

Allergy to insects in Japan

I. The reaginic sensitivity to moth and butterfly in patients with bronchial asthma

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A high frequency of reaginic sensitivity to moth and butterfly extracts was shown in 66 randomly selected patients with bronchial asthma. Thirty-seven (56.1%) of these 66 patients showed positive intradermal reactions to 1:10,000 dilution of moth allergen and 34 patients (51.5%) had positive reactions to 1:10,000 butterfly allergen. These positive responses were as frequent as those to Candida or house dust which are recognized as the most commonly reactive allergens in Japan. Radioallergosorbent tests (RAST) showed that 20 of 66 sera were positive to moth and 22 sera were positive to butterfly, which indicates that about 1/3 of patients with bronchial asthma had specific IgE to moth and butterfly in the sera. Bronchial testing revealed that skin reaction-positive and RAST-positive patients were commonly provoked by specific allergen but skin reaction-negative and RAST-negative patients were not. These data suggest that moth and butterfly emanations could cause allergy-induced bronchial asthma in certain patients. Since moths are attracted readily to artificial lights and often fly into houses, these insects are especially suspect as important factors in extrinsic asthma.

Human beings are surrounded by a huge variety of insect populations.¹ Moths and butterflies, especially, are insects which reside in close contact with human beings in green-rich countries such as Japan.

It has been noted that emanations of insects, especially those of moths and butterflies (*Lepidoptera*), can induce allergic rhinitis and allergic bronchial asthma in some patients. However, only a few illustrative case reports have been presented until now (Table I). In 1932, Parlato^{2, 3} reported on 6 patients, one of whom was a technical assistant who engaged in preparing moth and butterfly allergen. In the same year, Balyeat and co-workers⁴ presented 2 cases, and others were subsequently reported by Urbach and Gottlieb⁵ (one case) in 1941, Stevenson and Mathews⁶ (one case) in 1967, and Phanichyakarn and associates⁷ (one case) in 1969. The patient studied by Stevenson and Mathews had purely occupational exposure during raising and caring for moths (*Galleria*

mellonella), the larval stage of which is used as fish bait. Other subjects were mostly gardeners, either by occupation or hobby.

Moths and butterflies are characterized by wings covered on both surfaces with scales or hairs of varying shape. These appendages are easily rubbed off, either in flight or on the most gentle contact, forming a fine dust which is readily windborne. Furthermore, not only in the live stage but also after the death insect debris accumulates and becomes a part of dust in several seasons; however, identification of the debris as insect derived may be difficult.¹

Accordingly, we suspected that bronchial asthma in several patients might be attributable, in part, to moths and/or butterflies. To evaluate this possibility, we extracted allergens from moths and butterflies and tried some immunologic tests in randomly selected patients with bronchial asthma to see how many patients are sensitized by these insects.

MATERIALS AND METHODS

Subjects

The group with bronchial asthma consisted of 66 patients who were randomly selected from the Outpatient Asthma Clinic of the Chest Disease Research Institute, Kyoto University. The 37 subjects who participated in a control non-asthmatic group consisted of 7 normal subjects, 2 subjects

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TABLE I. Reports of bronchial asthma due to moth and butterfly

Author	No. of cases	Year reported
Parlato ^{2,3}	6	1932
Balyeat et al. ⁴	2	1932
Urbach et al. ⁵	1	1941
Stevenson et al. ⁶	1	1967
Phanichyakarn et al. ⁷	1	1969

with chronic bronchitis, 3 with emphysema, 11 with pulmonary tuberculosis, 3 with pneumonitis, 4 with lung cancer, 1 with spontaneous pneumothorax, 1 with vanishing tumor, 1 with multiple myeloma, 1 with silicosis, and 1 with sarcoidosis. The latter subjects were all nonatopic with serum IgE levels within normal limits. These two groups were well matched in age, ranging from 9 to 70 yr.

Allergens

Extraction of allergens from the whole bodies, including scaly wings, of both moths and butterflies was accomplished by completely identical procedures.

