

Occupational allergy to the common house fly (*Musca domestica*): Use of immunologic response to identify atmospheric allergen

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*A 48-year-old scientific worker developed rhinitis that occurred when she entered the housefly (*Musca domestica*) rearing room where she worked. She was found to have specific IgE antibody to *M. domestica* in her serum by RAST. She was relocated at work and avoided further occupational exposure to *M. domestica*. The level of specific IgE decreased in serial samples but subsequently increased after her inadvertent reexposure at work. Extracts of fly-cage dust and of high volume atmospheric samples from the fly-rearing room inhibited the *M. domestica* RAST in a dose-dependent fashion. After logit transformation the lines of inhibition of the fly cage dust and of the atmospheric samples were parallel and steeper than the self-inhibition by *M. domestica*, implying the cage dust and atmospheric samples shared antigens not present in the *M. domestica* extract. This method of monitoring atmospheric antigen has considerable potential for evaluating the effectiveness of environmental change in the workplace. (J ALLERGY CLIN IMMUNOL 76:826-31, 1985.)*

Inhaled *Musca domestica* fly dust can cause an allergic reaction. We report a case of a female scientific worker in whom *M. domestica* provoked rhinitis with specific IgE antibody in her serum. By use of the patient's serum we estimated the concentration of *M. domestica* allergen in the cage dust and atmosphere of the fly-rearing room where she worked.

CASE REPORT

A 48-year-old woman was referred because of sneezing, nasal discharge, and irritation of the eyes that occurred when she worked in the fly-rearing room at a research station. Her symptoms developed 4 months after starting this work, and 5 months later she was relocated away from the flies (Fig. 1). At her first visit to the Brompton Hospital, 4 months after ceasing exposure to the flies, she had imme-

diate skin prick test reactions provoked by extracts of *M. domestica* and fly dust, and specific IgE antibody to *M. domestica* was found in her serum.

We used the patient's serum to estimate the concentration of *M. domestica* allergen in the cage dust and atmosphere of the fly-rearing room where she worked.

MATERIAL AND METHODS

Description of the fly-rearing room

Colonies of different strains of house fly are reared in a purposely built room measuring 4.9 by 3.8 by 2.6 m. Ventilating air enters through a nuisance dust filter and diffusion ceiling that results in 40 air changes per hour. It is extracted at floor level through grilles on one side of the room, passes through a charcoal filter and heat exchanger, and is then discharged to the atmosphere. There is no recycling. The room is maintained at 28° C by heating the incoming air.

The flies are reared on a weekly cycle. Eggs are incubated in jars containing a nutrient medium of bran, dried milk, yeast, and water with intermittent stirring. After the pupae are formed, these are separated from the feed, which has, by this time, usually become moldy. The pupae are transferred to small rearing cages with muslin closures to provide access and ventilation, allowed to hatch, and then fed on sugar, water, and milk until required for phenotype determination and insecticide tests. Eggs are laid on dental rolls soaked in nutrient and introduced into the cages 2 to 3 days after emergence. The hatching jars and rearing cages are

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Received for publication Oct. 1, 1984.

Accepted for publication May 6, 1985.

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TABLE I. RAST (% binding)

	Fly-hypersensitive patient	Mold-hypersensitive patient
<i>A. flavus</i>	0.5	9.0
<i>R. stolonifer</i>	0.4	2.1
White actinomycetes	0.6	1.3
<i>A. corymbifera</i>	0.7	1.9

kept on shelves mounted on three walls of the room. Dust deposits, mainly fecal material, are often evident alongside the muslin closures of the rearing cages.

Preparation of extracts

Whole *M. domestica* flies and dust obtained from below the fly cages were defatted in three changes of ether during 24 hours and extracted for 16 hours in 10 volumes of Coca's solution (5 gm of sodium chloride, 2.75 gm of sodium bicarbonate, and 5 gm of phenol made up to 1 L with distilled water). Extracts were also prepared from broth cultures of four fungi (*Aspergillus flavus*, *Rhizopus stolonifer*, white actinomycetes, and *Absidia corymbifera*) isolated from air samples in the fly-rearing room by an Andersen sampler (Andersen Samplers, Inc., Atlanta, Ga.). The extracts were filtered through Whatman paper No. 1 followed by a 0.45 μ m membrane (Millipore Corp., Bedford, Mass.), dialyzed in Visking tubing (Medical International, London, U.K.) against three changes of distilled water during 24 hours, and lyophilized.

