

Characterization of mouse allergens

Reuben P. Siraganian, M.D., Ph.D., and Ann L. Sandberg, Ph.D. Bethesda, Md.

The major allergens present in mouse skin, serum, and urine have been identified. Skin extracts, serum, and urine were chromatographed, and the activities of the fractions were monitored by histamine release from the leukocytes of individuals sensitive to mice. Fractionation of skin extracts revealed two major allergens. The large allergen has a molecular weight of ~67,000 daltons and by biochemical and immunochemical criteria appears to be identical to mouse albumin. The smaller molecular weight allergen is ~17,000 daltons. The same two allergens are also found in mouse serum and mouse urine. Histamine release by leukocytes of individuals allergic to mice demonstrated that some individuals react predominantly to the large allergen, some to the small allergen, and one group of patients reacts to both allergens.

Hypersensitivity reactions to animals are an important occupational health problem among individuals who work with laboratory animals.¹⁻² Dander allergens have been demonstrated in skin extracts, serum, and urine.³⁻⁵ In contrast to the studies on dander from some animals (e.g., horses and cats), the allergens of laboratory animals have not been extensively examined.⁶⁻¹⁷ Some recent studies have suggested that mouse albumin is an important allergen¹⁸ and that allergenic activity is present in urine.¹⁹

The present study was initiated to characterize the allergens present in mouse skin, serum, and urine. Two major allergens were found; the leukocytes of individuals sensitive to mice reacted predominantly to one or the other allergens, one of which appears to be albumin.

MATERIALS AND METHODS

Mouse pelt allergen

Thirty mouse pelts were frozen on an inverted ice tray, scraped free of subcutaneous fat and connective tissue, and homogenized in 300 ml of phosphate-buffered saline (PBS) (0.15 M NaCl, 0.02 M PO₄, pH 7.2) containing 0.01 M ethylenediaminetetraacetic acid (EDTA). The homogenate was stirred overnight at 4° C, centrifuged, and the supernatant utilized as crude mouse allergens. Some preparations were dialyzed against PBS. Mouse allergenic extract was also obtained from a commercial source (mouse hair, 1:20 w/v; Hollister-Stier Laboratories, Yeadon, Pa.).

From the Laboratory of Microbiology and Immunology, National Institute of Dental Research, National Institutes of Health.

Received for publication Oct. 12, 1978.

Accepted for publication Jan. 15, 1979.

Reprint requests to: Dr. Reuben P. Siraganian, Building 10, Room 2B-12, NIH, Bethesda, MD 20505.

Mouse serum and urine, mouse albumin, and protein concentrations

Mice were bled from the retro-orbital sinus, and serum was obtained from the clotted blood. Urine was collected on ice from groups of mice placed in metabolism cages; pools were frozen every 24 hr and concentrated 10-fold by ultrafiltration.

Mouse albumin was obtained from Sigma Chemical Co., St. Louis, Mo., and Cappel Laboratories, Dowingtown, Pa. Protein concentrations were determined by the Lowry method.²⁰

Column chromatography

Ammonium sulfate gradient solubilization was carried out as described²¹: 88 mg of crude mouse pelt allergens in 28 ml were mixed with 72 ml Tris buffer (0.05 M Tris, 0.1 M NaCl, pH 7.97), 61 gm solid (NH₄)₂SO₄ (85% final concentration), and 10 gm Celite-545. After stirring for 45 min at room temperature, the mixture was poured into a column, washed with 0.05 M Tris saturated 85% with (NH₄)₂SO₄. The column was eluted with a gradient decreasing from 85% to 0% saturation of (NH₄)₂SO₄ in Tris (0.05 M). All samples were dialyzed against PBS.

Mouse pelt allergens were also chromatographed on Sephadex G-100 and Sephadex G-150 (Pharmacia Laboratories, Inc., Piscataway, N. J.) columns utilizing PBS as the elution buffer. The columns were calibrated with blue dextran, ovalbumin, and cytochrome C.

Crude mouse pelt allergens dialyzed against 0.075 M PO₄, pH 8.0 were chromatographed on DE-52 cellulose (Reeve-Angel, Clinton, N. J.) equilibrated in the same buffer. Stepwise elution was carried out utilizing this buffer, 0.15 M PO₄, pH 8.0, and 0.3 M PO₄, pH 8.0.