The composition of buffered saline (pH 8.0) used for extraction was that used at the University of Michigan Hospital,⁸ i.e., 5.0 gm of NaCl, 0.3 gm of KH₂PO₄, 7.0 gm of Na₂HPO₄ (anhydrous), 4.0 gm of phenol crystals, and water for injection to make 1,000 ml were mixed, adjusted to pH 8.0 with the use of 2 N NaOH or HCl, and filtered through a Millipore filter of 0.22μ pore size (Millipore Filter Corp., Bedford, Mass.). Analytic-grade reagents were used throughout.

Dry specimens were gently ground with a mortar and pestle. After being defatted with ethyl ether, 1 gm of resulting material was macerated for 3 days with occasional agitation in 49 ml of sterile buffered saline (pH 8.0) and 0.25 ml of toluene. The resulting extract was obtained as a supernatant by centrifugation (12,000 rpm for 15 min) and dialyzed against sterile buffered saline (pH 8.0) for 4 days in a cold room (4° C). Finally, the extract (1:50 by w/v) was sterilized by filtration through Millipore filters of 0.22μ pore size and checked for sterility. When it was necessary to dilute, diluent (Torii & Co., Ltd.) containing physiologic saline with 0.5% phenol was used.

The protein concentration, determined by micro-Kjeldahl method, was 140 μg/ml for 1:50 dilution of moth extract and 52 μg/ml for that of butterfly extract.

Skin tests

Tests were performed on the volar surface of the forearm by intradermal injection of 0.02 ml of 1:10³ and 1:10⁴ dilution (w/v) of allergen extracts. Readings were made after 15 min, and wheal and flare greater than 9 and 20 mm, respectively, were recorded as a positive reaction.

In addition to moth and butterfly allergens, *Candida* and house dust (Torii & Co., Ltd.), which are recognized as the most commonly reactive allergens in Japan, were tested

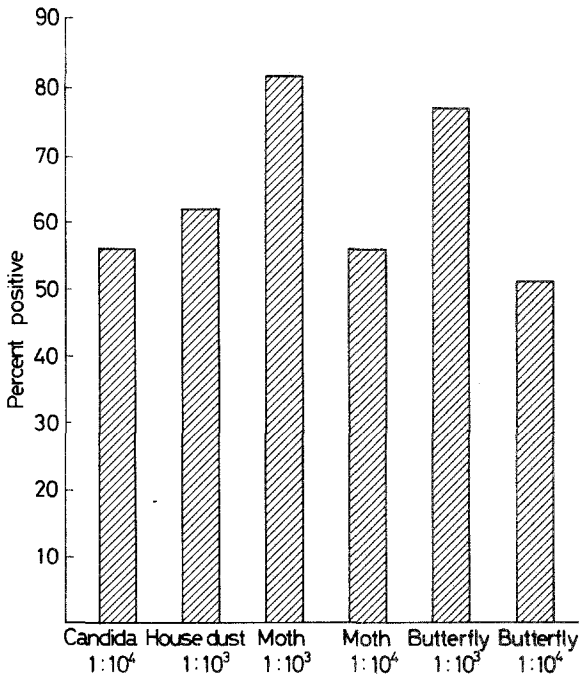


FIG. 1. Intracutaneous skin reactions in patients with bronchial asthma to *Candida* (1:10⁴), house dust (1:10³), moth (1:10³ and 1:10⁴), and butterfly (1:10³ and 1:10⁴).

simultaneously to compare the frequencies of positive reactions.

Passive transfer

A standard Prausnitz-Küstner (P-K) technique was used. That is, each 0.1 ml of patient serum was injected intradermally into persons nonatopic to moth and butterfly, and the sites were challenged 48 hr later with 0.02 ml of 1:10⁴ dilution (w/v) of moth and butterfly extracts. As controls, heat-inactivated patient serum (56° C, 2 hr) and nonatopic serum were used.