Skin prick testing

Skin prick test solutions of the fly and fly-dust extract were prepared in 50% Coca's solution and 50% glycerol at 0.1 mg/ml concentration of lyophilized powder and passed through a 0.22 μ m Millex GS filter (Millipore Corp., Bedford, Mass.) before use. This diluent and histamine dihydrogen chloride at 1 mg/ml were used as negative and positive control solutions, respectively. The patient was also skin prick tested with commercial extracts (Bencard, Brentford, Middlesex, U. K.) of mixed grass pollens, *Dermatophagoides pteronyssinus*, *Aspergillus fumigatus*, cat fur, and dog hair. Skin prick tests with the fly and fly-dust extracts at 1 mg/ml were made on five other patients attending the same clinic.

Environmental monitoring

Air sampling for microorganisms. Airborne microorganisms were isolated by use of an Andersen sampler operating at 25 L/min in the fly-rearing room. Organisms were collected on Petri dishes of either 2% malt extract agar plus penicillin and streptomycin or half-strength nutrient agar plus cycloheximide (Acti-Dione; Upjohn Co., Kalamazoo, Mich.). Incubation was at 25° and 40° C for fungi and at 25°, 40°, and 55° C for actinomycetes and bacteria.

Air sampling for allergens. A Staplex high volume

TABLE II. Inhibition of *M. domestica* RAST

Concentration of inhibitor (mg/ml)	RAST inhibition (%)		
	Fly extract	Fly-dust extract	Fly room-filter extract (8 hr)
0.001	11	ND	ND
0.005	24	ND	ND
0.01	39	ND	ND
0.1	69	1	ND
1.0	87	9	0
10.0	ND	43	9
25.0	ND	ND	15
50.0	ND	ND	25
100.0	ND	83	44

ND = not done.

air sampler (Staplex Co., Air Sampler Div., Brooklyn, N. Y.) was used to sample the atmosphere of the fly-rearing room for particulate antigens. This sampler maintains an airflow of 1.4 m³/min and is estimated to retain 95% of particles larger than 0.3 μ m on a fiberglass filter sheet, 22.7 by 17.7 cm.

Elution of filter sheet

Soluble material was eluted from the filter sheet by descending chromatography with a borate buffer (sodium citrate, 0.034 mol/L, and sodium borate, 0.0325 mol/L, in 0.16 mol/L of sodium chloride/distilled water, pH 8.2). Two eluates were collected, one after 8 hours and the other after a further 24 hours. These were then dialyzed separately against distilled water and lyophilized.

RAST

Paper discs (Schleicher & Schuell Inc., Keene, N. H., No. 589/3) were activated with cyanogen bromide. Ten milligrams of lyophilized *M. domestica* extract was coupled per 1 gm of activated discs by the method of Axen et al.¹ Paper discs were coupled similarly with the four molds.

Assay

Fifty microliters of serum per disc was incubated for 16 hours at room temperature. After washing, 50 μ l ¹²⁵I-labeled anti-IgE (Pharmacia Diagnostics, Uppsala, Sweden) was added to each tube for a second 16-hour incubation. After further washing, the tubes were counted in a gamma counter, and the percentage binding of the isotope was calculated. Cord-blood sera with no demonstrable IgE were used as negative controls. To investigate the possible interference of high total IgE in the assay, a serum from a nonexposed subject with a total IgE greater than 5000 IU was also assayed in the fly RAST. RAST assays for the four molds were performed similarly. A patient known to have specific IgE antibody to molds was tested against the mold extracts as a positive control for the assays.

