

### Allergy to insects in Japan

#### III. High frequency of IgE antibody responses to insects (moth, butterfly, caddis fly, and chironomid) in patients with bronchial asthma and immunochemical quantitation of the insect-related airborne particles smaller than 10 $\mu\text{m}$ in diameter

**Toshiya Kino, M.D., Junichi Chihara, M.D., Kouji Fukuda, M.D.,  
Yoshiyuki Sasaki, M.D., Yukio Shogaki, Ph.D., and  
Shunsaku Oshima, M.D.** *Kyoto, Japan*

*After recognizing that moth, butterfly, caddis fly, and chironomid were the insects predominantly found in our environment, reaginic sensitivities to these insects in patients with asthma were investigated with extracts of silkworm wing for the detection of moth and/or butterfly allergy, caddis fly wing for caddis fly allergy, and chironomid whole body for chironomid allergy. At least 50% of 56 randomly selected patients with asthma had positive intracutaneous reactions to these insect extracts, and at least 80% of the skin reaction positive patients had specific IgE antibodies. An immunochemical assay of these insect-related aeroallergens in the dust collected 18.6 m above ground revealed the presence in the air of insect-related particles <10  $\mu\text{m}$  in diameter with two peaks a year, one in the spring (silkworm wing, 3.18 ng protein equivalent to that of the crude extract per cubic meter of air; caddis fly wing, 3.08 ng/m<sup>3</sup>; and chironomid whole body, 5.21 ng/m<sup>3</sup>) and one in the autumn (silkworm wing, 8.24 ng/m<sup>3</sup>; caddis fly wing, 4.74 ng/m<sup>3</sup>; and chironomid whole body, 18.19 ng/m<sup>3</sup>). Although we have no idea whether or not such peak values are enough to cause asthmatic symptoms, seasonal fluctuations of specific IgE antibodies to these insects appeared to be similar to those of atmospheric insect allergens measured by this method. These findings suggest that insects are widespread and common inhalant allergens. (J ALLERGY CLIN IMMUNOL 1987;79:857-66.)*

We have emphasized the possible environmental distribution of insect allergens in previous articles<sup>1, 2</sup> in which it was noted that more than half of randomly selected patients with asthma had reaginic sensitivities to moths and butterflies and that these insect allergens were demonstrated by RAST-inhibition tests to cross-react almost completely with silkworm moth wing but not at all with mite. Additional examples of possible widespread inhalant insect allergy were presented by the frequent occurrence of positive skin test reactions

#### Abbreviation used

PBS: Phosphate-buffered saline

and positive RASTs to caddis fly wing extract<sup>3</sup> and to chironomid<sup>4, 5</sup> in Japan. A patient with rhinitis and bronchial asthma caused by caddis fly was originally reported in North America.<sup>6</sup> Widespread IgE-mediated hypersensitivity to chironomid has been reported in the Sudan,<sup>7</sup> and RAST studies of chironomid antigens demonstrated the allergic activity of the larvae to be in the hemoglobin molecule.<sup>8</sup>

However, we had no idea what kinds of insects were common in our environment, and quantitative aerometric data on insect allergens were lacking for the evaluation of the importance of this category of allergens.

From the Chest Disease Research Institute, Kyoto University, Kyoto, Japan.

Supported in part by grant-in-aid for scientific research from the Ministry of Education, government of Japan.

Received for publication June 24, 1986.

Accepted for publication Nov. 7, 1986.

Reprint requests: Toshiya Kino, M.D., Chest Disease Research Institute, Kyoto University, Sakyo-ku (606), Kyoto, Japan.

In this study, therefore, in order to clarify the importance of inhalant insect allergens in patients with bronchial asthma, we first counted the number of insects collected in a light trap or observed in daily life, then tested the reaginic sensitivities to the insects by skin tests and RAST in randomly selected patients with asthma without occupational exposure to any insects, and finally, tried to quantitate the insect-related airborne particles by an immunochemical method.

## MATERIAL AND METHODS

### Estimation of insect types and numbers in our environment

For 8 years, one of the authors (T. K.) recorded the number of butterflies he observed every morning at about 9 A.M. while he was driving about 5 km from his house in a residential quarter to the Chest Disease Research Institute, Kyoto University. During 1983, light-attracted insects were collected in a light trap every 5 days from 6 P.M. to 6 A.M. near the Midorogaike Pond in the northern part of Kyoto, and during 1985, twice a month from 6 P.M. to 6 A.M. in a light trap on the roof (18.6 m above ground) of the five-story Chest Disease Research Institute, about 70 m from the Kamo River.

### Detection of IgE antibody responses to the insects

**Allergen extraction.** The wings were removed from silkworm moths (*Bombyx mori* Linne), and a wing extract (silkworm wing) was made from the scaly wing. Silkworm wing extract was used for the detection of moth and/or butterfly allergy because of the cross-reactivity among the three and because the main allergen is in the wing, as demonstrated in a previous article.<sup>2</sup> Caddis fly allergen was made from *Macronema radiatum* MacLachlan, one of the main species around Kyoto, and the wing extract (caddis fly wing) was used for the detection of caddis fly allergy, since the main allergen is in the hairy wing.<sup>3</sup> Chironomid allergen was made from *Chironomus yoshimatsui*, which is one of the main species around Kyoto, and extract of the whole body, including the wing (chironomid whole body), was satisfactory as a test allergen.

The three extracts were prepared as described previously.<sup>1,2</sup>

The protein nitrogen content, determined by the micro-Kjeldahl method, was 35.5 µg/ml, 30.1 µg/ml, and 164 µg/ml in 1:50 dilutions of silkworm wing, caddis fly wing, and chironomid whole body, respectively.

**Subjects.** The bronchial asthma group consisted of 56 patients without any occupational exposure to insects who were randomly selected from the Outpatient Asthma Clinic of the Chest Disease Research Institute, Kyoto University. The 23 subjects in the control group without asthma consisted of 10 normal subjects, eight patients with pulmonary tuberculosis, three with emphysema, and two patients with lung cancer. These subjects were all nonatopic on the basis of clinical findings and multiple negative skin tests, and

their serum IgE levels were within the normal range. These two groups were well matched in age, ranging from 15 to 65 years.

