

Respiratory allergy to laboratory fruit flies (*Drosophila melanogaster*)

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Workers in a scientific laboratory, complaining of respiratory symptoms related to their contact with fruit flies, were tested to detect allergy to these insects. Seven of 22 of these individuals had respiratory symptoms, and six of these seven demonstrated both immediate-type skin reactions and positive RAST results. Nasobronchial challenge tests elicited responses also in six of the seven exposed workers with symptoms. Neither the majority of the exposed group without symptoms nor the nonexposed control group reacted to the skin test, RAST, or nasobronchial challenge. Specificity of the fruit-fly allergen and its presence in the dust of the laboratory cultivation room were confirmed by RAST-inhibition experiments. The present results reflect the high prevalence of occupational respiratory disease caused by an IgE-mediated allergy induced by the contact with laboratory fruit flies and fruit-fly cultures. (J ALLERGY CLIN IMMUNOL 77:108-13, 1986.)

Many articles have described and documented the role of insects in respiratory allergy. Roughly speaking, four types of situations can be distinguished in which contact with insects or insect material can lead to allergic sensitization: natural, domestic, occupational, and hobby exposure. Examples of insects reported recently to be sources of allergen are midges,^{1, 2} moths and butterflies,^{3, 4} beetles,⁵⁻⁸ crickets,⁹ locusts,¹⁰ and cockroaches,¹¹ as well as immature stages such as maggots¹² and mealworms.¹³

Relatively well-known are the extensive studies, performed some time ago in the United States, of inhalant sensitivities caused by natural exposure to insects commonly referred to as flies but belonging to different insect orders¹⁴: mayflies^{15, 16} (order Ephemeroptera) and caddis flies¹⁷⁻¹⁹ (order Trichoptera).

In this article we report clinical and immunologic studies in a group of laboratory workers, some of whom developed allergic respiratory symptoms that were believed to be associated with their work with the fruit fly *Drosophila melanogaster* (order Diptera).

PERSONS AND METHODS

Exposed workers and controls

Twenty-two persons (13 female and nine male), aged between 22 and 62 years and working with various laboratory strains of the fruit fly *D. melanogaster*, were studied. Some workers did mainly laboratory work and other workers, mainly office work, but all worked in the same building on the same floor in about 20 adjacent or connected rooms. In the laboratory there were office rooms where no fruit flies were held or studied, laboratory rooms where fruit flies were handled under CO₂ anesthetization, cultivation rooms where fruit flies were grown in large numbers of small glass bottles with cotton wool stoppers, and other rooms for the preparation of culture media, the cleaning of glassware, etc.

Fourteen nonexposed persons were included in the RAST studies; skin tests and inhalation tests were performed in eight persons.

Allergenic and immunologic tests

Extracts of a mixture of laboratory strains of *D. melanogaster* and extracts of dust collected from the floor of cultivation rooms were prepared in a phosphate-buffered saline solution in a strength 1:100 w/v. For the RAST-inhibition experiment extracts of house dust mite (*Dermatophagoides pteronyssinus*) and of nutrient-medium components were also prepared.

Skin tests were performed intracutaneously with extracts of fruit flies and cultivation-room dust in three serial dilutions of 1:1,000,000; 1:100,000; and 1:10,000 w/v. The reactions were recorded after 20 minutes, scored in plus signs (each corresponding with 10 mm diameter of the wheal and 30 mm diameter of the flare), and taken together for the three dilutions. A total score of <3 was considered as

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a negative skin test result. Results of control testing with saline solution were all negative. Reactivity of the skin was checked by testing with histamine (chloride) solution and found to lie within a normal range in all persons tested.

Allergen discs for RAST and RAST-inhibition studies were prepared as follows: Whatman No. 1 filter-paper discs (100 discs weighing approximately 250 mg) were activated by cyanogen bromide dissolved in acetonitrile (March et al.,²⁰ 1974). The activated discs were added to 6 ml of allergen extract (1% w/v in 0.1 mol/L NaHCO₃, pH 8.4) and incubated at 4° C overnight. After coupling of the allergens, the discs were washed as described by Ceska et al.,²¹ (1972) and stored in phosphate-buffered saline containing 0.3% w/v of human serum albumin and 0.05% sodium azide.

