

BRIEF COMMUNICATION

Asthma due to inhalation of moth flies (Psychoda)

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A case of allergic rhinitis and asthma due to sensitization to the moth fly (Psychoda) is reported. The patient was a 38-year-old white male who was employed on a rubber plantation in West Africa. For approximately 5 years he had had episodes of nasal congestion and asthma that occurred during March and April and were associated with exposure to large numbers of small flying insects which were present only during that time of the year. Direct scratch and intradermal tests with an extract of the flies were positive in the patient and negative in control subjects. The passive-transfer test gave a strong wheal and flare reaction, and the Shultz-Dale reaction was positive after the ileum had been sensitized by exposure to the patient's serum. Serum that had been inactivated by heat gave a negative result. PCA in the rhesus monkey was positive with the use of the patient's unheated serum only, but PCA in the guinea pig was negative. Precipitin and hemagglutination tests were negative, and lymphocyte cultures were not reactive. However, antibodies in this patient's serum fixed to human and primate skin but not to guinea pig skin and sensitized guinea pig ileum. Each of these properties was destroyed after heating at 56° C. for 30 minutes. Therefore this antibody had the characteristics of reaginic antibody.

Hypersensitivity reactions to insects are not extremely common. However, there are well-documented examples of allergic rhinitis, asthma, urticaria, and related disorders following stings or bites by insects. In 1929, Figley¹ described 4 cases of asthma and allergic rhinitis caused by inhalation of particles of may-fly (Ephemera). In other reports, similar illnesses have been associated with inhalation of particles or emanations of such insects as caddis fly or sandfly (Trichoptera),²⁻⁴ bed bug (*Cimex lectularius*),⁵ house fly (*Musca domestica*),⁶ mushroom fly (*Aphiochoeta agarici*),⁷ Mexican bean weevil (*Zabrotes subfasciatus*),⁸ water flea (Cladocera),⁹ and beetles (Coleoptera).¹⁰ Probably the first case

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of asthma due to sensitivity to the moth fly (*Psychoda*) was reported by Ordman¹¹ in 1946.

This is a report of a case of allergic rhinitis and asthma due to sensitization to emanations of the moth fly (*Psychoda*). Hypersensitivity to antigens of the moth fly was studied by several immunological techniques.

CASE REPORT

The patient was a 38-year-old white man who was employed on a rubber plantation in Harbel, Liberia, West Africa. For approximately 5 years, while living in Africa, he had had episodes of nasal congestion and itching which progressed in 3 or 4 weeks to cough, dyspnea, and wheezing. The illness occurred only during March and April and was associated with exposure to large numbers of small flying insects which were present only during that time of year. He had never experienced these symptoms during any season while living in the United States. Several of his associates in Liberia had identical symptoms following exposure to the flies. The attacks of asthma were severe enough to require hospitalization and treatment with corticosteroids. The remainder of his history was unremarkable. His son had rhinorrhea and cough following ingestion of milk, but the family history was otherwise unremarkable.

Physical examination revealed a well-developed man in no distress. The blood pressure was 160/110 mm. Hg, the pulse was 90 beats per minute and regular. Eyes, ears, nose, mouth, and throat were unremarkable. The neck was normal and the chest was clear. Heart tones were of good quality, and no murmurs were present. The abdomen was somewhat obese but contained no masses. The remaining physical examination was essentially normal.

The chest x-ray was normal. The hemoglobin was 16.7 Gm. per 100 ml., and the white blood cell count was 8,110 cells per cubic millimeter with 50 per cent neutrophils, 42 per cent lymphocytes, 6 per cent monocytes, and 2 per cent eosinophils. The sedimentation rate (Culter) was 6 mm. per hour, and the serum protein-bound iodine was 5.7 μ g per 100 ml. Serum protein electrophoresis showed a total protein of 7.7 Gm. per 100 ml., with 22 per cent gamma globulin. The urine was normal.

MATERIALS AND METHODS

Cutaneous testing

Intradermal skin tests were performed with commercial antigenic extracts of mixed tree pollens, mixed grass pollens, mixed weed pollens, mixed molds, mixed feathers, and house dust (Dome) in concentrations of 100 and 1,000 PNU per milliliter together with a diluent control. Skin tests with commercial extracts of insects were not done.