**Skin test.** Tests were performed on the volar surface of the forearm by intracutaneous injections of 0.02 ml of 1:10<sup>4</sup> dilutions (weight per volume) of silkworm wing, caddis fly wing, and chironomid whole body. Readings were made after 15 minutes, and a wheal and flare >9 and 20 mm, respectively, was recorded as a positive reaction.

**Preparation of allergen-paper disk complex for RAST.** Extracts of silkworm wing, caddis fly wing, and chironomid whole body were lyophilized. Lyophilized extracts, 130 mg for silkworm wing, 128 mg for caddis fly, and 162 mg for chironomid whole body, were dissolved in 10 ml of distilled water in which 200 pieces of CNBr-activated paper disk (Toyo Roshi Co., Ltd., Tokyo, Japan, filter paper No. 6, diameter, 6 mm) were immersed for 3 days to couple the allergen. In our preliminary experiments, the concentrations of aforementioned lyophilized extracts were found to be adequate for measuring maximum IgE antibodies in our patients' sera. All subsequent steps followed the method of Ceska et al.<sup>9</sup>

**RAST.** RAST was performed under the same procedures as described previously.<sup>1,2</sup> Portions of serum, 50 µl, and allergen-paper disks were incubated at room temperature for 2 days. After the paper disks had been washed three times with 2.5 ml of physiologic saline, 50 µl of <sup>125</sup>I-labeled anti-IgE (Pharmacia Diagnostics AB, Uppsala, Sweden) was added, and the mixtures were kept overnight at room temperature. The unbound <sup>125</sup>I-anti-IgE was washed away three times with 2.5 ml of physiologic saline, and the radioactivity bound to the disks was measured in a gamma counter.

The quantity of IgE antibodies was expressed as percent uptake of <sup>125</sup>I-anti-IgE added or permillage of a reference reaginic serum.

### Quantitation of airborne insect-related allergens <10 µm in diameter

**Collection of airborne particles.** A high-volume air sampler (HVS-1000; Shibata Kagaku Co. Ltd., Tokyo, Japan) was used for the present studies. This device collects airborne particles smaller and larger than 10 µm in diameter on lower and upper fiberglass filters (upper filter, size 12.6 by 16.6 cm; lower filter, size 20.3 by 25.4 cm; grade GB 100 R; Toyo Roshi Co. Ltd.), respectively. The lower filter is stipulated by the manufacturer to retain particles of 0.3 µm in diameter at 99.98% efficacy. During these studies, a constant and automatically adjusted airflow of 1 m<sup>3</sup>/min was maintained. The high-volume air sampler was placed on the roof (18.6 m above ground) of the Chest Disease Research Institute and operated for 3 days with a 100-minute break each day (actually operated for 4000 minutes) in the middle 10 days of every month of 1985.

**Elution of allergen.** Atmospheric allergens were extracted from the lower filter that retained particles <10 µm in diameter because it is said that only particles <10 µm in diameter can reach the bronchi.<sup>10</sup>

The filter was processed by descending elution<sup>11</sup> with 300 ml of PBS (0.139 mol/L NaCl, 0.00268 mol/L KC1,

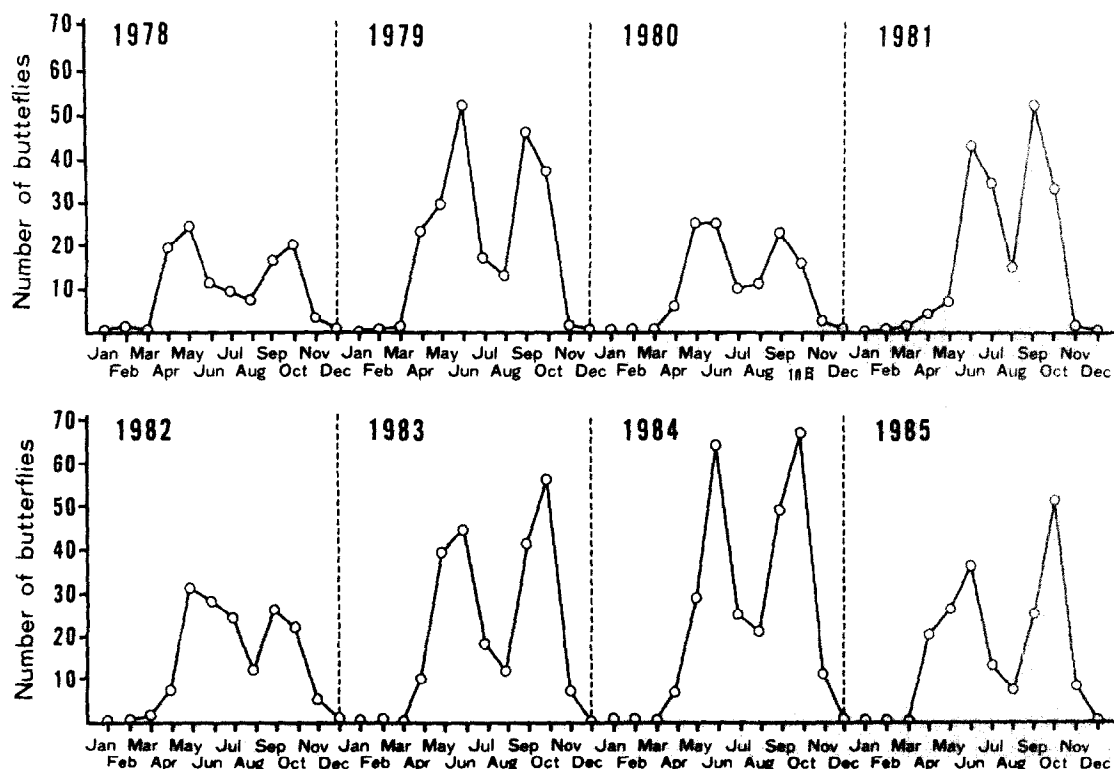


FIG. 1. Number of butterflies which one of the authors (T. K.) observed while he was driving 5 km at about 9 A.M. daily.