Polyacrylamide gel electrophoresis

Polyacrylamide gel electrophoresis was carried out as described.²² Fractions from gels run in the absence of so-

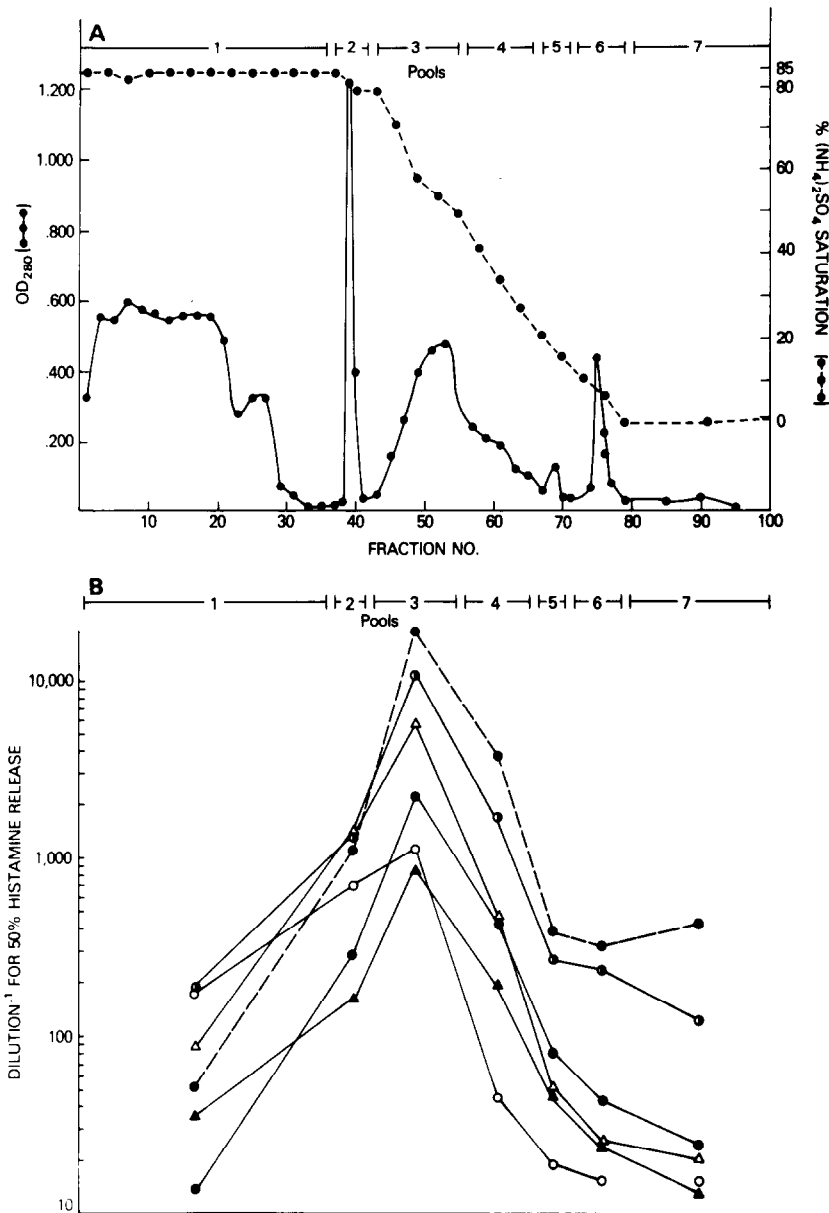


FIG. 1. Separation of mouse pelt extract by ammonium sulfate gradient solubilization on a Celite column. *A*, Dialyzed mouse pelt extract (28 ml, 88 mg protein) was mixed with 72 ml Tris (0.05 M, pH 7.97), 61 gm of solid (NH₄)₂SO₄ (85% final saturation), and 10 gm of Celite-545. After stirring for 45 min at room temperature, this mixture was poured into a column. The column was washed with Tris (0.05 M) saturated 85% with (NH₄)₂SO₄. The column was eluted with a gradient, decreasing from 85% to 0% saturation of (NH₄)₂SO₄ in 0.05 M Tris. Ten-milliliter fractions were collected and pooled as indicated. *B*, Aliquots of pools were dialyzed and serial dilutions were tested for their ability to induce histamine release. Each symbol represents histamine release by leukocytes from one allergic individual.

dium dodecyl sulfate were crushed and eluted overnight in PBS. Some analytical gels were also run in the presence of sodium dodecyl sulfate.

Immuno-electrophoresis and immunodiffusion

Agarose (0.8%) in veronal buffer, 0.05 M, pH 8.6 was employed. Electrophoresis was carried out at room temperature for 2 hr at 220 V. Patterns were developed with hyper-

immune rabbit antisera raised against one purified mouse pelt allergen (large allergen) and mouse albumin. These antisera were obtained by immunization of rabbits with 0.5 mg of large allergen or of chromatographically purified mouse albumin in complete Freund's adjuvant. Animals were boosted twice at 4-wk intervals and bled 7 days after the last injection. The large allergen used for immunization was purified by DE-52 cellulose chromatography followed by Sephadex G-100 column chromatography as described in

"Results." Polyacrylamide gel electrophoresis of this allergen yielded a single line.

Patient population

Leukocytes were obtained from donors with positive immediate skin reactivity to mouse dander and histories of allergic reactions to mice.