Preparation of allergen–paper disc complex

Extracts of moth and butterfly, 1:50 (w/v), were lyophilized. Lyophilized material, 80 mg, was dissolved in 10 ml of distilled water, in which 200 pieces of CNBr-activated paper disc (Toyo filter paper No. 6, diameter, 6 mm) were immersed to couple the allergen. All subsequent steps followed the method of Ceska and co-workers.⁹

Radioallergosorbent test (RAST)

Portions of serum, 50 μl, and an allergen–paper disc were incubated at room temperature for 2 days. After the paper discs were washed 3 times with 2.5 ml of physiologic saline, 50 μl of ¹²⁵I–anti-IgE (Pharmacia, Sweden) was added and the mixture was incubated overnight at room temperature. The excess of ¹²⁵I–anti-IgE was washed away 3 times with 2.5 ml of physiologic saline and the radioactivity bound to the discs was determined in a gamma counter.

TABLE II. Intracutaneous skin reactions in asthmatic and nonasthmatic groups to moth and butterfly

Allergen	Asthmatic		Nonasthmatic		χ^2 test
	No. positive	% positive	No. positive	% positive	
Moth					
1:10 ³ *	54/66	81.8	11/37	29.7	p < 0.001, χ^2 = 27.63
1:10 ⁴	37/66	56.1	3/37	8.1	p < 0.001, χ^2 = 22.96
Butterfly					
1:10 ³	51/66	77.3	10/37	27.0	p < 0.001, χ^2 = 24.77
1:10 ⁴	34/66	51.5	3/37	8.1	p < 0.001, χ^2 = 19.40

*Dilution expressed in weight/volume.

TABLE III. RAST in asthmatic and nonasthmatic groups in relation to skin reactions

Group of subjects	Moth		Butterfly	
	RAST+*	RAST-	RAST+*	RAST-
Asthmatic				
Skin test +†	20	17	22	12
Skin test -	1	28	2	30
Nonasthmatic				
Skin test +	0	2	1	1
Skin test -	1	28	1	28

*More than 2 times cpm of negative control was considered as positive RAST.

†Skin test was done by use of 1:10⁴ dilution of moth and butterfly.

Provocation tests

When the patients became symptom free, the provocation test was performed according to a slight modification of the method outlined by Chai and associates.¹⁰ Oral or inhaled bronchodilators were omitted at least 12 hr before testing, and in patients who had used corticosteroids testing was done 7 days after cessation of therapy.

The tests were accomplished by the following procedure: First, 10 min following inhalation of 0.5 ml of diluent, it was confirmed that FEV₁ was not significantly decreased from the baseline value. Then the patient inhaled 0.5-ml portions of moth or butterfly extracts from a level 100 times more concentrated than the end point of the intracutaneous tests to a final concentration of 1:100 (w/v). These were nebulized through a bird respirator for 3 min, and FEV₁ was measured immediately, 15 min, and 5 hr later. A greater than 20% fall in FEV₁ from the diluent control test was considered a significant decrease. When no significant decrease of FEV₁ was observed even at the final concentration of 1:100 (w/v), the provocation test was considered negative.

RESULTS
Skin test

The results of intracutaneous tests with 1:10⁴ dilution (w/v) of moth and butterfly extracts are noted in Table II. Thirty-seven (56.1%) of 66 patients with

bronchial asthma displayed positive intracutaneous reactions to moth extract and 34 (51.5%) were positive to butterfly extract. In 37 subjects of the nonasthmatic group, only 3 subjects (8.1%) showed positive intracutaneous reactions to moth and butterfly extracts, respectively. The frequencies of positive reactions in these two groups (bronchial asthma and nonasthmatic) were significantly different at p < 0.001. Although tests with 1:10³ dilution of the allergens also showed a highly significant difference between asthmatic and nonasthmatic groups, the percent positive to moth and butterfly in the nonasthmatic group increased to 29.7% and 27.0%, respectively. Thirty-seven (56.1%) of 66 patients with bronchial asthma exhibited positive intracutaneous reactions to *Candida* and 41 (62.1%) were positive to house dust (Fig. 1). These data show that the frequencies of positive intracutaneous reactions to moth and butterfly were as high as those to *Candida* and house dust which are recognized as the most common sensitizers in Japan.