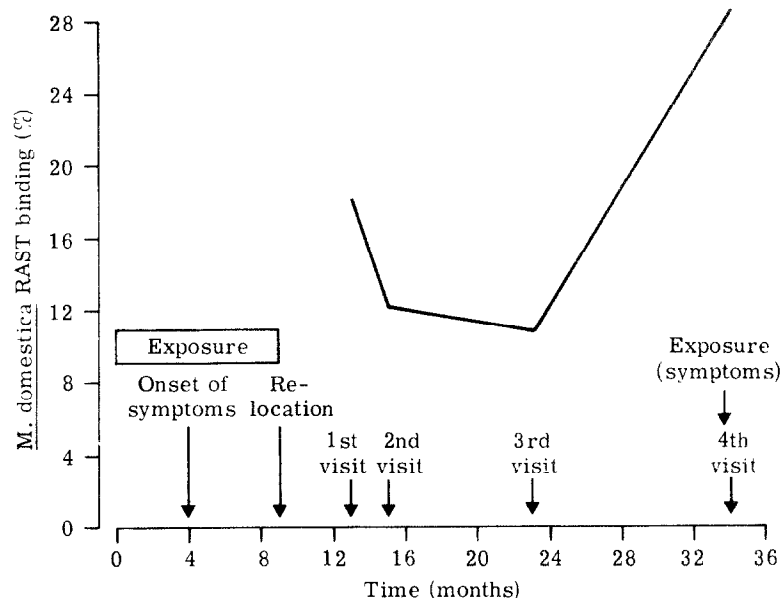


FIG. 1. Time course of patient's exposure, clinic visits, and specific IgE antibody levels to *M. domestica*.

RAST inhibition

RAST-inhibition studies were performed by a 16-hour preincubation of the positive serum (100 μ l) with the following concentrations of lyophilized powder of the three antigens (100 μ l): (1) fly extract 0.001, 0.005, 0.01, 0.1, and 1.0 mg/ml; (2) fly dust extract 0.1, 1.0, 10, and 100 mg/ml; and (3) high air volume filter extract 1, 10, 25, 50, and 100 mg/ml.

The fly-extract RAST was performed, and percentage binding of the ^{125} I-anti-IgE compared with that of the unabsorbed serum.

Cord-blood serum was similarly preincubated with fly extract at 0.01, 0.1, 1, and 10 mg/ml to examine for non-specific inhibition of the fly RAST.

RAST-inhibition analysis

When percentage inhibition (p) is plotted against log concentration, the relationship is that of a sigmoid, s-shaped curve that tends to flatten out at either end. A logit transformation, $\log_e(p/[1-p])$ changes this into a straight line and enables comparison to be made between the allergen content of different extracts.

We required a minimum of 4 points on the inhibition curve to define the regression line, and a minimum acceptable correlation coefficient was arbitrarily set at 0.90. Points of percentage inhibition of greater than 95 and less than 5 were excluded because they fall on the flattened ends of the sigmoid curve.

The straight lines obtained by the logit-log transformation were analyzed for parallelism, and a common slope was fitted where it was possible. Parallelism is understood to imply that there is evidence of antigenic identity, whereas a significant deviation from parallelism implies at best partial

antigenic identity. Relative antigen content can only be compared for those extracts with parallel regression lines.

RESULTS

Skin prick testing

Solutions of 0.1 mg/ml of the *M. domestica* and fly-dust extracts elicited wheals of 6 and 3 mm in diameter in the patient. Histamine dihydrogen chloride at 1 mg/ml and cat fur antigen provoked a 4.5 mm wheal and 3 mm wheal, respectively. No reaction was elicited by the negative control solution nor by extracts of mixed grass pollens, *D. pteronyssinus*, *A. fumigatus*, and dog hair. Skin prick tests with the fly and fly-dust extracts at 1 mg/ml provoked no reactions in four patients with (atopic) and one patient without (nonatopic) one or more skin prick-test reactions to these common inhalant allergens, all without occupational exposure.

Environmental monitoring

Air sampling for microorganisms. *A. flavus*, *A. coenobifera*, *R. stolonifer*, and white actinomycetes were cultured from the Andersen sampler isolates. Sampling was undertaken when feed was being prepared and pupae harvested and gave up to 137,000 colony-forming units per cubic meter of air.

Air sampling for allergens. The Staplex high-volume air sampler was run in the fly-rearing room for 82 hours. Approximately 3800 m³ of air was sampled, and 265 mg of dust was retained.

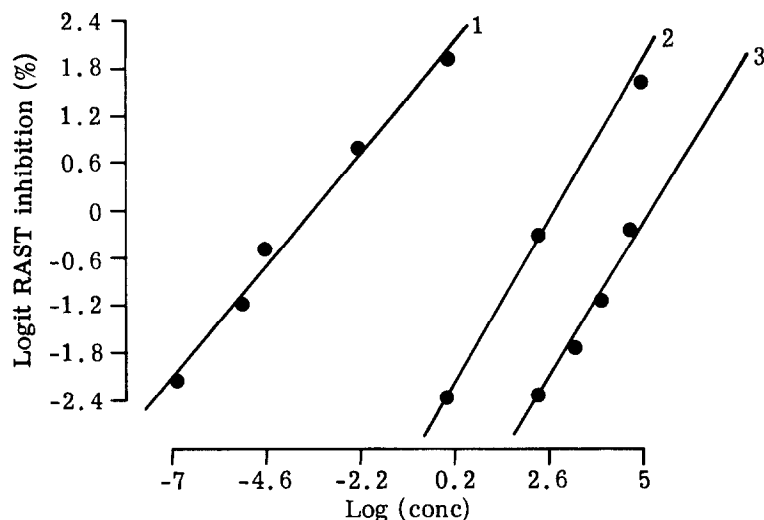


FIG. 2. Logit transformation of fly (*M. domestica*) RAST inhibition: fly extract (1); fly room-dust extract (2); and fly room-filter extract (3).

