

Immediate hypersensitivity to cockroach

Isolation and purification of the major antigens

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Crude cockroach extract elicited positive skin tests in 50% of patients with positive and in 4% with negative environmental history for cockroach exposure, suggesting a possible role of cockroach in perennial atopic disease. Three major allergens in crude American and German cockroach extracts have been identified using sequential purification steps on Sephadex G-75, diethylaminoethyl (DEAE) cellulose, and agarose gel electrophoresis. Cr-I elicits positive skin tests in 70% of patients sensitive to the crude extracts. It has a molecular weight of approximately 25,500 daltons, is highly acidic, and resists boiling for four hours. Boiling in 4 N acetic acid completely abolishes its allergenicity. The purified allergen elicits positive skin tests at a concentration of 3 µg/ml and is capable of inducing >50% histamine release from sensitive leukocytes at 0.05 ng/ml. A second antigen, Cr-II, elicits positive skin tests also in approximately 70% of cockroach-sensitive individuals, has a molecular weight of approximately 63,000 to 65,000 daltons, and has similar heat stability and acid hydrolysis characteristics to Cr-I. A third, less well-characterized antigen, Cr-III, has a molecular weight <10,000 daltons and elicits positive skin tests in 30% of individuals sensitive to the crude extract.

Immediate hypersensitivity to house dust is recognized as an important cause of perennial allergic rhinitis and asthma, but house dust is a poorly defined mixture of allergens of varying complexity and antigenicity.¹ Its composition in relation to individual allergens varies both quantitatively and qualitatively from one location to another, thus making precise characterization of its component antigens difficult. Recently the mite (*Dermatophagoides pteronyssinus*) has been identified as a significant factor in house dust sensitivity in some areas of the world.² Allergens rec-

ognized as part of house dust include kapok,³ feathers,⁴ molds,⁵ cotton linters,⁶ and others, including cockroach body parts and feces. Evidence has been presented that cockroach sensitivity may be an etiologic agent in perennial allergic rhinitis and asthma.⁷⁻¹⁴ Of approximately 55 species of cockroach in the United States, only 5 appear to infest dwellings to any significant degree.¹⁵ In the Boston area, the major infestation is with American cockroach, *Periplaneta americana* (Linnaeus), and German cockroach, *Blattella germanica* (Linnaeus).

The present studies were undertaken in order to define the frequency of cockroach sensitivity in a patient population with allergic diseases and to identify, purify, and characterize the principal allergens in crude extracts of cockroach body parts and feces. Crude cockroach extracts were initially separated into two major allergenic groups on the basis of molecular weight. The larger proteins, after further purification on agarose electrophoresis, yielded two distinct allergens designated Cr-I and Cr-II with molecular weights of approximately 25,500 and 69,000 daltons, respectively, which elicit positive immediate skin reactions in 70% of patients sensitive to the crude extract. A smaller antigen, Cr-III, with a molecular weight of <10,000 daltons, elicits positive skin reactions in approximately 30% of patients sensitive to crude extract.

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MATERIALS AND METHODS

Source of reagents

Crude cockroach extracts. Glycerinated crude extracts of American cockroach (Lots B26-12-1x2 and B26-13-1x5) and German cockroach (Lot GB46-B-1A) were kindly supplied by Greer Laboratories, Lenoir, N. C. Cockroaches used to prepare the crude extracts were raised by Insect Control and Research Inc., Baltimore, Md. They were grown on Purina dog chow, and adult insects were killed by freezing. The frozen insects were ground, defatted with acetone, and powdered in a ball mill; the powder was then extracted in Coca's solution for 24 hr. Crude extract was filtered through Whatman No. 4 filters and then further clarified by use of a Cox No. 200-100-70 filter. The pH was adjusted to 7.40, phenol added to a final concentration of 0.4%, and sterile filtration accomplished with a Seitz filter.

House dust extracts. Glycerinated extracts of house dust were obtained from Greer Laboratories, Lenoir, N. C.; Hollister-Steir Laboratories, Yeadon, Pa.; Center Laboratories, Port Washington, Long Island, N. Y.; and Endo Laboratories, Garden City, Long Island, N. Y.