Passive transfer

Among 5 patients with bronchial asthma who showed positive intracutaneous tests to moth and butterfly, the P-K tests were successfully accomplished in 4. In control experiments, negative P-K reactions were observed with heat-inactivated serum (56° C, 2 hr) and with serum from a nonatopic subject. These results confirm the presence of skin-sensitizing antibody to moth and butterfly in the sera of the skin-reactive patients.

Radioallergosorbent test (RAST)

RAST is more useful than passive transfer to detect specific IgE in sera since it facilitates testing of a considerable number of sera at once without the danger of transferring hepatitis virus.

According to the procedure described in the section on materials and methods, specific IgE to moth and butterfly was sought in 66 patients with bronchial

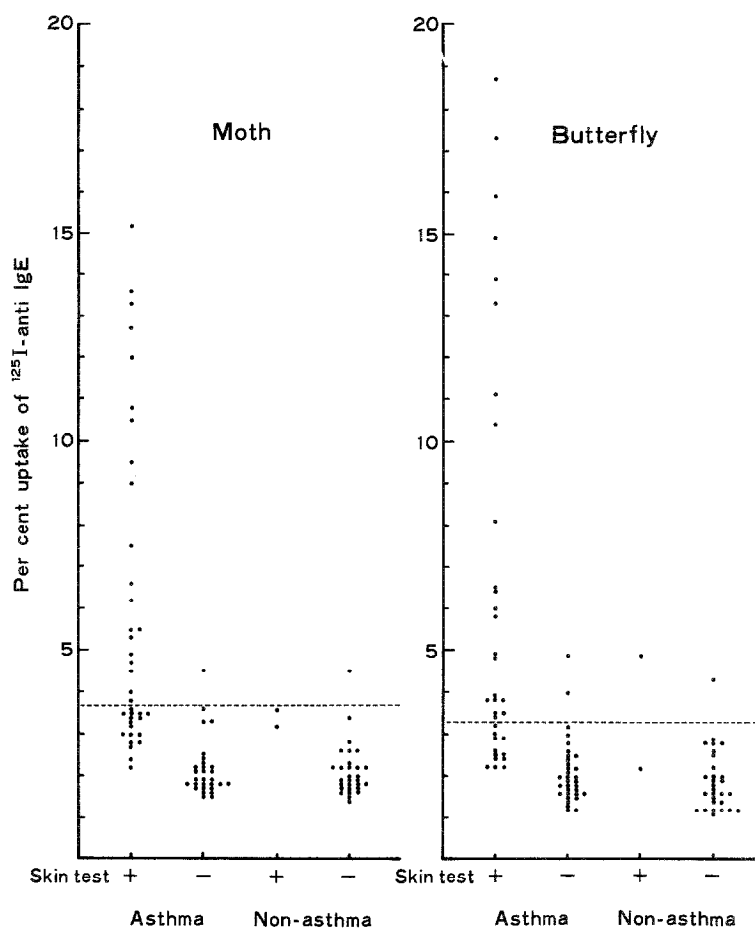


FIG. 2. Distribution of ^{125}I -anti-IgE uptake in RAST. Total activity added was 56,926 cpm/50 μl (10 ng of anti-IgE). *Dashed lines* show 2 times average uptake of 5 nonatopic normal sera, 3.7% and 3.3% in the RAST of moth and butterfly, respectively.

asthma (asthmatic group) and 31 subjects without asthma (nonasthmatic group). When the bound radioactivity exceeded twice that of nonatopic normal serum, it was considered to indicate specific IgE in the serum.

The results of the RAST and the distribution of ^{125}I -anti-IgE uptake for each subject are shown in Table III and Fig. 2. In the group of asthmatic subjects, specific IgE to moth extract was detected in 20 of 37 skin-reactive patients, and 22 of 34 patients skin reactive to butterfly extract were shown to have butterfly-specific IgE in their sera. On the other hand, only 1 and 2 patients with negative skin reactions showed specific IgE in the sera to moth and butterfly, respectively, with low uptake of ^{125}I -anti-IgE. In the nonasthmatic group the subjects who had specific IgE to moth and butterfly were as few as in the skin reaction-negative patients with bronchial asthma. These data show that approximately 1/3 of non-selected patients with bronchial asthma had specific IgE to moth and butterfly, a result which was

significantly different from that in nonasthmatic patients.