The RAST procedure was carried out according to the instructions of the manufacturer of the radiolabeled IgE specific antibodies (Pharmacia Diagnostics AB, Uppsala, Sweden). RAST results were considered as positive when the count rates, expressed as percentage of the total activity added, exceeded twice the mean value of the negative control group. Binding percentages of positive RAST results for *Drosophila* and cultivation-room dust were above 1.0% and 0.7%, respectively.

With the sera of nine exposed workers and nine nonexposed control subjects, RAST was performed with house dust-mite discs prepared in our laboratory as described above. The results were compared with those obtained with commercially available *D. pteronyssinus* discs, by use of enzyme-labeled IgE specific antibodies (Phadezym, Pharmacia Diagnostics AB), according to the instructions of the manufacturer.

RAST-inhibition studies were performed with pooled sera of three persons with strong RAST positivity by a method essentially according to Arbesman et al.²² (1977). In brief, serial dilutions of allergen extracts in buffered saline containing 1% of human serum albumin were prepared. To 50 µl of a mixture of three *Drosophila* RAST positive sera (Nos. 3, 4, and 8), 150 µl of allergen dilution was added. After overnight incubation at room temperature, an allergen disc was added, and the subsequent steps were performed as described for the normal RAST procedure. The results were expressed as

$$\frac{\text{radioactive counts uninhibited} - \text{radioactive counts inhibited}}{\text{radioactive counts uninhibited}} \times 100$$

Inhalation tests were performed with fruit-fly extract (1:10,000, and 1:1,000) in 14 exposed workers and eight nonexposed control subjects. The extract was aerosolized in a Wiesbadener Doppel inhalator (Wiesbadener Inhalatoren-Vertrieb, Wiesbaden, W. Germany), which disperses about 1 ml in 10 minutes and inhaled via the nose, so that precipitation occurred on nasal as well as bronchial mucosa. Both nasal and bronchial resistance was measured during a 7-minute period before inhalation (15 successive measurements) and during a 15-minute period after inhalation (20 measurements).

This was done by the technique of interruption of the airflow with measurement at opening.²³ The measurements were made during mouth as well as nasal respiration, and the value after subtraction of the two was regarded as the nasal resistance.

Peak flow values were measured during the 24 hours before and after inhalation (hourly, but not during sleep), and complaints were noted during the 48 hours before and 24 hours after the inhalation. Inhalation tests with the solvent (phosphate-buffered saline) under a single blind condition were performed on separate days in most of the patients tested. The early bronchial reaction was considered positive if the resistance (mean of 15 values) rose by 25% and complaints were present. The late reaction was considered positive if the peak flow value fell at any time by 10% and the drop was associated with complaints. The early nasal reaction was called positive if resistance rose 100% or complaints were present. The late nasal reaction was called positive if complaints were reported but were absent after inhalation of the solvent.

RESULTS

Symptoms

The 22 exposed workers were interviewed concerning the occurrence of allergic respiratory symptoms such as wheezing, dyspnea, coughing, stuffy nose, and itchy eyes related to their work. Special attention was focused on the relation between the onset of complaints and starting work as well as symptom-free periods during weekends and/or holidays. Interviewing was continued until an unequivocal temporal relation between symptoms and exposure could be confirmed or excluded.

In the first column of Table I, a relationship between exposure and symptoms is indicated qualitatively by plus signs. For seven workers a positive relation was found. Fifteen workers reported no symptoms related to contact with fruit flies.

All seven persons with complaints reported nasal symptoms, whereas the lower respiratory tract and the eyes were involved in four and three cases, respectively.

Skin test and RAST

Table I demonstrates the results of skin tests and RAST with extracts of fruit flies and cultivation-room dust in the 22 exposed workers, in nine of whom both an experimental and a routine *D. pteronyssinus* RAST was done to check the experimental technique. The results of these tests in nonexposed control persons are demonstrated in the lower part of Table I.