Column chromatography. Sephadex G-75 was obtained from Pharmacia, Uppsala, Sweden; DEAE-cellulose from Brown Co., Berlin, N. H.

Separation procedures

Dialysis and concentration. Extracts were dialyzed and concentrated by repeated dilution and concentration using an Amicon ultrafiltration apparatus (Amicon Corporation, Lexington, Mass.). Membranes of appropriate pore size were chosen for individual procedures as noted below.

Column chromatography. Gel filtration was performed on 2.5 × 100 cm Sephadex G-75 columns. Elution by gravity flow at 25 to 30 ml/hr was performed at 4° C, and 3 to 4 ml fractions were collected. The eluant was either saline or phosphate-buffered saline, 0.15 M, pH 7.4. Fractions were monitored for absorption at 280 nm. Analytical columns were calibrated for use with markers of known molecular weights including bovine serum albumin (BSA) (MW, 68,000), egg albumin (MW, 45,000), carbonic anhydrase (MW, 29,000), and chymotrypsin (MW, 11,000). DEAE was activated, washed in starting buffer (0.005 M phosphate buffer, pH 7.4), and the sample (in the same buffer) then applied. A linear salt gradient (limit buffer: 0.005 M phosphate, 0.5 M NaCl pH 7.4) was used to elute fractions (4 cc) from the DEAE-cellulose column (2 × 20 cm). Protein concentration of fractions was monitored as above.

Electrophoretic procedures. Electrophoretic separation of proteins was performed in 0.8% agarose prepared in Veronal buffer 0.05 M, pH 8.6. Analytical agarose gels were 1.0 mm thick, whereas preparative gels were 3.0 to 4.0 mm thick. Samples were generally electrophoresed 6 to 7 cm on 20 × 11 cm plates run at 75 ma (350 volts) for 2 to 3 hr. The protein front was estimated by a marker of human serum and bromphenol blue. Preparative gels were cut into 2-mm strips perpendicular to the direction of electrophoresis. Elution of fractions was accomplished by freeze-thawing the gel strips.

TABLE I. Environmental history and immediate skin test response to house dust and crude cockroach antigen (American)

Environ- mental history	Number of patients	Skin test-positive			
		House dust		Cockroach	
		No.	%	No.	%
HD+* CR+†	65	43	(66)	33	(50.8)
HD+ Cr-	63	39	(61.9)	3	(4.8)
HD- Cr+	18	9	(50)	9	(50)
HD- Cr-	54	28	(51.8)	2	(3.7)
Total	200	119	(59.5)	47	(23.5)

*A history of perennial symptoms or symptoms exacerbated during the winter months was considered suggestive of house dust sensitivity.

†History was considered positive for significant cockroach exposure if patients reported cockroach infestation of the home.

Molecular weight determinations were performed according to the method of Weber and Osborn¹⁶ in sodium dodecyl sulfate (SDS) polyacrylamide gels.¹⁶ β -galactosidase (MW, 130,000), phosphorylase (MW, 94,000), serum albumin (MW, 68,000), actin (MW, 45,000), chymotrypsinogen (MW, 25,700) and cytochrome C (MW, 11,700) served as markers of known molecular weights.

Stability of antigens. Purified cockroach antigen preparations were mixed with glacial acetic acid (final concentration 4 N acetic acid) and boiled for 4 hr according to the method of Bunn and co-workers.¹⁷ Heat stability was estimated by boiling a similar sample for 4 hr at neutral pH. Purified cockroach allergens were exposed to ultraviolet light at 365 nm in a fluorimeter as described previously.¹⁸

Bioassay for antigenic activity.

Skin testing: Immediate hypersensitivity to crude cockroach extract or fractions thereof was measured by the scratch method. Antigens used for scratch testing were 50% glycerol preparations. A reaction was considered positive if the wheal exceeded 5 mm and/or the flare was greater than 15 mm.

Histamine release: Antigen-mediated release of histamine in vitro from leukocytes of individual donors was estimated using the method of May and co-workers.¹⁹ Leukocyte suspensions were incubated at 37° C with appropriate antigen dilutions; buffer blanks served as controls for total histamine content and nonspecific release.