0.00958 mol/L  $\text{NaH}_2\text{PO}_4$ , and 0.00141 mol/L  $\text{Na}_2\text{HPO}_4$ , pH 7.4). This elution method confirmed in a preliminary experiment that the recovery of insect-derived allergens was >80%. All eluates were dialyzed with 3500 molecular weight cutoff casing (Spectrapor 3; Spectrum Medical Industries, Inc., Los Angeles, Calif.) against distilled water, then lyophilized, and finally reconstituted in 1 ml of PBS.

**Measurement of allergenic activity.** The activities of insect-related allergens (silkworm wing, caddis fly wing, and chironomid whole body) in the eluates were measured by RAST-inhibition assay by use of the insect allergen-paper disk complexes described previously.

A serum pool prepared by mixing equal volumes of serum from five untreated patients, i.e., without specific immunotherapy and highly allergic to silkworm wing, caddis fly wing, and chironomid whole body, was used for this study. The serum pool was also used as a reference reaginic serum to draw standard RAST dilution curves.

Serial threefold dilutions of the filter eluates and each of the three insect reference allergens (each crude extract of 10 ~ 10,000 ng of protein nitrogens per milliliter) in PBS were prepared, and 25  $\mu\text{l}$  of each was placed in duplicate rows of seven polystyrene test tubes. The last tube in each row contained the diluent (PBS) alone. Twenty-five microliters of the serum pool was then added to each tube. The mixtures were shaken vigorously, kept at 4° C overnight, and then subjected to the RAST procedure for measurement of uninhibited IgE antibodies against the three insect reference allergens, as previously described. The ti-

ters of uninhibited IgE antibodies against silkworm wing, caddis fly wing, and chironomid whole body per milligram of a reference reaginic serum were obtained from each standard RAST dilution curve, and eluate RAST-inhibition lines and insect reference allergen RAST-inhibition lines were drawn.

The insect-related allergen content equivalent to insect crude extract was calculated as follows: nanogram of protein per cubic meter of air equals the amount of the insect reference allergen (nanogram of protein nitrogen per milliliter) that produced 50% RAST inhibition, which was obtained from the insect reference allergen RAST-inhibition line times the reciprocal of the eluate dilution ratio that produced 50% RAST inhibition, which was obtained from the eluate RAST-inhibition line times 6.25 (the protein factor) divided by 4000 (total filtered air volume, cubic meter).

## RESULTS

### Insects found in our environment

The number of butterflies was counted every morning during several years to observe the seasonal fluctuation. Although the total number of butterflies observed differed from year to year, the seasonal fluctuation pattern was consistently the same every year. Butterflies increased in number from April to October with two peaks, one in the spring and the other one in the autumn (Fig. 1).

When a light trap was placed during the year 1983

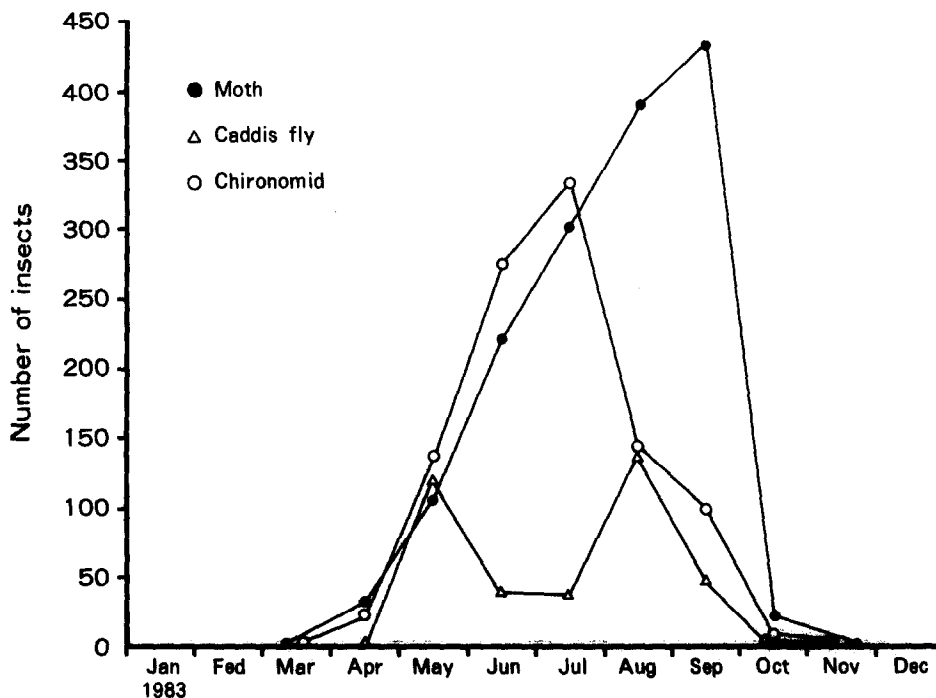


FIG. 2. Seasonal fluctuations of insects collected in a light trap near the Midorogaike Pond, Kyoto. Insects were collected every 5 days.

near the Midorogaike Pond, which is located about 6 km north of the Chest Disease Research Institute, Kyoto University, and during the year 1985 on the roof of the five-story building of the Institute, three kinds of insects were mainly collected: moths, caddis flies, and chironomids. Other insects, such as mayflies and crane flies, were sometimes captured in a light trap, but they were much less in number than moths, caddis flies, and chironomids at both places. At the former place, moths and chironomids were the predominant insects with a single peak each, moths in September and chironomids in July (Fig. 2), whereas at the latter place, caddis flies and chironomids were predominant with two peaks each, caddis flies in June and September and chironomids in July and October (Fig. 3).

These findings imply that the kinds of predominant insects and their seasons of occurrence differ slightly from place to place or from year to year even in a small area of Kyoto but that at least moths, butterflies, caddis flies, and chironomids are the predominant insects in our environment and that they are generally present in increased numbers in the spring and autumn.

#### Frequency of IgE antibody responses to the insects

On the basis of these ecologic features of the insects, the possibility was tested whether some patients

with asthma were sensitized by airborne emanations of these four insects by intracutaneous skin tests and RASTs in 56 randomly selected patients with asthma without any occupational exposure to insects.

As described in Material and Methods, silkworm wing was used for the detection of moth and/or butterfly allergy, caddis fly wing for caddis fly allergy, and chironomid whole body for chironomid allergy.