Histamine release

Histamine release from washed leukocytes was performed as described previously.²³ Briefly, serial dilutions of mouse allergens at different purification steps were tested for their histamine-releasing activity. Usually 2 or 3 different concentrations per each $\log_{10}$ interval were tested. Reaction mixtures consisted of 0.25 ml of washed leukocytes and 0.25 ml of the allergen preparations. After 45 min at 37° C, the tubes were centrifuged and the supernatants analyzed for their histamine content.²⁴ Cells incubated with buffer alone consistently released less than 10% of their total histamine. All experiments were performed at least twice. The ascending portion of the histamine release curve was used to calculate the dilution required for 50% release.

RESULTS

Effect of dialysis

Several serial dilutions of dialyzed and undialyzed mouse pelt allergen were tested for histamine-releasing activity on the cells of six different allergic individuals. Dialyzed allergens had about 65% (50% to 78%) of the activity of the undialyzed allergens.

Column chromatography

Crude mouse pelt allergen was fractionated by $(\text{NH}_4)_2\text{SO}_4$ gradient solubilization (Fig. 1, A). Fractions were pooled and, after dialysis, tested for their ability to release histamine from the cells of six donors allergic to mice (Fig. 1, B). The allergenic activity was predominantly in pool 3, which eluted at 60% to 80% $(\text{NH}_4)_2\text{SO}_4$ saturation. After dialysis, 47% of the total optical density (OD) eluted was in pool 3, which also contained 70% of the histamine-releasing activity. Pools 2 and 4 contained less allergen.

Pool 3 was concentrated and applied to a Sephadex G-150 column. Most of the protein was eluted in fractions 60 to 70 (Fig. 2, A). Serial dilutions of the fractions were assayed for their histamine-releasing activity (Fig. 2, B). The results demonstrate heterogeneity in the reactivity of individuals to different fractions. The leukocytes from 2 of the donors reacted predominantly to a large molecular weight allergen. The leukocytes from two other donors were most reactive with a smaller molecular weight allergen, although in one case there was also release with the large allergen.

Several protein peaks were obtained when crude

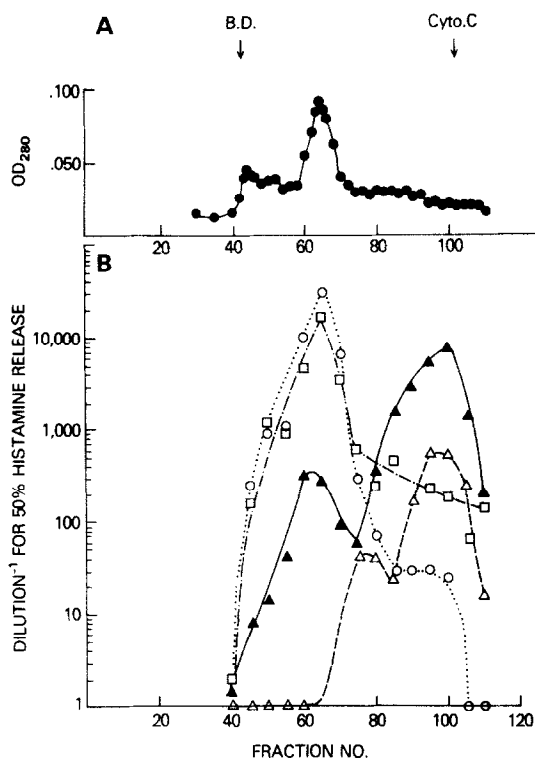


FIG. 2. Sephadex G-150 column chromatography of pool 3 from $(\text{NH}_4)_2\text{SO}_4$ Celite-545 column were concentrated by ultrafiltration to 22 ml and 2.5 ml applied to a Sephadex G-150 column calibrated with blue dextran (B.D.) and cytochrome C (Cyto.C). The column was eluted with PBS. B, Serial dilutions of the fractions were assayed for their ability to induce histamine release. Each symbol represents histamine release by leukocytes from one allergic individual.

mouse pelt allergen was eluted stepwise from a DE-52 cellulose column (Fig. 3, A). One major and three minor protein peaks were eluted with the starting buffer (0.075 M PO_4 , pH 8.0). Increasing the molarity of the phosphate buffer to 0.15 M resulted in the elution of a single protein peak (pool 5), and one additional peak was eluted by further increasing the phosphate to 0.3 M. These peaks were assayed for their ability to induce histamine release from the leukocytes of allergic individuals (Fig. 3, B). Pools 1 to 4 were inactive, whereas pools 5 and 6 contained biologic activity. The leukocytes from two patients were maximally responsive to pool 5. This pool induced 50% histamine release from these patients' leukocytes at a dilution of 1:100,000. In contrast, the leukocytes from two additional allergic individuals were slightly more responsive to pool 6 than to pool 5, while the cells from one donor were significantly more reactive with pool 6 than with pool 5. Thus it is apparent that although complete fractionation of pelt allergens was not achieved by DE-52 cellulose chromatography, the cells from some individuals were in