An extract was prepared from flies that had been captured by the patient, dried, and carried to the United States without preservative. The extraction process was according to the method of Phillips.¹² Briefly, the flies were defatted with diethyl ether at room temperature. The ether was decanted and the residue collected by filtration. The defatted flies were then extracted at room temperature in Coca's solution for 48 hours. Bacterial fermentation was prevented by the addition of toluene. Clarification was accomplished by allowing the material to stand overnight in the refrigerator. The extract was then dialyzed for 24 hours against 40 volumes of sterile water and sterilized by Millipore filtration. The nitrogen content of the extract was measured by the Kjeldahl method,¹³ and a qualitative carbohydrate assay was carried out by Molisch's test.¹⁴

Direct skin testing with the undiluted fly extract was done by the standard

scratch technique. Coca's solution provided the control. Intradermal testing was done with aqueous extracts in dilutions of 1:100, 1:1,000, and 1:10,000 and an inoculum of 0.02 ml. Intradermal studies were also done on 12 normal persons and 12 patients with pollinosis. However, no subjects known to be allergic to insects were available for study. A wheal and flare of over 15 mm. within 15 minutes was considered to be a positive reaction.

Passive transfer of reactivity¹⁵ to human skin was performed by intradermal injection of 0.1 ml. of serum from the patient and a normal saline control. Forty-eight hours later the skin sites were challenged with 0.02 ml. of the undiluted fly extract. Reactions were evaluated in the same manner as the direct skin tests.

Passive transfer of hypersensitivity to rhesus monkey skin was carried out by the method of Layton and associates¹⁶ with 0.1 ml. of serum. This test was accomplished by injection of unheated serum and serum that had been inactivated by heating at 56° C. for 30 minutes or 4 hours. Forty-eight hours later a mixture of 4 ml. of 0.5 per cent Evans blue dye and 1 ml. of the undiluted moth fly extract was injected intravenously. The response was considered positive when an area of blue discoloration of the skin was observed over the site of the intradermal injection 15 minutes after the intravenous challenge.

Passive cutaneous anaphylaxis in the guinea pig was carried out according to the method described by Terry.¹⁷ The test was accomplished by the intradermal injection of 0.1 ml. of both the patient's serum and normal serum before and after heating the sera to 56° C. for 4 hours. Four hours later a mixture of 0.5 ml. of Evans blue dye and 0.5 ml. of undiluted fly extract was injected intravenously. After 15 minutes the guinea pig was killed and areas of blueness were sought in the dermis under the test sites. In both passive-transfer experiments involving intravenous administration of antigen and Evans blue dye, normal saline and 15 µg of histamine phosphate in a volume of 0.1 ml. were injected intradermally in separate sites to provide positive and negative controls to facilitate interpretation of the test.

The Schultz-Dale reaction¹⁸ (in vitro anaphylaxis) was performed with the use of a guinea pig ileum. The following test materials were added to a 10 ml. bath containing a strip of ileum with careful washing between each step: (1) 0.2 ml. of undiluted fly extract; (2) 0.2 ml. of unheated serum of the patient; (3) 0.2 ml. of unheated serum incubated for 15 minutes, followed by the addition of 0.2 ml. of fly extract; (4) 0.2 ml. of serum that had been heated to 56° C. for 30 minutes, incubated with the ileum for 15 minutes followed by 0.2 ml. of fly extract; (5) 0.2 ml. of serum heated to 56° C. for 4 hours and incubated with the ileum for 15 minutes, followed by 0.2 ml. of fly extract.

Thirty micrograms of histamine phosphate were added to the bath at the beginning and end of each experiment to evaluate the contractile properties of the ileal strip.

Agglutinating antibodies

Hemagglutination studies were carried out with the use of group O, Rh negative human red blood cells. Two milliliters of washed, packed erythrocytes

were added to 2 ml. of veronal buffer, pH 8.3, incubated with 0.5 ml. of the fly extract for 60 minutes at 37° C. and then stored overnight at 4° C. An aliquot part of cells was then washed 4 times with phosphate-buffered isotonic saline, pH 7.2, and diluted to a 3 per cent suspension. Serial twofold dilutions (0.3 ml.) of test serum were prepared in phosphate-buffered saline, pH 7.2, containing 1:100 normal rabbit serum. One tenth milliliter of antigen-coated erythrocytes as added to each tube, and after 10 minutes of incubation at room temperature the tubes were centrifuged at 1,000 r.p.m. for 2 minutes. Agglutination was read immediately.

Erythrocytes of the same group were tanned with 1:20,000 tannic acid and coated with undiluted fly extract according to the method described by Arbesman and associates.¹⁹ The cells were added to doubling dilutions of the patient's and normal sera. Other controls included tanned, uncoated cells and the same sera. The tubes were mixed and allowed to stand at room temperature for 2 hours and read. They were then resuspended and stored overnight at 4° C., and the patterns were read again. The degree of hemagglutination was recorded from 0 to 4 plus.