The results of intracutaneous tests with 1:10<sup>4</sup> dilutions (weight per volume) of silkworm wing, caddis fly wing, and chironomid whole body are presented in Table I. Thirty-nine (69.6%) and 30 (53.6%) of 56 patients with bronchial asthma had positive intracutaneous reactions to silkworm wing and caddis fly wing, respectively. The frequencies of positive reactions to these allergens were as high as those previously reported.<sup>2,3</sup> Moreover, 32 (57.1%) of these patients with asthma had positive intracutaneous reactions to chironomid whole body. In 23 of the group without asthma, only two (8.7%) patients had positive intracutaneous reactions to these three allergens. The atopic status, other than insect allergies of this group with asthma, as demonstrated by intracutaneous skin test reactivities to common inhalant allergens (Torii & Co., Ltd., Tokyo, Japan), is illustrated in Fig. 4. These data demonstrated that the frequencies of positive intracutaneous reactions to these insect allergens were as high as reactions to house dust,

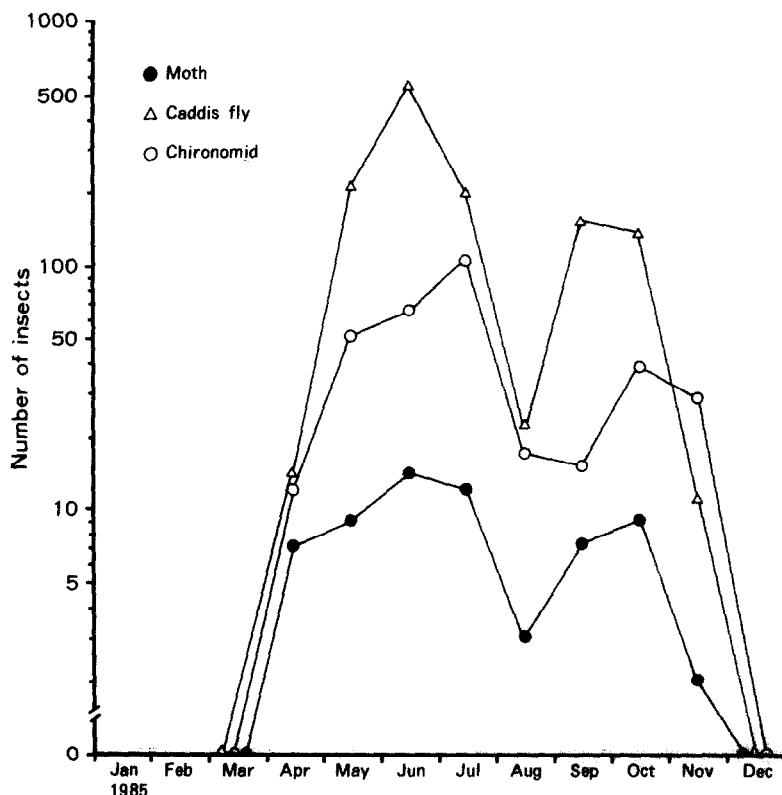


FIG. 3. Seasonal fluctuations of insects collected in a light trap on the roof of the Chest Disease Research Institute, Kyoto University. Insects were collected two times each month.

which is recognized as the most common sensitizer in Japan.

In order to confirm further the reaginic sensitivities to these insect allergens in detail, specific IgE antibodies to silkworm wing, caddis fly wing, and chironomid whole body were examined by RAST in the same groups with and without asthma. Bound radioactivity more than twice that of nonatopic normal serum was considered to indicate the presence of specific IgE antibody in the serum or positive RAST. The results of the RAST and the distribution of  $^{125}\text{I}$ -anti-IgE uptake for each group with positive or negative skin reactions are presented in Fig. 5. In the group with asthma, specific IgE antibodies to silkworm wing, caddis fly wing, and chironomid whole body were detected in 32 (82.1%) of 39 patients with positive skin reactions to silkworm wing, 25 (83.3%) of 30 sensitive to caddis fly wing, and 26 (81.3%) of 32 patients sensitive to chironomid whole body. By contrast, only two of the skin reaction-negative patients with asthma and none of the group without asthma had IgE antibodies. These data illustrate that even if the frequencies of positive skin reaction in patients with asthma were underestimated as much as 50%, at least 40% ( $50\% \times 0.8 = 40\%$ ) of nonselected patients with asthma without any occupational exposure

TABLE I. Intracutaneous skin tests against silkworm wing, caddis fly wing, and chironomid whole body in groups with and without asthma

| Insect extract<br>(1:10 <sup>4</sup> ) | Asthmatic |            | Nonasthmatic |            |
|----------------------------------------|-----------|------------|--------------|------------|
|                                        | No.       | % Positive | No.          | % Positive |
| Silkworm wing                          | 39/56     | 69.6       | 2/23         | 8.7        |
| Caddis fly wing                        | 30/56     | 53.6       | 2/23         | 8.7        |
| Chironomid whole body                  | 32/56     | 57.1       | 2/23         | 8.7        |

to insects had specific IgE antibodies against these insect allergens.

#### Quantitation of airborne insect-related allergens <10 $\mu\text{m}$ in diameter

Although one study<sup>12</sup> has demonstrated that some intact pollens >10  $\mu\text{m}$  in diameter can be found in the bronchi and lung parenchyma of resected and post-mortem lungs, it is usually considered that each allergen must be in fragments <10  $\mu\text{m}$  in diameter inhaled into the bronchi to cause sensitization.