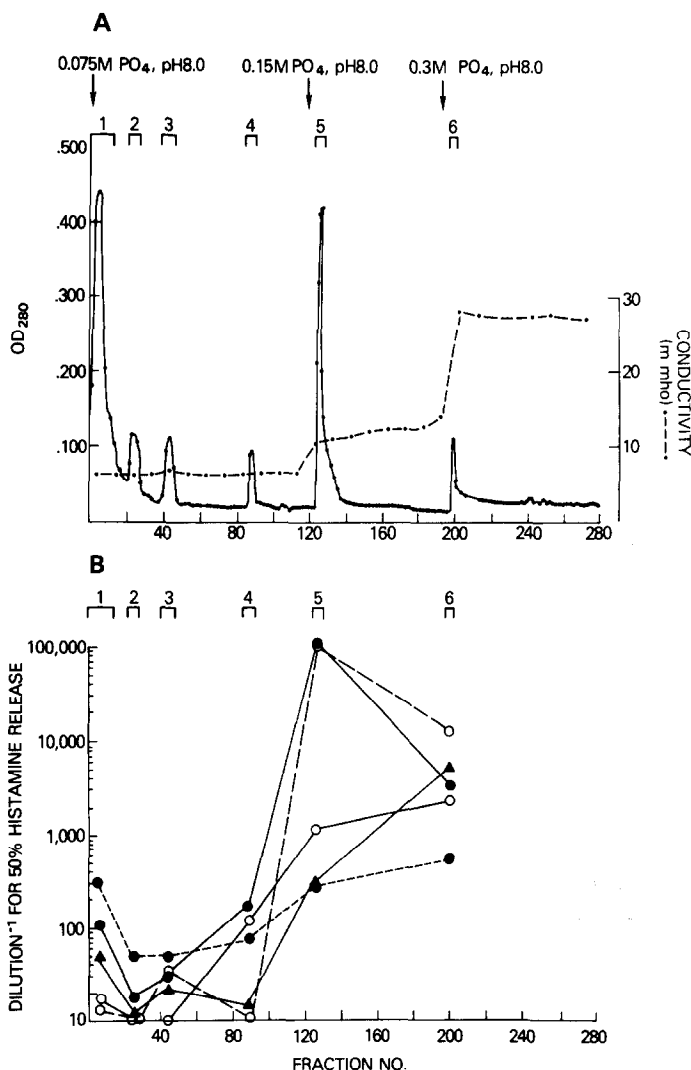


FIG. 3. DE-52 cellulose chromatography of crude mouse pelt extract. *A*, Crude mouse pelt extract was dialyzed against 0.075 M PO₄ buffer, pH 8.0, and applied to a DE-52 cellulose column equilibrated with the same buffer. Stepwise elution was carried out utilizing this buffer, 0.15 M PO₄, pH 8.0, and 0.3 M PO₄, pH 8.0. Fractions were pooled as indicated. *B*, Serial dilutions of the pools were tested for their ability to release histamine. Each symbol represents histamine release by leukocytes from one allergic individual.

general more responsive to pool 5 or pool 6. These results suggested the presence of both the large and small allergens in pools 5 and 6. Identical results were obtained when the pools contained all the DE-52 fractions rather than only the protein peaks. When pool 5 or 6 was subjected to Sephadex G-100 column chromatography, the large and small allergens were separated. Pool 5 contained more large allergen, whereas pool 6 was predominantly small allergen (data not shown).

Since these two procedures (Celite-545 and DE-52 cellulose chromatography) designed for the processing of large quantities of proteins did not result in

adequate separation of the allergenic material, whole pelt allergens were applied directly to a Sephadex G-150 column. The protein profile is shown in Fig. 4, *A*. The leukocytes from fourteen individuals were assayed for their ability to release histamine in response to the column fractions. A distinct pattern emerged, with the allergic donors falling into three major categories. One group of patients exhibited maximum reactivity to the material eluted in fractions 50 to 80 with a peak at fraction 65 (Fig. 4, *B*). The leukocytes from a second group of donors were most responsive to the material eluted in fractions 80 to 110, with peak reactivity in fraction 95 (Fig. 4, *C*). The histamine

release pattern of the leukocytes from a third group of allergic individuals demonstrated activity in both of the two previously designated regions of the column (Fig. 4, *D*). Calculated on the basis of OD for 50% histamine release, the leukocytes from individuals reactive with the large allergen were 2,000 to 10,000 times more sensitive to this allergen than to the small molecular weight allergen. The leukocytes from donors predominantly reactive with the small allergen were 100 to 2,000 times more sensitive to this allergen than to the large allergen. These findings clearly demonstrate that two major allergens are present in crude mouse pelt extracts and that these two are distinguishable by molecular weight. The molecular weight of the large allergen is approximately 67,000 daltons as determined by Sephadex chromatography, while that of the small allergen is approximately 17,000 daltons. The large allergen elutes from Sephadex G-150 in the same position as albumin. Analogous results were obtained utilizing the commercial mouse allergenic extracts.