Other studies

The Ouchterlony method of detecting precipitins was used.²⁰ Dilutions of the patient's serum, control serum, and fly extract were placed in appropriate wells of the agar slide, incubated for 48 hours at room temperature, and observed for precipitin bands.

The *in vitro* lymphocyte transformation test was carried out with the use of the method described by Kirkpatrick and Ruth.²¹ Blood was drawn under sterile conditions into a heparinized syringe, and through a process of differential centrifugation, sedimentation, and adherence to glass, a lymphocyte-rich supernatant fluid was obtained. The cells were divided into a final suspension containing 3×10^6 lymphocytes in 3 ml. of Eagle's minimal essential medium supplemented with 15 per cent autologous plasma, 100 units of penicillin, and 100 μ g of streptomycin. Each test was run in triplicate with the use of unstimulated cells as a negative control, phytohemagglutinin-stimulated cells as a positive control, and 0.2 ml. of fly extract undiluted and in dilutions of 1:10, 1:100, 1:1,000. The tubes were incubated at 37° C. in an atmosphere of 95 per cent O₂ and 5 per cent CO₂. At the end of the fourth day, 1 μ c per milliliter of tritiated thymidine (specific activity 6.7 c. per millimole, New England Nuclear Corp., Boston, Mass.) was added to each of the culture tubes. Twenty-four hours later the cells were harvested, washed with saline, and the protein was precipitated with cold 5 per cent trichloroacetic acid. The trichloroacetic acid was extracted with cold methanol. The pellet was dried and dissolved in 0.5 ml. of NCS (Nuclear-Chicago Corp., LaGrange, Ill.) base solution, and an aliquot part was counted in a liquid scintillation spectrometer.

RESULTS

The protein nitrogen content of the fly extract was 90 μ g per milliliter. There was no carbohydrate found in the extract.

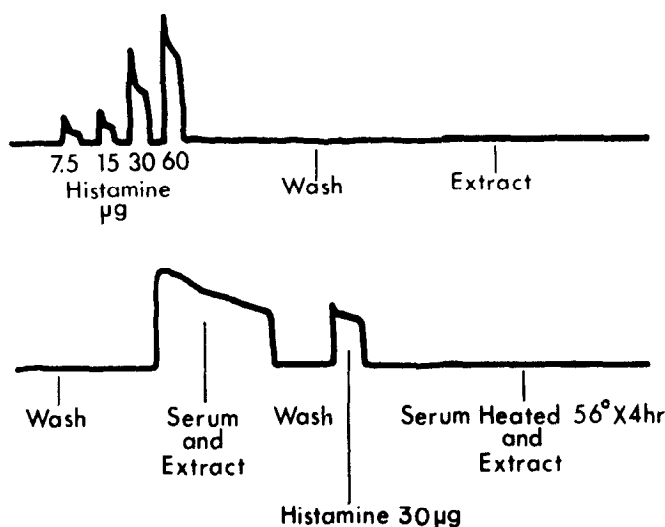


Fig. 1

Schultz-Dale test with the use of guinea pig ileum. No response was obtained with serum or the fly extract alone, but brisk contraction occurred after the ileum had been sensitized with the patient's serum. This activity was lost after the serum had been heated at 56° C. Although not shown in the figure, the tissue was still responsive to histamine after these experiments. Redrawn from original kymogram.

All scratch and intradermal tests with a variety of commercial antigenic extracts commonly used in this area were negative. However, the patient had markedly positive cutaneous responses to the scratch tests with the undiluted fly extract and the intradermal tests with all dilutions of the antigen. No reactions were observed with the diluent controls or in the allergic and normal subjects tested with the fly extracts.

The positive response was passively transferred to a skin test-negative volunteer with the patient's serum, but this property was lost after the serum had been heated to 56° C. for 4 hours. Passive cutaneous anaphylaxis in the rhesus monkey was also positive with the patient's serum but was negative when the heat-inactivated serum was used. Passive cutaneous anaphylaxis in the guinea pig was negative.

In the Schultz-Dale test, contraction of the strip of ileum was observed only after the tissue had been sensitized by exposure to the patient's serum and challenged with the fly extract. Neither the serum nor the extract alone produced contraction (Fig. 1). Heating the serum at 56° C. for 30 minutes or 4 hours completely abolished the sensitizing property. Although it is not shown in the figure, when histamine was added to the strip after these studies, brisk contraction occurred indicating that the tissue was still responsive. Because this heat-labile property was unexpected and not compatible with the recognized properties of human IgG, this experiment was repeated several times; and the same results were obtained. The sensitizing property of the serum was also unchanged after the serum had been stored at 4° C. for 2 months.