The reactions to *D. pteronyssinus* in the experimental and the routine RAST demonstrate complete concordance: seven sera were positive with both tech-

TABLE I. Clinical and immunologic data of exposed workers and nonexposed control subjects

Subject No.	Symptoms*	Skin reactionst		RAST binding percentages‡			Routine scores§		Inhalation
		<i>D. melano-gaster</i>	Cultivation-room dust	<i>D. melano-gaster</i>	Cultivation-room dust	<i>D. pteronys-sinus</i>	<i>D. pteronys-sinus</i>		
Workers									
1	+	12½	11	7.5	5.3	ND	0	+	
2	+	10½	6½	4.2	1.0	0.2	0	+	
3	+	11	9	9.9	3.1	0.9	0	+	
4	+	8	3½	5.3	3.2	ND	0	+	
5	+	7½	4½	1.1	1.7	ND	0	+	
6	+	7	3½	4.5	4.2	ND	2	—	
7	+	0	0	0.4	0.5	ND	0	+	
8	—	11½	9	8.1	6.0	ND	2	+	
9	—	9	8	4.6	2.3	ND	0	—	
10	—	0	0	0.6	0.5	ND	0	+	
11	—	0	0	0.5	0.5	0.6	0	+	
12	—	5	4	1.2	0.8	23.8	3	ND	
13	—	1	½	1.6	0.8	22.3	4	ND	
14	—	0	0	0.5	0.4	0.4	0	—	
15	—	0	0	0.5	0.4	ND	0	—	
16	—	0	0	0.4	0.3	ND	0	—	
17	—	½	0	0.6	0.4	0.5	0	ND	
18	—	0	0	0.6	0.4	0.3	0	ND	
19	—	0	0	0.4	0.3	0.3	0	ND	
20	—	½	0	0.5	0.5	ND	0	ND	
21	—	0	0	0.4	0.3	ND	0	ND	
22	—	0	0	0.4	0.3	ND	0	ND	
Control subjects									
23		8	3½	1.6	2.1	ND	0	—	
24		3	3	0.8	1.1	ND	3	—	
25		2	½	2.6	1.8	22.9	3	—	
26		0	0	0.7	0.7	27.5	3	—	
27		0	0	0.7	0.6	0.5	ND	—	
28		2	1	0.4	0.4	ND	ND	—	
29		0	0	0.5	0.5	ND	ND	—	
30		½	0	0.4	0.4	ND	ND	—	
31		ND	ND	1.4	1.8	36.7	4	ND	
32		ND	ND	1.2	0.8	34.1	4	ND	
33		ND	ND	0.7	0.8	37.9	4	ND	
34		ND	ND	1.1	1.4	0.8	0	ND	
35		ND	ND	0.8	1.1	0.9	0	ND	
36		ND	ND	0.5	0.6	0.4	ND	ND	

ND = not done.

*Qualitative score of symptoms related to laboratory work.

†Sums of plus signs as scores of diameters of wheal and flare after intracutaneous tests with three serial dilutions.

‡RAST binding percentages as determined by radioactive counting.

§Routine score as determined by the Phadezym immunosorbent test (Pharmacia Diagnostics AB) by use of scoring in classes according to the instructions of the manufacturer.

||Early and/or late nasal and/or bronchial reaction after inhalation.

niques, and nine were negative. This control result provides certainty that the experimental technique in question is effective.

The correspondence between the immediate-type skin reactions and the RAST responses to both fruit-

fly and cultivation-room extracts is very good. Of the 22 exposed workers, eight reacted positively to both RAST and skin test with the two extracts (Nos. 1 to 6, 8, and 9; No. 5 eliciting a weak RAST result), 12 had a negative response (Nos. 7, 10, 11, and 14 to

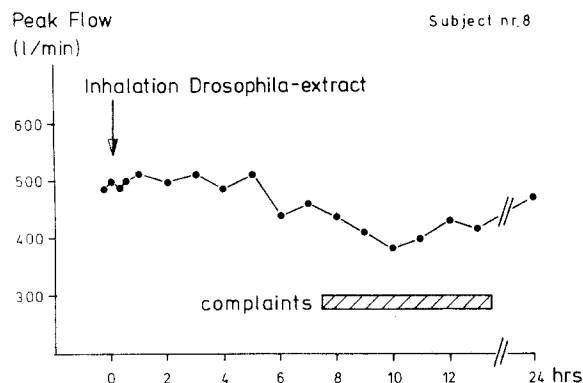


FIG. 1. Example of late bronchial reaction after nasobronchial inhalation of fruit-fly extract.

22), whereas results for No. 12 were weakly positive in both tests. No. 13 was negative for the skin test and weakly positive for the RAST. These findings indicate that eight of 22 exposed workers had become sensitized to an allergenic substance occurring both in fruit flies and in the cultivation-room dust, 12 had not become sensitized, and two demonstrated a weak sensitization.