Polyacrylamide gel electrophoresis

Experiments were performed to characterize the small and large allergens and to establish the relationship between the large allergen and albumin.

Crude pelt extract, large allergen purified by Sephadex G-150 chromatography, and mouse albumin were subjected to polyacrylamide gel electrophoresis (Fig. 5). Gels were electrophoresed in duplicate, and one gel was stained. The second gel containing albumin or crude pelt extract was sliced as indicated, eluted with PBS, and the eluate assayed for histamine-releasing activity. The major band of protein in the pelt extract co-electrophoresed with the purified large allergen and the monomeric form of albumin. The leukocytes from one individual reactive with large allergen released histamine in response to the eluate of fraction 4 from both the albumin and pelt extract gels. The leukocytes from a second individual reactive with the small allergen released histamine when incubated with fraction 5 of the pelt extract gel. Analytical gels were also run in the presence of sodium dodecyl sulfate. The migration of the large allergen remained identical to that of albumin. Sufficient quantities of small allergen have not been obtained to observe its electrophoretic behavior by protein staining.

Allergens in mouse serum

Mouse serum was fractionated on Sephadex G-150 and the fractions tested with the cells of two individuals known to be reactive to either the large or

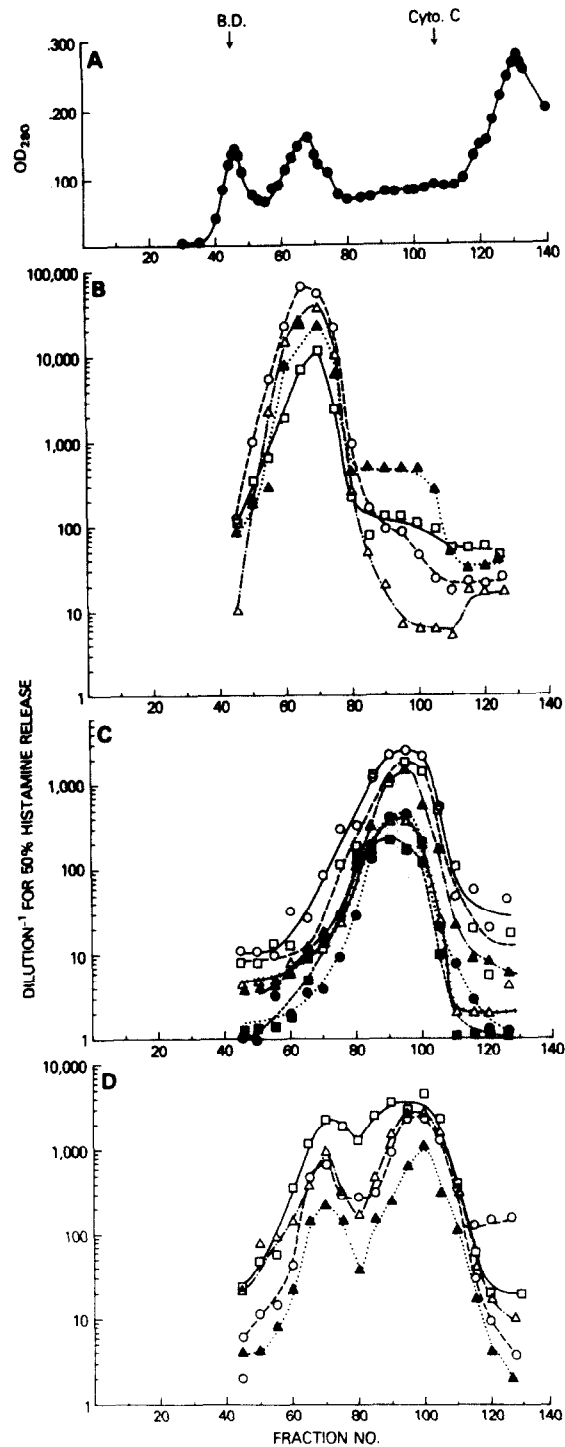


FIG. 4. Sephadex G-150 column chromatography of 2.5 ml crude mouse pelt extract. *A*, The column was eluted with PBS. *B*, *C*, and *D*, Serial dilutions of indicated fractions were tested for their ability to release histamine. Each symbol represents histamine release by leukocytes from a different allergic individual. A total of fourteen patients was studied.