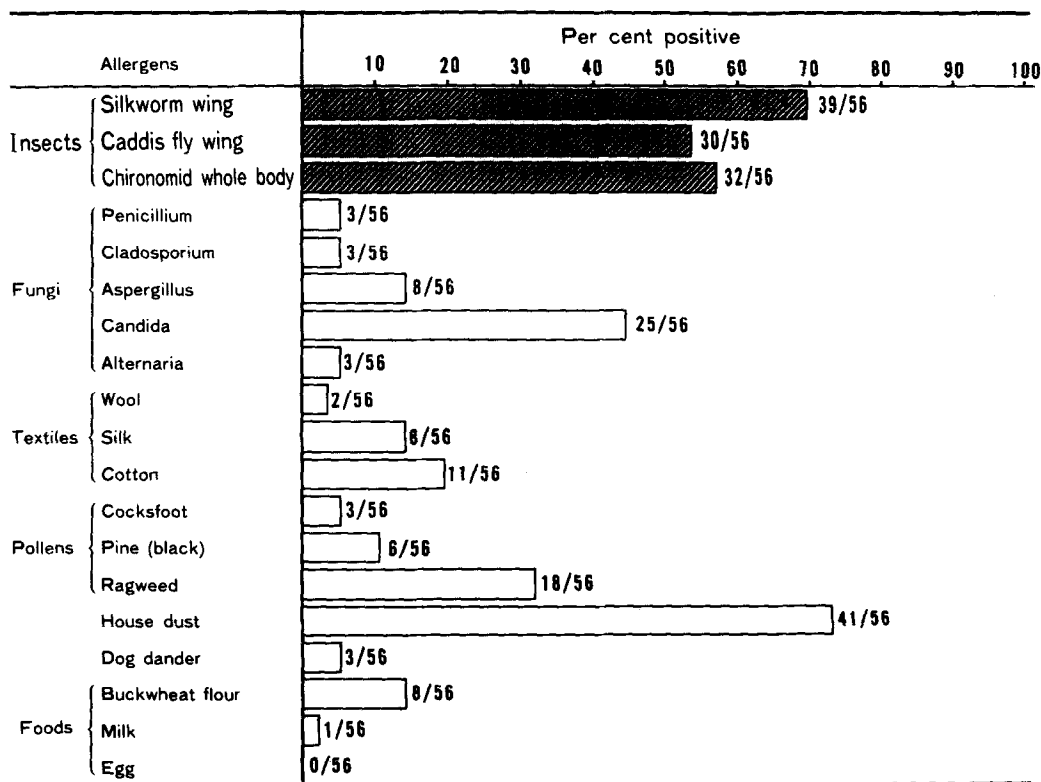


FIG. 4. Intracutaneous skin reactions against common inhalant allergens and insects in the patients with asthma.

In order to determine whether or not the insects (moth, butterfly, caddis fly, and chironomid) that were found in our environment fulfill this criterion, airborne dust  $<10 \mu\text{m}$  in diameter was collected through a high-volume air sampler placed on the roof of our five-story institute, and the filter sheet eluates from January to December were assayed by silkworm wing, caddis fly wing, and chironomid whole body RAST-inhibition tests to quantitate the atmospheric insect-related allergen content.

All filter-sheet eluates inhibited the three insect RASTs in a dose-related fashion, as illustrated, e.g., in Fig. 6. According to the method described in the section on Material and Methods, protein contents equivalent to contents of the crude silkworm wing, caddis fly wing, and chironomid whole body extracts were calculated. The seasonal fluctuations demonstrated two peaks, one in spring (June or July) and the other in autumn (October); the insect-related allergen contents in the air equivalent to the protein content of the crude extracts increased gradually from April, reaching the first peaks in June (caddis fly wing,  $3.08 \text{ ng/m}^3$  of air) or in July (silkworm wing,  $3.18 \text{ ng/m}^3$ , and chironomid whole body,  $5.21 \text{ ng/m}^3$ ), and they decreased in August, the hottest season in Japan, but sharply increased again in October, making the

second peaks (silkworm wing,  $8.24 \text{ ng/m}^3$  of air; caddis fly wing,  $4.74 \text{ ng/m}^3$ ; and chironomid whole body,  $18.19 \text{ ng/m}^3$ ), and continued in considerable amounts in November and in measurable amounts even in December (Fig. 7). In general, it appeared that chironomid particles were the most abundant throughout the year.

#### Seasonal fluctuations of IgE antibodies against the insects

Since the insects (moths, butterflies, caddis flies, and chironomids) and their airborne debris tended to increase with two peaks in a year, one in the spring and one in the autumn, we were interested to observe whether or not IgE antibodies against these insects might also fluctuate in the same way. Furthermore, one could determine whether the antigenicities of silkworm wing, caddis fly wing, and chironomid whole body were identical from observations of the seasonal fluctuations of these insect IgE antibodies. If the antigenicities of these three insect allergens are completely identical, their fluctuation patterns should be the same, and if their fluctuation patterns are not the same, their antigenicities are not necessarily identical.<sup>2</sup>

Therefore, seasonal fluctuations of IgE antibodies

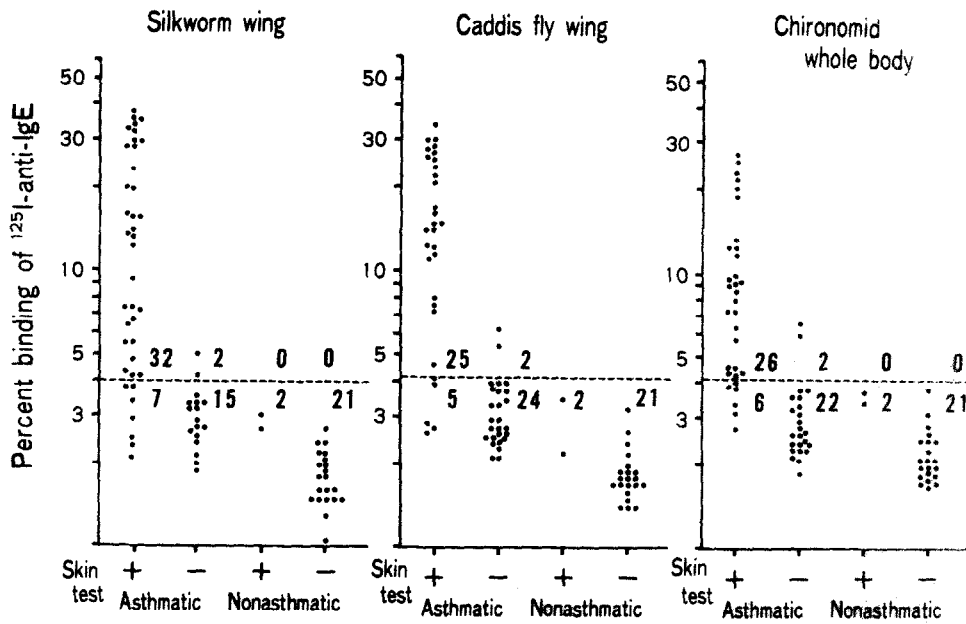


**FIG. 5.** Percent binding of <sup>125</sup>I-anti-IgE in RAST against silkworm wing, caddis fly wing, and chironomid whole body in the patients with asthma compared with control subjects without asthma. Broken lines illustrate twofold average <sup>125</sup>I-anti-IgE binding of five nonatopic control sera. Number of patients with positive or negative RAST are presented above or below the broken lines.