RAST

When the patient was observed initially, 4 months after avoidance of exposure to flies, the percentage binding of the ^{125}I -anti-IgE by the patient's serum in the *M. domestica* extract RAST was 18%, and her total IgE was 1173 IU. Two months later the percentage binding had fallen to 12.1%, and, after a further 8 months, to 10.8%. Two years after her last contact with flies, she was inadvertently reexposed. While she was away on holiday, fly cages had been left in a basement room where she occasionally worked. Within minutes of entering the room following her return from holiday, she started sneezing. She was observed in the clinic 5 days later when the RAST percentage binding had increased to 28.6% (Fig. 1). Cord-blood sera ranged from 0.7% to 1.3% binding. The mean percentage binding of the unexposed individuals was 0.97%, and the serum with total IgE greater than 5000 IU had 1.2% binding. The patient had a negative RAST to the four molds tested, whereas a known mold-hypersensitive person had positive RAST results (Table I).

RAST inhibition

Preincubation of the patient's serum with increasing concentrations of the fly, fly dust, and filter extracts inhibited the *M. domestica* RAST in a dose-dependent fashion (Table II).

RAST inhibition with 1 mg/ml concentration of inhibitor was 87% with the fly extract, 9% with the fly-dust extract, and 0% with the 8-hour filter extract (Table II). The second eluate collected by descending chromatography from this filter sheet after a further

24 hours produced only one seventh of the RAST inhibition of the 8-hour eluate with 100 mg/ml of inhibitor and was not used further. There was no alteration in RAST binding when a cord-blood serum was preincubated with fly antigen from 0.01 to 10 mg/ml. The percentage binding of isotope remained at 1%.

RAST-inhibition analysis

When a logit transformation of the RAST-inhibition curves was made, those of the fly extract and the filter extract fulfilled the conditional criteria for the regression lines (see Material and Methods section). With the fly-dust extract, however, only 3 points on the inhibition curve fulfilled these criteria, but because they were all very close to the same straight line (the correlation coefficient was 1.0), it was considered acceptable to include the regression line for comparison with those of the fly and filter extracts. The lines for the fly dust and the filter extracts were parallel with slopes of 0.87 and 0.90, whereas the slope of the whole fly extract was significantly less steep with a slope of 0.58 (Fig. 2) ($p < 0.01$ for overall differences between the slopes). It can be observed clearly that the quantitative responses of the fly dust and filter extracts were considerably lower than that of the fly extract. A common slope was fitted to the lines for fly dust and filter extract. The concentration of inhibitor required to obtain 50% RAST inhibition was 14.9 mg/ml for the dust extract and 157.8 mg/ml for the filter extract, demonstrating a greater than ten-fold difference in potency between the dust and filter extracts.

DISCUSSION

M. domestica fly dust inhaled at work can cause an allergic reaction. Our patient developed rhinitis when she worked in the fly-rearing room and had positive skin prick test reactions and specific IgE antibody to *M. domestica*.

Skin test reactions to *M. domestica* have been reported from various parts of the world²⁻⁵ with prevalences of between 10% to 29% in studies testing groups with a panel of insect allergens.⁶⁻¹⁰ Jamieson¹¹ has reported the only previous case of allergy to *M. domestica* in a 49-year-old atopic woman who developed rhinorrhea and asthma when flies flew around her at home.

The level of specific IgE antibody to *M. domestica* in the patient's serum fell as the interval increased since avoidance of exposure at work to the flies but increased markedly following her reexposure. It appears possible that IgE antibody level may be a sufficiently sensitive response to allergen exposure for serial measurements to be useful as a biologic monitor of exposure after changes in work practice, or, as presented here, to measure the effectiveness of relocation. Preliminary data from Busse et al.¹² that ragweed allergen might be carried in particles smaller than pollen grains led Agarwal et al.¹³ to suggest that airborne allergen concentration might be a more relevant measure of the cause of hypersensitivity than counts of pollen grains in the atmosphere. They developed a specific immunochemical method to estimate airborne allergen concentration that involved the use of a high-volume air sampler, elution of the filter sheet, and analysis of eluate allergen content by RAST-inhibition assays. Of three methods tested, they found that an 8-hour descending elution of the filter gave maximum allergen yield.