Season

The patients with bronchial asthma were divided into two groups depending on whether or not they had specific IgE to moth and/or butterfly in sera. The symptomatic seasons of 23 patients with positive RAST and 30 patients with negative RAST who were observed clinically through the year at the Outpatient Asthma Clinic were plotted in Fig. 3, *A* and *B*. The symptoms, which consist of attacks of bronchial asthma, sneezing, and nasal discharge occurred most frequently from April to October in the RAST-positive patients, which closely corresponds to the activity periods of moths and butterflies; while the RAST-negative patients, 12 of whom had perennial and nonatopic type of bronchial asthma, showed less symptoms during insect flying seasons. As about 60% of RAST-positive patients also showed sensitivity to house dust and/or caddis fly, we cannot imply that all

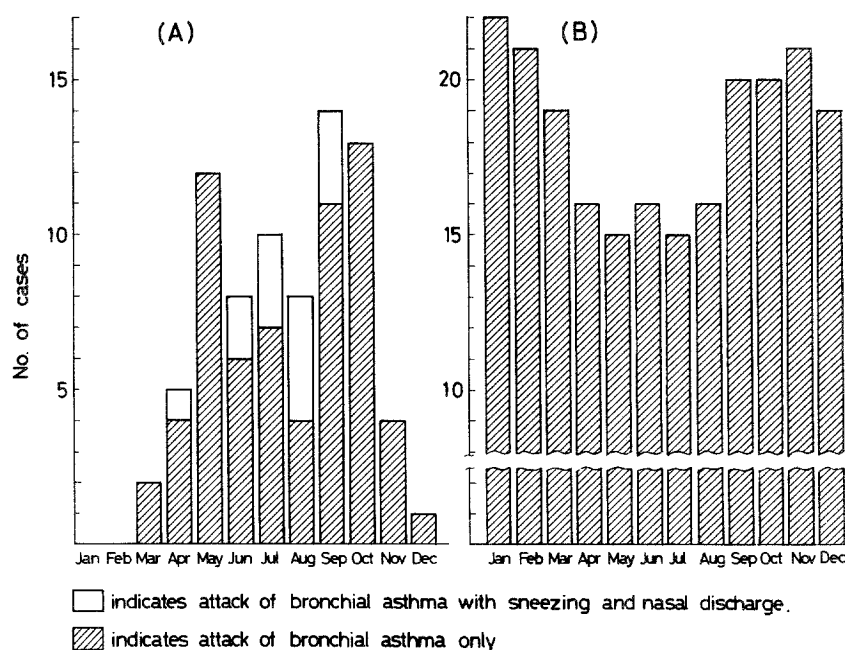


FIG. 3. Seasons of attack of bronchial asthma in 23 RAST-positive (A) and 30 RAST-negative (B) patients with bronchial asthma to moth and/or butterfly.

symptoms during insect flying seasons could be attributable solely to these insects. However, as all 23 patients with positive RAST could be described as atopic on the basis of disease pattern, multiple skin test positivity, and suggestive family history, these data do not deny the clinical relevance of moth and butterfly exposure.

Provocation test

The inhalation tests were performed in 6 patients with bronchial asthma to directly evaluate respiratory reactivity to moth and butterfly. Three patients (R. T., K. K., and E. E.) with negative skin tests and negative RASTs showed neither significant reduction of FEV₁ nor clinical symptoms even when maximum doses (1:100 dilution) of moth and butterfly allergens were inhaled (Tables IV and V). The remaining 3 patients (K. T., K. M., and K. O.) with positive skin tests and positive RASTs, whose end points of intracutaneous testing were, respectively, 1:10⁵, 1:10⁴, and 10⁵ to moth and 1:10⁵, 1:10⁵, and 1:10⁵ to butterfly, showed airway responses. In these, a reduction of more than 20% in FEV₁ was observed 15 min after extracts 100 times the concentration of each end point of the intracutaneous tests (data were not shown in the tables). In Tables IV and V, positive provocations of these 3 patients at the concentration of 1:100 and each baseline value are shown to compare with the negative provocation tests of the patients with negative skin tests and negative RASTs. These data sug-

gest that the provocation was allergy induced rather than caused by nonspecific irritant reactions.