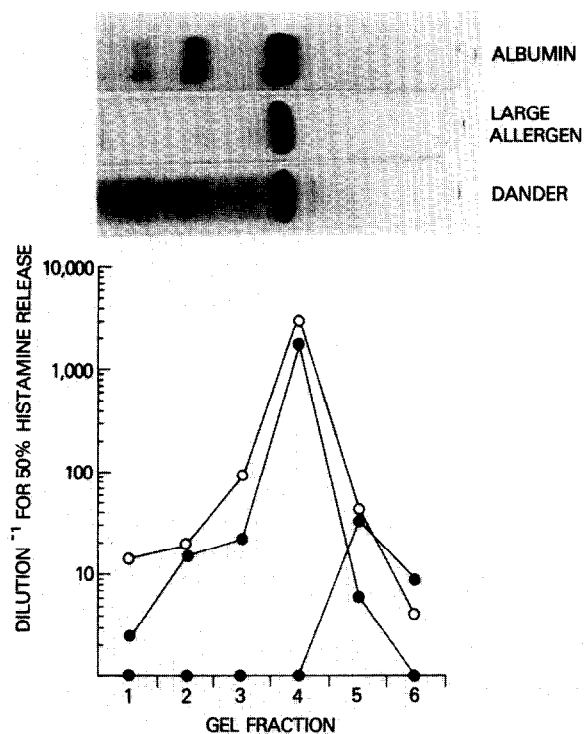


FIG. 5. Polyacrylamide gel electrophoresis of albumin, large allergen, and crude mouse pelt extract and histamine release induced by the eluates of gel fractions. ●—●, Histamine release with eluates from the crude pelt extract gel. Leukocytes were from two individuals, one of whom reacted with the large and the other with the small allergen. ○—○, Histamine release with eluates from the albumin gel. Leukocytes were from an individual reactive to large allergen.

small allergens (Fig. 6). The results demonstrate that both large and small allergens are present in the serum; the large allergen reactivity corresponds to the albumin elution peak.

Allergens in mouse urine

Allergenic activity was found in dialyzed urine assayed with the cells of donors sensitive to mice. After separation on a Sephadex G-150 column, both large and small molecular weight activity could be demonstrated (data not shown).

Allergic reactions to albumin preparations

On testing several commercial mouse albumins, it was found that the leukocytes from individuals reactive to both large and small allergens released histamine in response to some albumin preparations. Sephadex G-100 fractionation of these albumin preparations demonstrated the presence of both large and small molecular weight allergens (data not shown).

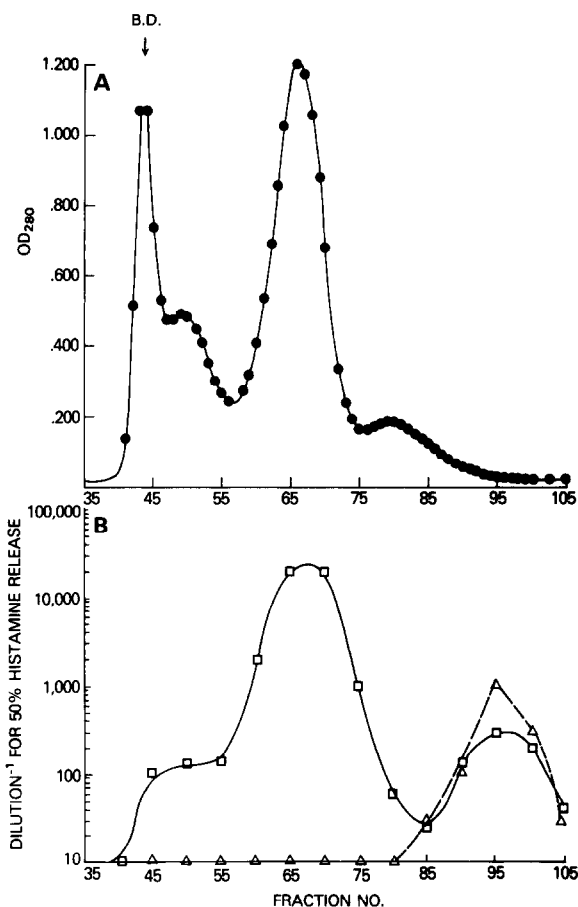


FIG. 6. Sephadex G-150 column chromatography of 1.3 ml mouse serum. *A*, The column was eluted with PBS. *B*, Serial dilutions of indicated fractions were tested for their ability to release histamine from the leukocytes of two individuals, as represented by the symbols.

Ouchterlony analysis and immunoelectrophoresis

Precipitation in gel revealed identity between albumin, large allergen, serum, and urine (Fig. 7). Albumin and large allergen also migrated identically in immunoelectrophoresis. These patterns were developed with rabbit anti-large allergen serum. The same results were obtained when the patterns were developed with rabbit anti-albumin serum.

DISCUSSION

The results clearly indicate that there are at least two major mouse allergens. The large allergen has a molecular weight of ~67,000 daltons, and by biochemical and immunochemical criteria is identical to mouse albumin. The small allergen has a molecular weight of ~17,000 daltons. Although the large allergen could be purified to homogeneity as deter-

mined by polyacrylamide gel electrophoresis, the small allergen could not be obtained in sufficient quantities for extensive characterization.