Five of the 14 nonexposed control subjects (Nos. 23, 25, 31, 32, and 34) were weakly positive in the RAST for fruit-fly and/or cultivation room-dust extracts. Three of these had become strongly sensitized to *D. pteronyssinus* like the two weak reactors in the exposed group (Nos. 12 and 13). The possible significance of a suggestion of some degree of cross-reactivity was investigated in a RAST-inhibition experiment, the results of which are presented below.

The results of skin tests in seven of the eight nonexposed subjects (Nos. 24 to 30) were almost or completely negative. Only nonexposed subject No. 23 reacted positively in the skin test.

Inhalation tests

Combined nasal and bronchial inhalation of an aerosol of fruit-fly extract was performed in 14 of the 22 exposed workers and in eight nonexposed control subjects (Table I). None of the eight control subjects, three of whom (Nos. 24 to 26) were allergic to house dust mites (*D. pteronyssinus*), demonstrated any reduction of respiratory function after the inhalation.

Of the 14 exposed persons tested, nasal reactions were observed in nine (four early, two late, and three dual); three of them also demonstrated a bronchial reaction (late). A representative case of a late bronchial reaction is illustrated in Fig. 1.

Concordance was found between the results of the skin test or RAST and the results of the inhalation test for nine of 14 exposed persons who received the inhalation test. Six persons demonstrated a positive

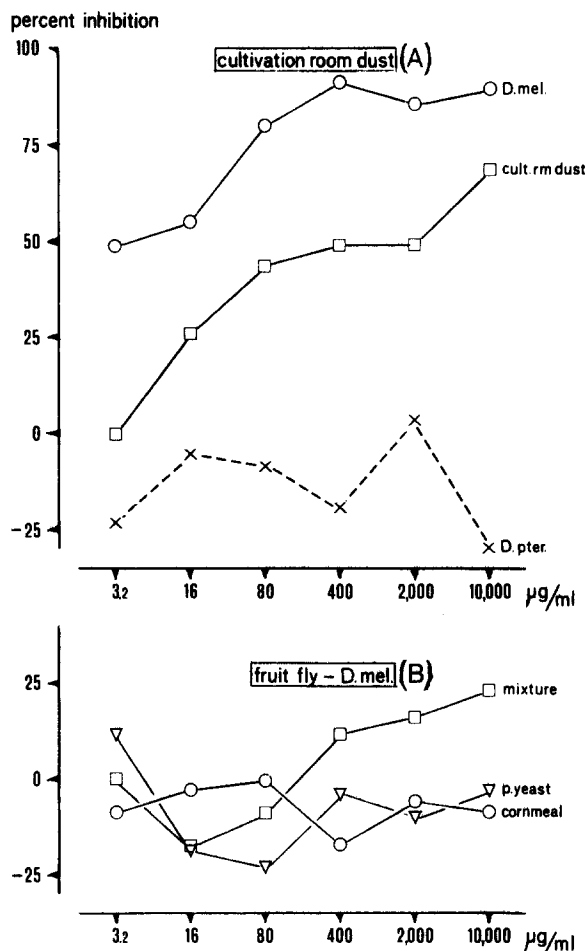


FIG. 2. RAST inhibition of IgE binding (A) to discs coupled with cultivation room-dust allergen after preincubation with extracts of fruit flies, cultivation-room dust, and house dust mite and (B) to discs coupled with fruit-fly allergen after preincubation with extracts of three nutrient-medium constituents.

reaction in both types of test (Nos. 1 to 5 and 8), and three did not react in either test (Nos. 14 to 16). The results for five subjects of the respective tests were divergent, Nos. 6, 7, and 9 to 11.

Relation between symptoms and test results

Seven of the 22 exposed persons reported respiratory symptoms related to their work in the laboratory, and six (Nos. 1 to 6) had a positive skin test or RAST result (Table I). In one person (No. 6) this result was not correlated with the inhalation-test result. One subject (No. 7) in the group with symptoms did not react in either the skin test or RAST, but the result of the inhalation test was positive.