DISCUSSION

For insects to merit attention as allergen sources, it is necessary that the insects reside in close contact with patients and that a considerable number of patients are sensitized by them.

From our study it was realized that the frequencies of positive intracutaneous reactions to moth and butterfly were as high as those to house dust and *Candida* which are recognized as the most commonly sensitizing allergens in Japan and approximately 1/3 of non-selected patients with bronchial asthma had specific IgE to moth and butterfly in their sera. Furthermore, bronchial responsiveness to these insects was suggested by appropriate provocative testing. These data suggest that allergic asthma due to moth and butterfly may be widespread in view of frequent sensitization. In addition, moths and butterflies make their appearance from spring to autumn with several emergences. While butterflies are active diurnally around flowers or vegetables, moths appear in the late afternoon, flying into houses and buildings, attracted by artificial lights. Their scales and hairs are easily rubbed off, either in flight or on gentle contact, to form fine and windborne dusts. Although the detection of these insects' emanations in our environment was not the scope of this study, Parlato² and Balyeat and associates⁴ both reported that scales and hairs were found on

TABLE IV. Provocation test in patients with bronchial asthma by moth allergen

Patient	Intracutane- ous test	RAST	Baseline	Diluent	Provocation test		
					0.5 ml of 1:100 moth allergen		
					Immediate	15 min	5 hr
K. T.	+	+	1,900†	1,910	1,450 (24.1↓‡)	1,170 (38.7↓)	1,780 (6.8↓)
K. M.	+	+	4,350	4,350	4,120 (5.2↓)	3,410 (21.6↓)	4,240 (2.6↓)
K. O.	+	+	2,940	2,920	2,160 (26.0↓)	1,610 (44.9↓)	2,650 (9.2↓)
R. T.	—	—	3,360	3,410	3,480 (2.1↑)	3,340 (2.1↓)	3,470 (1.8↑)
K. K.	—	—	2,760	2,750	2,770 (0.7↑)	2,610 (5.1↓)	2,740 (0.4↓)
E. E.	—	—	4,110	4,030	4,110 (2.1↑)	3,970 (1.5↓)	3,980 (1.2↓)

*Positive (+) or negative (—) tested at the concentration of 1:10⁴ dilution of moth allergen.
†FEV₁ (ml).
‡% loss (↓) or gain (↑) from FEV₁ at diluent inhalation.

TABLE V. Provocation test in patients with bronchial asthma by butterfly allergen

Patient	Intracutane- ous test	RAST	Baseline	Diluent	Provocation test		
					0.5 ml of 1:100 butterfly allergen		
					Immediate	15 min	5 hr
K. T.	+	+	1,850†	1,830	1,410 (23.0↓‡)	1,090 (40.4↓)	1,800 (1.6↓)
K. M.	+	+	4,110	4,120	2,900 (29.6↓)	2,780 (32.5↓)	4,090 (0.7↓)
K. O.	+	+	2,940	2,970	1,920 (34.2↓)	1,580 (45.9↓)	2,610 (10.6↓)
R. T.	—	—	3,460	3,470	3,450 (0.6↓)	3,460 (0.3↓)	3,490 (0.6↑)
K. K.	—	—	2,750	2,740	2,760 (0.7↑)	2,650 (3.3↓)	2,750 (0.4↑)
E. E.	—	—	4,100	4,050	4,030 (0.5↓)	4,110 (1.2↑)	4,060 (0.2↑)

*Positive (+) or negative (—) tested at the concentration of 1:10⁴ dilution of butterfly allergen.
†FEV₁ (ml).
‡% loss (↓) or gain (↑) from FEV₁ at diluent inhalation.

glass slides which had been exposed on the top of an office building, several parks, and hospitals in amounts corresponding closely to those of pollens.