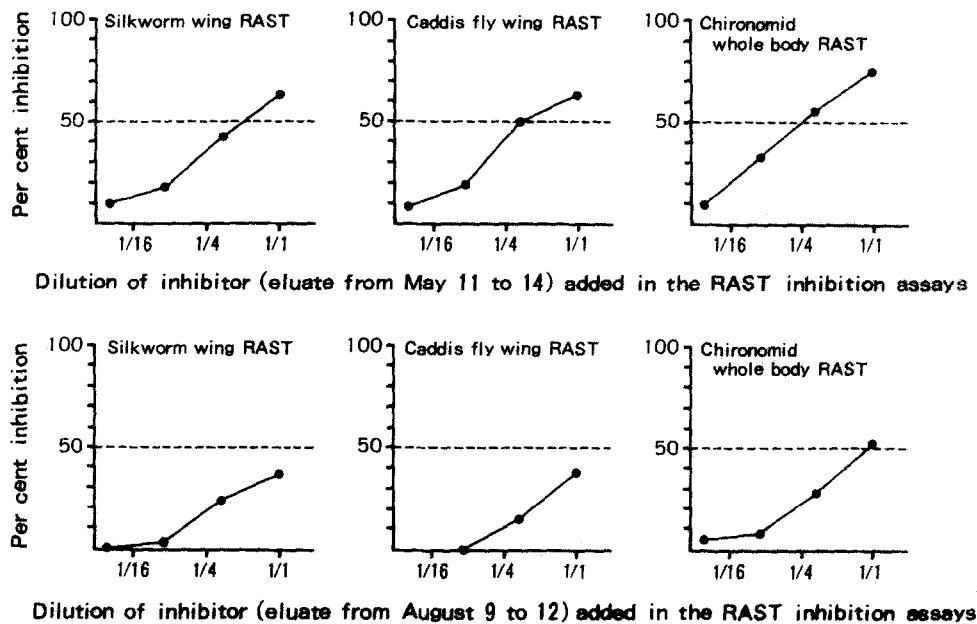


**FIG. 6.** RAST-inhibition lines produced by inhibitors from atmospheric filter eluates containing particles <10  $\mu$ m in diameter.

against silkworm wing, caddis fly wing, and chironomid whole body were determined throughout the year 1985 in two subjects with asthma (Fig. 8).

The first subject (case A) was an 18-year-old schoolboy who had no special outdoor activity, such as collecting insects. Increases in both IgE antibodies

against silkworm wing and caddis fly wing were observed starting in April, and a high level of IgE antibody against silkworm wing was maintained until the beginning of November with a slight decrease in August, but the IgE antibody against caddis fly wing began to decrease in August. In contrast, IgE antibody

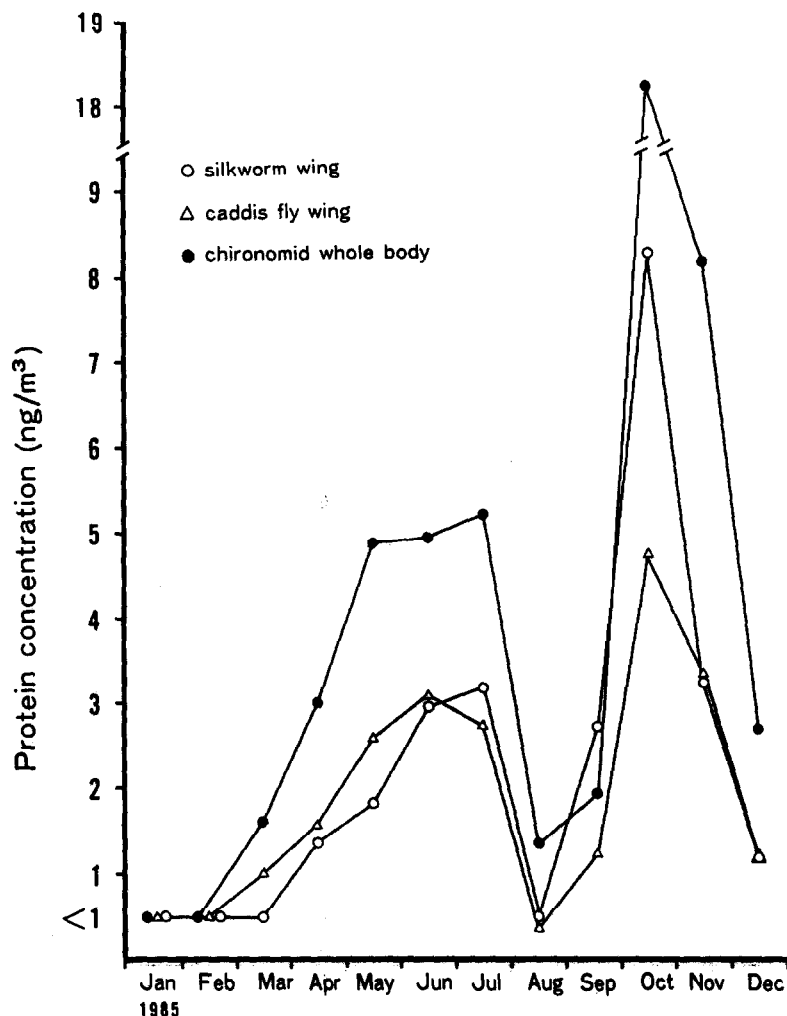


FIG. 7. Seasonal fluctuations of atmospheric insect-related allergens  $<10 \mu\text{m}$  in diameter.

against chironomid whole body did not demonstrate any significant increases during the year.

The second case (case B) was a 22-year-old woman working in an office. Until August there were only slight increases in the insect IgE antibodies, but IgE antibodies against silkworm wing, caddis fly wing, and chironomid whole body all increased from September on, making steep peaks of IgE antibodies against silkworm wing and chironomid whole body in November and a broad peak of IgE antibody against caddis fly wing in November and December.