In the 15 exposed persons without respiratory symptoms associated with their laboratory work, one (No. 8) reacted positively in both skin test/RAST and in the inhalation test; one reacted positively only in the skin test/RAST (No. 9); another person (No. 12) re-

acted weakly in the skin test/RAST (inhalation not done); and two other persons (Nos. 10 and 11) demonstrated a reaction after inhalation despite a negative skin test and RAST result. The remaining 10 symptom-free exposed workers did not respond in either the skin or RAST, and three of them did not demonstrate a reaction after inhalation. No inhalation test was performed in seven individuals.

Identification of the allergen source

To identify the main source or carrier of the allergenic activity in the laboratory, RAST-inhibition experiments were carried out. IgE binding to the cultivation room–dust extract proved to be strongly inhibited by the fruit-fly extract (Fig. 2, A). Significant inhibition by the cultivation room–dust extract (as a positive control) was also observed. The same experiment demonstrated that a house dust–mite (*D. pteronyssinus*) extract had no inhibitory activity.

Furthermore, the nutrient-medium constituents were tested for possible allergenic activity (Fig. 2, B). Corn meal, powdered yeast, and a mixture of agar, brown sugar, yeast cells, and nipagin did not inhibit IgE binding to fruit-fly allergen discs.

DISCUSSION

A review of the literature on allergic reactions related to fruit flies yielded only one article²⁴ in which *D. melanogaster* as a potential source of ingestant allergens was suggested. The present study had demonstrated unequivocally that fruit flies are a source of allergens, and the absence of articles on respiratory allergy must be considered highly surprising because a very large number of life science research laboratories have used various species and many strains of fruit fly for many years.

The prevalences mentioned in the available articles on allergic respiratory symptoms in people with occupational or domestic contact with other insects differ, but the published results cannot be compared because of differences in the techniques and criteria used to determine the presence of symptoms and/or sensitization. In the present study seven of 22 persons exposed (albeit probably to different degrees) to substances originating from fruit flies of the species *D. melanogaster* reported respiratory symptoms connected with their work in a laboratory, and in six of these seven persons, skin tests, RAST, and/or inhalation tests demonstrated that the fruit fly was the cause of the allergic symptoms. No relationship was observed between the degree of contact with the flies (hours per day spent in the laboratory or in the office) or the overall duration of the exposure (years em-

ployed in the institute) and the risk of developing allergic symptoms and/or sensitization. All persons with respiratory complaints could continue the work, some of them under symptomatic medication. Also localization of the symptoms in nose, bronchi, and/or eyes was not related to severity of complaints or with duration of exposure.

The correlation between the symptoms and the results of various allergenic tests, which holds for many such biologic tests (such as the skin test, RAST, and inhalation test), is not complete. However, there is concordance in most cases, and the disagreement is attributable to individual variation in the hyperreactivity properties of the nasal and bronchial mucous membranes and the skin as well as, for instance, differences between "natural" exposure to corpuscular allergen carriers and "artificial" exposure to aerosolized allergen extract and differences in subjective experience of symptoms.

Although the irritative or nonspecific threshold in the various tests was not determined, the differences in the individual skin test scores suggested a hypersensitivity rather than a toxic reaction. Moreover, exposure by inhalation of toxic doses of unfamiliar agents implies serious risks.

The RAST-inhibition experiments performed in this study demonstrated that allergenic extracts of both the fruit flies themselves and the cultivation-room dust are cross-reactive, which strongly suggests that the allergenic property of the dust is due to the presence of the fruit-fly constituents. If this is the case, it would mean that the exposure to small inhalable airborne particles making up a large part of the cultivation-room dust and potentially present everywhere in a laboratory complex is probably the main cause of the allergic sensitizations and symptoms rather than contact with the intact flies.

The application of strict hygienic measures in laboratories working with fruit flies could be expected to reduce exposure to airborne fruit-fly material and thus prevent sensitizing concentrations.

Dr. J. Maassen brought the work-related respiratory symptoms to the attention of the Department of Pulmonology; Dr. J. C. J. Ecken provided the information about the laboratory work, supplied the laboratory strains of fruit flies, and allowed us to collect dust from the cultivation rooms; Miss A. H. Nikkels prepared the allergen extracts and the RAST discs; Mr. A. C. van der Linden and Miss Ch. van Twisk performed the RAST and RAST inhibition; Mrs. I. Seeger-Wolf corrected the English text; and Miss A. Midderham typed the manuscript. Their skillful help is acknowledged by the authors.

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