The following evidence indicates that the large allergen is albumin: the molecular weight of this fraction is ~67,000 daltons; rabbit antibodies to the large allergen show lines of identity between the immunizing antigen and mouse albumin; rabbit antibodies to mouse albumin reveal identity between the albumin and the large allergen; the immunoelectrophoretic patterns of albumin and large allergen are identical; by disc electrophoresis the large allergen and mouse albumin co-migrate, and histamine release activity parallels the protein band.

The small molecular weight allergen is ~17,000 daltons. There is no clear relationship between the small and large allergens; the predominant reactivity of different individuals to one or the other allergen indicates that both have unique antigenic determinants. It is theoretically possible that the large allergen is a tetramer of the small molecule with all its small type allergenic sites hidden in the tetramer. However, disc electrophoresis of large allergen under strongly dissociating conditions exhibited a single band which migrated in the region of albumin.

There were fourteen leukocyte donors who were used repeatedly in this study. Among these, four (29%) were clearly reactive to the large allergen and six (43%) to the small allergen. Cells from these individuals did, however, release some histamine when exposed to high concentrations of the allergen to which they were not principally reactive. Whether this is due to cross-contamination or true reactivity to both antigens cannot be determined from the present results. The leukocytes from four additional donors reacted with both allergens. These donors had received desensitization injections with mouse allergenic extract. No data are available to indicate if this responsiveness to both allergens is a direct result of the immunotherapy. The importance of using leukocytes from multiple donors in assaying the allergenic activity of all column fractions is clear from the present study.

Animal danders are multiallergenic systems.³⁻¹⁶ Individuals sensitive to animal danders are also often sensitive to the serum from these animals,^{6, 8, 11, 12} and these findings were verified in the present studies. Albumins have been thought to be important allergens in several dander systems. Sensitivity to horse serum albumin has been reported among horse dander allergic individuals.^{5, 11} Serum albumin of other mammalian species (e.g., dogs, cats, mice) have been shown to be allergenic in man,^{12, 15, 18} although it appears to

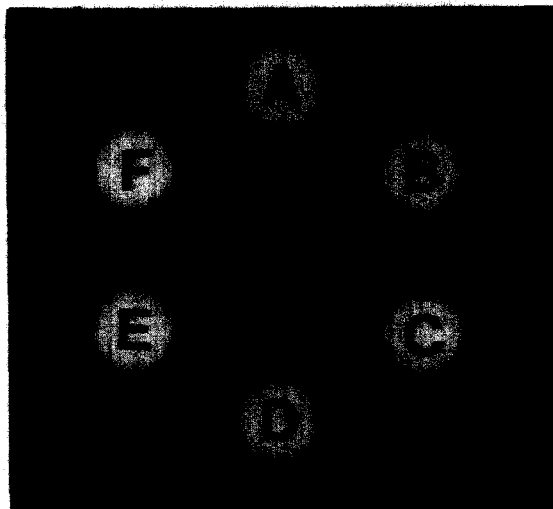


FIG. 7. Precipitation in gel between antiserum to large allergen and several allergens of murine origin. Center well contains antiserum. A, albumin; B, large allergen; C, serum; D, albumin; E, urine; F, crude pelt extract.

be of minor importance in hypersensitivity reactions to cat dander.⁹

The present results clearly demonstrate the presence of at least two major allergens present in mouse skin, mouse serum, and mouse urine. Individuals sensitive to mice may react predominantly with either or both of these two allergens.

We acknowledge the excellent technical help of Ms. Pamela Pazoles, Ms. Suzanne Chan, and Ms. LuAnn Basciano and the secretarial help of Ms. Patricia Noyes. This research would not have been possible without the collaboration of the mouse-allergic individuals who repeatedly donated blood for this project.

REFERENCES

1. Lincoln TA, Bolton NE, Garrett AS, Jr: Occupational allergy to animal dander and sera, *J Occupational Med* **16**:465, 1974.
2. Lutsky II, Neuman I: Laboratory animal dander allergy. I. An occupational disease, *Ann Allergy* **35**:201, 1975.
3. Marsh DG: Allergens and the genetics of allergy, in Sela M, editor: *The antigens*, New York, 1975, Academic Press, Inc., vol. 3, p. 271-359, 1975.
4. King TP: Chemical and biological properties of some atopic allergens, *Adv Immunol* **23**:77, 1976.
5. Berrens L: *The chemistry of atopic allergens*, Basel, 1971, S. Karger AG.
6. Ohman JL, Jr, Lowell FC, Bloch KJ: Allergens of mammalian origin: Characterization of allergen extracted from cat pelts, *J ALLERGY CLIN IMMUNOL* **52**:231, 1973.
7. Ohman JL, Jr, Lowell FC, Bloch KJ: Allergens of mammalian origin. III. Properties of a major feline allergen, *J Immunol* **113**:1668, 1974.
8. Ohman JL, Jr, Lowell FC, Bloch KJ: Allergens of mammalian origin. II. Characterization of allergens extracted from rat,