No antibody activity was demonstrable by hemagglutination tests with nor-

mal or tanned erythrocytes or by the Ouchterlony technique, but the lack of a positive control serum makes definitive interpretation of these results impossible. The patient's lymphocytes were stimulated to synthesize DNA by phytohemagglutinin in a normal manner, but all cultures that were exposed to the fly extract were less active than the controls.

DISCUSSION

The moth fly belongs to the genus *Psychoda*, family *Psychodidae*, order *Diptera*. The *Psychoda* are small, slender moth-like flies about 3 to 4 mm. in length with long legs and a wing span of about 6 mm. The body and wings are thickly covered with hairs, the antennae are long and hairy, and the short proboscis not adapted to biting. The flies make their appearance from the hibernating larvae and pupae at the beginning of warm weather, and the insects pair on the walls of buildings and cluster in shady places. *Psychoda* are ubiquitous, occurring in Europe, Asia, Africa, both Americas, and Australia.

Allergy to insects, although not commonly observed, is nevertheless a well-documented cause of asthma, hay fever, urticaria, and other allergic disorders. Well-known examples are associated with *Hymenoptera*, where stings have produced severe urticaria, constitutional symptoms, and death.²²

The subject of this report had a positive direct skin test to the whole fly extract, and this antigen did not produce wheal and flare reactions in healthy subjects or patients with pollinosis. Ordman¹¹ has reported that positive responses occurred only with extracts of the bodies of the flies but not the wings.

The patient's serum contained antibodies that could passively sensitize human and monkey skin, but not guinea pig skin, and would sensitize a guinea pig ileum to produce a positive Schultz-Dale reaction. Each of these properties was lost after the serum had been heated to 56° C. Neither the serum nor the extract alone was capable of inducing contraction of the ileum. No precipitating or agglutinating antibodies were detected.

Several investigations have demonstrated that human immunoglobulin classes differ in their ability to sensitize animal skin. Human skin-sensitizing antibodies (reagins) reside in a unique immunoglobulin class that has been designated as IgE.²³ These antibodies also sensitize monkey skin and produce P-K and PCA reactions parallel to those found by direct testing.^{16, 24} In contrast, the capacity to passively sensitize guinea pig skin is limited to certain subclasses of IgG.¹⁷ Buckley and Metzgar²⁹ have shown that human precipitating antibodies gave positive PCA reactions in the guinea pig but not the monkey, and Dale³⁰ and Halpern³¹ have shown that precipitins will also sensitize a guinea pig ileum.

Arbesman and associates³² have found that sera from untreated atopic patients can sensitize the ilea of both monkeys and guinea pigs, but several differences were apparent. Sera that were able to sensitize monkey ileum in high dilutions were not active in guinea pigs. Heating the serum to 56° C. for 2 hours destroyed the capacity to sensitize monkey tissue but had little effect on the guinea pig. It was concluded that two species of antibodies were involved: a heat-labile reagin that was active with monkey tissue and a heat-stable molecule that sensitized guinea pig ileum. The latter antibody may have been identical to those responsible for agglutination of sensitized erythrocytes.¹⁹

The observations described in this report are also compatible with antibody activities in two classes of immunoglobulins but differ somewhat from those of Arbesman and co-workers. One antibody sensitized human and monkey skin and was heat labile, and therefore it probably was IgE. However, the heat lability of the sensitizing factor in the Schultz-Dale reaction was unexpected and was not consistent with the current knowledge of the properties of IgG molecules. The possibility that this activity resided in a unique immunoglobulin class or subgroup cannot be resolved from these studies. Unfortunately the patient has returned to Liberia, and additional serum for study has not been obtainable.

Another heat-labile component of some immunological reactions is complement.³³ Although the Schultz-Dale response is not known to be complement dependent, a fragment from C'5 has anaphylatoxic activity and causes contraction of a guinea pig ileum.³⁴ An attempt to restore the response of the guinea pig ileum by adding complement (fresh guinea pig serum) to heat-inactivated patient's serum was not successful.

SUMMARY

This is a case report of respiratory allergy due to moth flies. The seasonal pattern of allergic rhinitis and asthma occurred only during the time of appearance of moth flies. Antibody activity was demonstrable in the patient's serum by direct skin tests and passive transfer to human and monkey skin but not to guinea pig skin. The capacity to sensitize skin was heat-labile. The patient's serum also sensitized a guinea pig ileum in the Schultz-Dale system, and, contrary to expectations, this activity was also heat labile. This feature is not compatible with current knowledge of the properties of human immunoglobulins, but it has not been possible to further characterize these molecules.

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