In these two cases, insect IgE antibodies increased, in general, in spring and autumn, with two peaks when asthmatic symptoms frequently occurred, but the seasons when the peaks occurred differed from case to case and from insect to insect, suggesting that the amounts of each insect allergen might differ from place to place and further indicating that the antigenicities of silkworm wing, caddis fly wing, and chi-

ronomid whole body might not be completely identical.

## DISCUSSION

Our previous studies<sup>1-3</sup> and studies of others<sup>4-7</sup> have called attention to the possibility of widespread insect inhalant allergy and the importance of inhalant insect allergens as one of the environmental factors. However, we had no idea what insects were most numerous in our environment, and quantitative aerometric data on insect allergens were lacking.

We found at least four types of insects in large numbers throughout a year, butterflies, moths, caddis flies, and chironomids, with seasonal fluctuations but with slightly different population patterns from place to place and from insect to insect.

In order to confirm the reaginic sensitivity to these four insects in randomly selected patients with asthma without occupational exposure to the insects, we per-

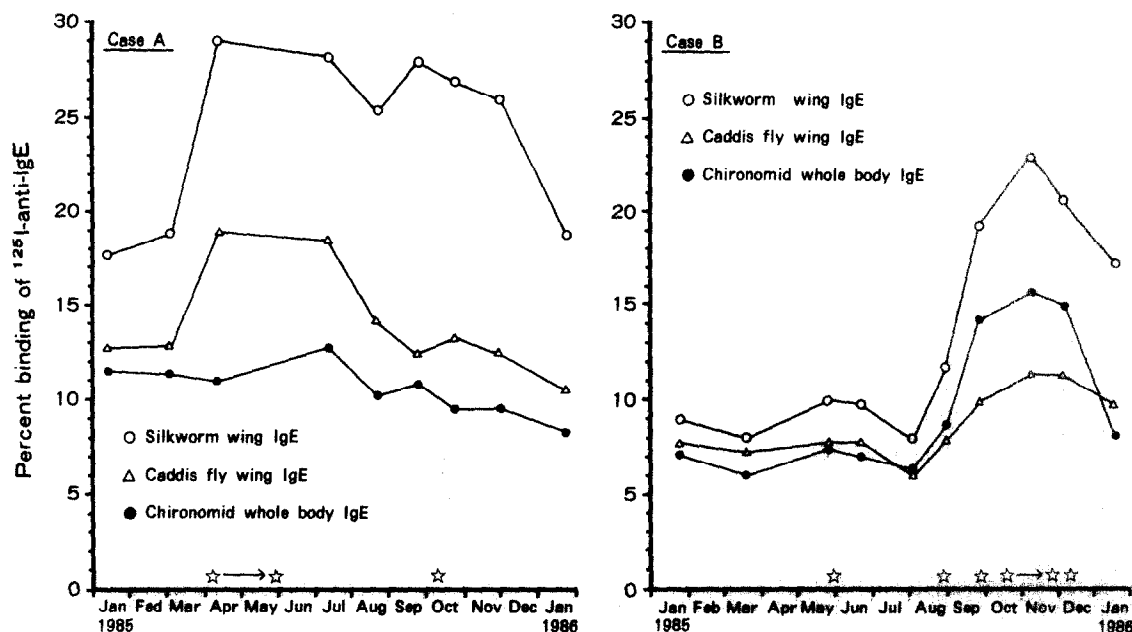


FIG. 8. Seasonal fluctuations of IgE antibodies against silkworm wing, caddis fly wing, and chironomid whole body. \*Indicates occurrence of asthmatic symptoms.

formed intracutaneous skin tests and RASTs with silkworm wing extract for the detection of moth and/or butterfly allergy, caddis fly wing extract for caddis fly allergy, and chironomid whole body extract for chironomid allergy. We found that the frequencies of positive intracutaneous reactions to these three insect allergens (silkworm wing 69.6%, caddis fly wing 53.6%, and chironomid whole body 57.1%) were as high as reactions to house dust (73.2%), which is recognized as the most common sensitizer in Japan, and at least 80% of the skin reaction positive patients had IgE antibodies against each insect allergen. These findings confirmed the findings in our previous studies<sup>1-3</sup> and the probable distribution of moth, butterfly, caddis fly, and chironomid allergens in our environment. The clinical relevance of IgE antibodies against these insect allergens detected in patients' sera has also been suggested by bronchoprovocation tests<sup>1-3</sup>; three patients with positive skin tests and positive RASTs against moth, butterfly, and/or caddis fly wing demonstrated significant reduction of FEV<sub>1</sub> (with symptoms of bronchial asthma) when moth, butterfly, and/or caddis fly wing allergens were, respectively, inhaled, whereas, for patients with negative skin tests and negative RASTs, these allergens demonstrated no airway responses.

Moths and butterflies are characterized by wings covered on both surfaces with scales of varying shapes, approximately 40 to 80  $\mu$ m in area, and the wings of caddis flies are covered on both surfaces with

microscopic hairs, approximately 80 to 100  $\mu$ m in length. We have already reported that IgE antibodies against silkworm moths and caddis flies were most effectively detected when wing extracts were used in the RASTs but not when whole body extract or body only extract was used.<sup>2,3</sup> These findings suggest that microscopic scales and hairs of moths, butterflies, and caddis flies may be the major vectors in airborne exposure. By contrast, although chironomids have neither microscopic scales nor hairs, IgE antibody against chironomids was detected in 26 (81.3%) of 32 skin reaction-positive patients by the use of whole body extract. These findings indicate that chironomid bodies and even scales and hairs of moths, butterflies, and caddis flies must be subdivided into small pieces so that they become airborne particles to be inhaled into the bronchi where they can sensitize patients with asthma.

Most methods used until now for quantitation of airborne allergens have involved morphologic identification and enumeration such as used for pollen grains or mold spores. In 1932, Balyeat et al.,<sup>13</sup> using a morphologic method, reported that the count of butterfly scales was higher than that of grass pollens in June and July. However, morphologic methods are limited to scales of moths and butterflies or hairs of caddis flies, and it is of little clinical value to count insects and their appendages because insects themselves and even their microscopic appendages (scales, 40 to 80  $\mu$ m, and hairs, 80 to 100  $\mu$ m) appear to be

too large to reach the bronchi and cause symptoms.<sup>10</sup> Perlman<sup>14</sup> stated in 1958 that identification of insect debris in air or dust samples was disappointing.