Despite these ecologic features, reports of allergy due to moth and butterfly have been presented only rarely until now (Table I). Balyeat and co-workers⁴ extracted the allergens from scales of moth and butterfly by use of glycerine and Coca's solution and tried to routinely test 79 patients with asthma and hay fever. Then, only 2 of 79 patients showed positive intracutaneous reaction. The reason for this major difference from our results is unknown because neither the protein concentration of the allergens tested nor the precise method of extraction was described in his paper. One factor may relate to differences in levels of the insect exposure around patients in Japan and the United States; another reason might be that Balyeat and co-workers⁴ extracted allergens from moth and butterfly scales while we used whole insect bodies including scaly wings. The latter possibility has little to support it, however, because of scales

being well recognized as a major vector of airborne exposure. Rather, the most important factor seems to be the way in which the principal allergenic components are extracted. In fact, another series of allergen extracts was made from the same specimens of moths and butterflies we had studied with the use of D.P. solution (4.5 gm of dextrose, 0.2 gm of NaHCO₃, 0.5 gm of phenol, and water for injection to make 100 ml) by Torii & Co., Ltd., Tokyo, Japan. Although certain details of processing are confidential, the resulting extracts were tested on the same patients as had been tested previously. The frequencies of positive intracutaneous reactions obtained both to moth and butterfly were reduced to approximately 1/3 of the results shown in this report, and the diameter of the wheal and flare was smaller in each patient. Furthermore, the number of the patients with positive RAST and the amounts of specific IgE detected were less than those we obtained with our own extracts (unpublished data).

None of the patients sensitive to moth and/or but-

terfly had notable histories of exposure to these insects, except for one whose hobby was collecting moths and butterflies. This suggests that a majority of our patients were sensitized involuntarily by windborne emanations of these insects or those which are incorporated in the house dust.

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REFERENCES

1. Perlman, F.: Insects as inhalant allergens. Consideration of aerobiology, biochemistry, preparation of material, and clinical observations, *J. ALLERGY* **29**:302, 1958.
2. Parlato, S. J.: Emanations of flies as exciting causes of allergic coryza and asthma. III. Hypersensitiveness to moths and butterflies (Lepidoptera), *J. ALLERGY* **3**:125, 1932.
3. Parlato, S. J.: Emanations of flies as exciting causes of allergic coryza and asthma. V. A study of the atopic reagins of the caddis fly, butterfly and moth, *J. ALLERGY* **3**:459, 1932.
4. Balyeat, R. M., Stemen, T. R., and Taft, C. E.: Comparative pollen, mold, butterfly and moth emanation content of the air, *J. ALLERGY* **3**:227, 1932.
5. Urbach, E., and Gottlieb, P. M.: Asthma from insect emanations. Report of a case due to moths, *J. ALLERGY* **12**:485, 1941.
6. Stevenson, D. D., and Mathews, K. P.: Occupational asthma following inhalation of moth particles, *J. ALLERGY* **39**:274, 1967.
7. Phanichyakarn, P., Dockhorn, R. J., and Kirkpatrick, C. H.: Asthma due to inhalation of moth flies (*Psychoda*), *J. ALLERGY* **44**:51, 1969.
8. Phillips, G. L.: Preparation of allergenic extracts for testing and treatment, *in* Sheldon, J. M., Lovell, R. G., and Mathews, K. P., editors: *A manual of clinical allergy*, Philadelphia and London, 1967, W. B. Saunders Co., p. 507.
9. Ceska, M., Eriksson, R., and Varga, J. M.: Radioimmunosorbent assay of allergens, *J. ALLERGY CLIN. IMMUNOL.* **49**:1, 1972.
10. Chai, H., Farr, R. S., Froehlich, L. A., Mathison, D. A., McLean, J. A., Rosenthal, R. R., Sheffer, A. L., Spector, S. L., and Townley, R. G.: Standardization of bronchial inhalation challenge procedures, *J. ALLERGY CLIN. IMMUNOL.* **56**:323, 1975.