By use of the same methods Twigg et al.¹⁴ and Reed¹⁵ described measurements of airborne mouse allergens, and Edwards et al.¹⁶ extended their findings to measure airborne urinary allergens from mice, rats, guinea pigs, and rabbits and examined the effects of environmental changes on airborne allergen levels.

We have followed the immunochemical method of Agarwal et al.¹³ and used the patient's serum containing specific IgE antibody to measure atmospheric fly-allergen concentration. Logit transformation of the percentage RAST inhibition allows comparison of atmospheric concentration of antigens of similar specificity. The lack of parallelism between the inhibition curves when all three fly extracts were analyzed together (Fig. 2) demonstrates only partial allergenic identity between them. The slopes suggest that the fly-cage dust had additional antigens that were not present in the whole fly extract but that were airborne.

We have investigated IgE antibody production to four molds isolated from the air in the fly-rearing room as possible antigen sources to account for this finding but have found no evidence of specific mold antibody in the patient's serum. The logit plots obtained for the fly dust and the filter extracts alone had a common slope, identifying allergens with identity of immunologic behavior. It can be said that they were qualitatively comparable although, not surprisingly, the potency of the filter extract was considerably less.

Methods that use specific IgE antibody have considerable potential for sampling atmospheric allergen. They allow the identification of airborne, and therefore inhalable, allergens and evaluation of intervention designed to decrease allergen exposures. Such methods may include increasing ventilation and relative humidity, both found to decrease atmospheric concentrations of urinary allergens in a laboratory animal house.¹⁶

With further experience of atmospheric sampling in different work places, it may become possible to determine the critical concentrations of airborne allergens necessary to cause allergic disease, enabling control limits to be recommended.

We thank Mrs. Valerie Church for her helpful advice and Miss Cathi Gray for her secretarial assistance in the preparation of this paper.

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Clinical evaluation of patients with complaints related to formaldehyde exposure

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*Formaldehyde is a very widely used chemical in our present society and one with which every physician has had a first-hand experience in his early days of training in the anatomy laboratory. The National Institute of Occupational Safety and Health lists 52 occupations that expose people to formaldehyde.¹ In recent years, however, the increasing use of formaldehyde resins in the production of building materials such as particleboard and urea-formaldehyde foam insulation has resulted in exposures of large numbers of people in nonoccupational settings. Consumer products such as cosmetics, cigarettes, textiles, furniture, draperies, and preservatives release formaldehyde. It is present in the outdoor atmosphere from products of combustion and automobile exhaust and likewise in the home from such things as gas cooking. These more widespread and increased exposures have resulted in concern regarding potential health effects.²⁻⁴ Therefore, it is likely that physicians have or will encounter patients who wish evaluations of a present or potential health effect from formaldehyde. This article is for the purpose of providing assistance in such evaluation. (*J ALLERGY CLIN IMMUNOL* 76:831-40, 1985.)*

A system of classification of the toxic effects of formaldehyde on humans is essential for proper evaluation of symptoms and findings. Unlike known toxic effects on animals, human complaints, such as fatigue or headache can be very difficult to classify in accordance with any known mechanism of pathogenesis. Since numerous adverse effects have been recently attributed to formaldehyde exposure, a closer attention to location and kind of effect will be helpful.

CLASSIFICATION OF TOXIC EFFECTS OF FORMALDEHYDE

Site of action—local versus systemic

Most known effects of formaldehyde occur at the site of contact² in contrast to systemic toxins that are absorbed and distributed by the blood to distant organs

Abbreviations used

NIOSH: National Institute of Occupational Safety and Health

ACD: Allergic contact dermatitis

where the chemical or its metabolite manifests its effect.

Duration of exposure and acute versus chronic effect

Since formaldehyde is a rapidly acting toxin, any effects after a brief exposure are manifested soon afterward.⁵ Primary effects of brief airborne low-level exposure are eye and mucous membrane irritation² that are self-limited and of short duration. Prolonged or repeated exposure in the workplace or home may result in recurring acute symptoms that may masquerade as chronic disease but that quickly clear when the affected individual leaves the environment.⁶

Received for publication Feb. 21, 1984.

Accepted for publication May 6, 1985.

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