In order to explain why so many subjects with asthma have IgE antibody responses to these insects and to overcome some of the above difficulties, we used the specific immunochemical method originally developed by Agarwal et al.<sup>11</sup> to quantitate atmospheric insect allergens <10 µm in diameter, collected in a high-volume air sampler. In the fraction of particles <10 µm in diameter, there existed activities to inhibit silkworm wing, caddis fly wing, and chironomid whole body RASTs with seasonal fluctuations, but eluate from an unexposed filter could not inhibit these insect RASTs. These findings indicate the existence of airborne insect-related allergens <10 µm in diameter with peak values equivalent to protein contents of crude extracts of silkworm wing, 3.18 ng/m<sup>3</sup> of air, caddis fly wing, 3.08 ng/m<sup>3</sup>, and chironomid whole body, 5.21 ng/m<sup>3</sup> in spring, and of silkworm wing, 8.24 ng/m<sup>3</sup> of air, caddis fly wing, 4.74 ng/m<sup>3</sup>, and chironomid whole body, 18.19 ng/m<sup>3</sup> in autumn. We have no idea whether or not such peak values of insect allergens can cause asthmatic symptoms. However, in comparison with the previously reported peak values<sup>15</sup> of airborne antigen E (approximately 3 ng/m<sup>3</sup> of air) and of airborne Alt-I allergen (approximately 2 ng/m<sup>3</sup> of air), the contents of insect-related allergens <10 µm in diameter measured in this study did not appear to be much less than those of antigen E and Alt-I allergens. Although precise symptom scores of the insect-sensitive patients were, unfortunately, unavailable to correlate with the content of the airborne insect allergens in this study, the increments of IgE antibodies against silkworm wing, caddis fly wing, and chironomid whole body were found, in general, in spring and/or autumn, when insect-related allergen contents increased in the air and asthmatic symptoms frequently occurred for which oral medications of β-agonists and theophyllines were required. However, the fact that the patterns of seasonal fluctuations of IgE antibodies against the insect allergens were not completely the same from patient to patient and from insect to insect suggests that seasonal fluctuations of the insect allergens in the air vary from place to place.

Although it is possible that the immunochemical procedure used in this study measures several cross-reacting allergens, this method of quantitating atmospheric insect allergens is probably a valuable tool for determining the presence of common inhalant allergies caused by insects.

We thank Daiichi Radioisotope Laboratories, Tokyo, Japan, for generous supply of <sup>125</sup>I-anti-IgE, and Torii & Co., Ltd., Tokyo, Japan, for the determination of the protein concentration of allergens.

## REFERENCES

1. Kino T, Oshima S. Allergy to insects in Japan. I. The reaginic sensitivity to moth and butterfly in patients with bronchial asthma. *J ALLERGY CLIN IMMUNOL* 1978;61:10.
2. Kino T, Oshima S. Allergy to insects in Japan. II. The reaginic sensitivity to silkworm moth in patients with bronchial asthma. *J ALLERGY CLIN IMMUNOL* 1979;64:131.
3. Kino T, Oshima S. Studies on bronchial asthma due to insects. 2. Caddis fly as an inhalant allergen and detection of IgE antibody by RAST. *Jap J Allergol* 1978;27:31.
4. Ito K, Miyamoto T, Shibuya T, Kamei K, Mano K, Taniat T, Sasa M. Skin test and radioallergosorbent test with extracts of larval and adult midges of *Tokunagayusurika akamusi* Tokunaga (Diptera: Chironomidae) in asthmatic patients of the population of metropolitan area of Tokyo. *Ann Allergy* 1986; 57:199.
5. Igarashi T, Saeki Y, Matsuno M, Adachi Y, Murakami G, Suzuki Y, Okada T, Kumagai A, Sasa M. Widespread occurrence in Japan of the bronchial asthma induced by chironomid midges. *Ann Allergy* 1985;55(2 PtII):375.
6. Parlato SJ. A case of coryza and asthma due to sand flies (caddis flies). *J ALLERGY* 1929;1:35.
7. Gad El Rab MO, Kay AB. Widespread immunoglobulin E-mediated hypersensitivity in the Sudan to the "green nimitti" midge, *Cladotanytarsus lewisi* (diptera: Chironomidae). I. Diagnosis by radioallergosorbent test. *J ALLERGY CLIN IMMUNOL* 1980;66:190.
8. Bauer X, Dewair M, Fruhmman G, Aschauer H, Pfletschinger J, Braunitzer G. Hypersensitivity to chironomids (non-biting midges): localization of the antigenic determinants within certain polypeptide sequences of hemoglobins (erythrocrucorins) of *Chironomus thummi thummi* (Diptera). *J ALLERGY CLIN IMMUNOL* 1982;69:66.
9. Ceska M, Eriksson R, Varga JM. Radioimmunosorbent assay of allergens. *J ALLERGY CLIN IMMUNOL* 1972;49:1.
10. Bates DV, Fish BR, Hatch TF, Mercer TT, Morrow PE. Deposition and retention models for internal dosimetry of the human respiratory tract. *Health Phys* 1966;12:173.
11. Agarwal MK, Yunginger JW, Swanson MC, Reed CE. An immunochemical method to measure atmospheric allergens. *J ALLERGY CLIN IMMUNOL* 1981;68:194.
12. Michel FB, Marty JP, Quet L, Cour P. Penetration of inhaled pollen into the respiratory tract. *Am Rev Respir Dis* 1977; 115:609.
13. Balyeat RM, Stemen TR, Taft CE. Comparative pollen, mold, butterfly, and moth emanation content of the air. *J ALLERGY* 1932;3:227.
14. Perlman F. Insects as inhalant allergens: consideration of aerobiology, biochemistry, preparation of material, and clinical observations. *J ALLERGY* 1958;29:302.
15. Agarwal MK, Swanson MC, Reed CE, Yunginger JW. Immunochemical quantitation of airborne short ragweed, *Alternaria*, antigen E, and Alt-I allergens: a two-year prospective study. *J ALLERGY CLIN IMMUNOL* 1983;72:40.