- mouse, guinea pig, and rabbit pelts, *J ALLERGY CLIN IMMUNOL* **55**:16, 1975.
9. Ohman JL, Kendall S, Lowell FC: IgE antibody to cat allergens in an allergic population, *J ALLERGY CLIN IMMUNOL* **60**:317, 1977.
 10. Varga JM, Ceska M: Characterization of allergen extracts by polyacrylamide gel isoelectrofocusing and radioimmunosorbent allergen assay. II. Dog and cat allergens, *Int Arch Allergy Appl Immunol* **42**:438, 1972.
 11. Ponterius G, Brandt E, Yman L: Comparative studies on the allergens of horse dandruff and horse serum, *Int Arch Allergy Appl Immunol* **44**:679, 1973.
 12. Brandt R, Ponterius G, Yman L: The allergens of cat epithelia and cat serum. Comparative studies based on the radioallergosorbent technique (RAST), *Int Arch Allergy Appl Immunol* **45**:447, 1973.
 13. Ceska M: Characterization of allergen extracts by dose-response studies and by polyacrylamide gel isoelectrofocusing using the paper disc radioallergosorbent test as the assay method. III. Horse dandruff allergen, *Int Arch Allergy Appl Immunol* **43**:419, 1972.
 14. Ceska M, Hultén E: Characterization of allergen extracts by dose-response studies and by polyacrylamide gel isoelectrofocusing using the paper disc radioallergosorbent test as the assay method. IV. Cow dandruff allergen, *Int Arch Allergy Appl Immunol* **43**:427, 1972.
 15. Yman L, Brandt R, Ponterius G: Serum albumin—an important allergen in dog epithelia extracts, *Int Arch Allergy Appl Immunol* **44**:358, 1973.
 16. Aronsson T, Wide L: Studies on allergens of horse epithelium with two variants of RAST, *Int Arch Allergy Appl Immunol* **47**:224, 1974.
 17. Løwenstein H, Markussen B, Weeke B: Identification of allergens in extract of horse hair and dandruff by means of crossed radioimmunoelectrophoresis, *Int Arch Allergy Appl Immunol* **51**:38, 1976.
 18. Levy DA: Allergenic activity of proteins from mice, *Int Arch Allergy Appl Immunol* **49**:219, 1975.
 19. Taylor AN, Longbottom JL, Pepys J: Respiratory allergy to urine proteins of rats and mice, *Lancet* **2**:847, 1977.
 20. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ: Protein measurement with the folin phenol reagent, *J Biol Chem* **193**:265, 1951.
 21. King TP: Separation of proteins by ammonium sulfate gradient solubilization, *Biochemistry* **11**: 367, 1972.
 22. Davis BJ: Discelectrophoresis. II. Method and application to human serum proteins, *Ann NY Acad Sci* **121**:404, 1964.
 23. Siraganian RP: Histamine release and assay methods for the study of human allergy, in Rose NR, Friedman H, editors. *Manual of clinical immunology*, Washington, D. C., 1976, American Society for Microbiology, p. 603.
 24. Siraganian RP: An automated continuous-flow system for the extraction and fluorometric analysis of histamine, *Anal Biochem* **57**:383, 1974.

Announcements

Tenth International Congress of Allergology

The Tenth International Association of Allergology will be held in Jerusalem, Israel, on November 4 to 11, 1979, at the invitation of the Israel Society of Allergology and Clinical Immunology. The comprehensive scientific program of the Congress will cover advances in immunological and clinical aspects of allergic and related diseases geared to clinical immunologists, practicing allergists, and related specialists, e.g., otolaryngologists, pneumologists, and dermatologists. Opportunities will be provided within the framework of the Congress for visiting medical centers and research institutions, and for sightseeing in this ancient-modern country presenting many contrasts and exciting accomplishments.

Colleagues wishing to present a paper are kindly requested to submit an abstract on a form which will be provided on request. Due to a prolonged postal strike in Israel, the deadline for submitting abstracts or free communications has been extended to June 15, 1979. Please address all correspondence to: Dr. Israel Glazer, Chairman, Organizing Committee, X International Congress of Allergology, P.O.B. 16271, Tel Aviv, Israel.

This continuing medical educational activity fulfills 26 credit hours for Category I requirements.

Eleventh Congress of the European Academy of Allergology and Clinical Immunology

The Eleventh Congress of the European Academy of Allergology and Clinical Immunology will be held in Vienna, Austria, from October 6 to 10, 1980. Please address communications to: Dr. H. Ludwig, c/o Wiener Medizinische Akademie, Alser Straße 4, A-1090 Vienna, Austria.

Thirteenth Annual Congress of American Association for Clinical Immunology and Allergy

The American Association for Clinical Immunology and Allergy has scheduled its Thirteenth Annual Congress for July 22 to 27, 1979, at Denver, Colo.