one direction than the other.

Our findings are comparable to other prospective studies of airway responsiveness. Among persons with asthma, the response is reproducible within one to two twofold dilutions over months^{21,22} to years.⁷ In general population studies²³ or in studies of occupations with a high prevalence of occupational asthma,⁶ the response is generally less reproducible than we found. In part this may be explained by the problem faced by population studies in which the response to the test for HRA is positive in a minority of the population. To allow analysis for change, the data can be censored by excluding unresponsive subjects or can be dichotomized into positive and negative response on the

basis of arbitrary limits defining an abnormal response. We chose to use dichotomized data because we wanted to analyze changes among those participants who were originally unresponsive and because changes that occurred between observations at 6 and 12 months could be analyzed and discussed. The effect of this decision was to reduce the variability in this group of persons with widely varying HRA. This effect was first discussed by Redline et al.²³ in their study of eucapneic hyper-ventilation.

The relatively loose relationship between symptoms and HRA agrees with previous reports. In cross-sectional studies of patients with asthma^{21, 24} and of general populations,²³ about half those with HRA have symptoms and about half those with symptoms have HRA. It has been argued that the *point* prevalence of HRA in these studies has been compared with the *cumulative* prevalence of symptoms, so that symptomatic asthmatic episodes might have been associated with transient HRA that had resolved by the time the methacholine challenge is performed.²³ In the present study methacholine responsiveness and symptoms were reassessed repeatedly throughout a year but HRA still could be demonstrated in only 24% of those who consistently had symptoms during a year of observation. This agrees with previous longitudinal studies in subjects with asthma,⁷ in working populations,⁶ and in general populations.²³

We also showed that 10 of 22 persons with consistent HRA in the asthmatic range¹⁹ had no symptoms. This proportion (55%) is in agreement with earlier reports⁹ and is especially difficult to explain. It is unlikely that the 12 persons without symptoms did not encounter an appropriate stimulus for symptoms in their environment; for example, in the case of allergy all but two subjects had positive skin test reactions to inhalant allergens. The possibility that symptoms were underreported cannot be excluded, because we depended on recall at 6-month intervals. These inconsistencies lead us to agree with others^{6, 8, 23} that, as a surrogate for asthma in epidemiologic studies, HRA is no better than questionnaire responses. Until a "gold standard" is developed, the application of methacholine challenge results does not improve the case definition of asthma.

In summary, we have shown that HRA among a group of workers exposed to laboratory animal allergens is remarkably stable during a year's observation. The presence of atopy and to some extent asthmatic symptoms are risk factors for the

presence of HRA but do not increase the ability of the measure.

This work is dedicated to the memory of Dr. Harold A. Menkes, physician, scientist, and teacher, under whose guidance the concept of this manuscript was developed. We acknowledge Alice Rodman for her contribution to the data collection and processing.

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