Patient population

Two hundred consecutive new patients presenting to the Allergy Clinic at the Children's Hospital Medical Center, Boston, Mass., were studied in the initial screening for frequency of cockroach hypersensitivity. Approximately 10% of these patients were referred from private physicians, generally from Boston suburban areas, and 90% were urban residents. Children tested were all more than 2 years of age and had asthma and/or rhinitis. History was considered positive for significant cockroach exposure if patients re-

TABLE II. Positive immediate skin reactivity to commercial extracts of house dust

Number of patients studied		Number of patients with positive skin tests to commercial house dust extracts			
		No. 1	No. 2	No. 3	No. 4
Negative history	136	62	26	6	8
History of:					
Perennial symptoms alone	41	19	12	7	3
Winter exacerbation with or without perennial symptoms	115	55	26	10	9
Total	292*	136	64	23	20

*Total of 292 patients were skin-tested with each of four extracts; of these, 160 had a positive skin test to one or more of the commercial dust extracts used.

ported cockroach infestation of the home and for dust sensitivity if perennial symptoms were present or were exacerbated following onset of the heating season.

Testing for sensitivity to fractions of crude cockroach extract was performed in patients known to have skin test reactivity to the crude material.

RESULTS

Frequency of cockroach sensitivity

Of 200 consecutive patients included in the initial screening, 38 had a positive history of exposure to cockroach (Table I). Skin tests for immediate hypersensitivity performed by the scratch method with crude American cockroach extracts revealed a positive wheal-and-flare reaction in 51% of those with positive history of cockroach exposure. Only 4% of the 117 patients with negative history of exposure had positive immediate skin reactions to crude cockroach antigen.

In the same population, we compared frequency of positive skin tests to cockroach and house dust as a function of clinical history suggestive of dust sensitivity. Greer, Center, Hollister-Stier, and Endo house dust extracts were used to determine immediate skin test reactivity. Regardless of environmental history, approximately 50% to 60% of patients had positive skin tests to one or more of the house dust extracts (Table I).

Comparison of skin test results with the four house dust extracts was extended to a total of 292 patients. No clear correlation between results of scratch testing and a history of either perennial symptoms or exacerbation of symptoms during the winter season could be established for any of the preparations tested in this group of patients (Table II). Intradermal skin testing was not routinely performed on those having negative scratch tests.

Another group of patients were tested with both American and German cockroach extracts. Of 30 patients with positive skin reactions to either cockroach

preparation, 21 reacted to extracts of both species, 4 reacted only to German cockroach, and 5 had positive reaction only to American cockroach. Intradermal testing of patients with negative scratch tests was not performed.

Purification of the antigen

Initial fractionation of crude American cockroach extract was performed on Sephadex G-75. The extract was concentrated on a UM05 filter prior to fractionation. Fig. 1 illustrates a typical elution pattern for crude cockroach extract. Antigenic activity of fractions was assayed by skin testing in 16 individuals known to have positive reactions to the crude extract. Every second or third fraction collected was assayed in this manner. This procedure succeeded in separating two major allergenic peaks. Positive reactions were obtained in 11 of 16 patients (approximately 70%) with fractions corresponding to the first small protein peak (Fig. 1). Two separate allergens were subsequently isolated from a pool of these fractions and were designated Cr-I and Cr-II. Fractions 110 through 130 elicited positive reactions in 5 of the 16 patients (approximately 33%). The allergen eluted in this area was called Cr-III. It was not further purified. Calibration of the Sephadex column revealed that the Cr-I and Cr-II antigens have molecular weights between 29,000 and 68,000 daltons, while the Cr-III antigen has a molecular weight of <10,000 daltons.

Following identification of two major antigenic peaks in preliminary studies, further purification and isolation of the larger molecular weight allergens (Cr-I and Cr-II) was undertaken in the following manner. Initial dialysis and concentration on an Amicon PM-10 filter (molecular weight exclusion, 10,000 daltons) served to partially separate the two major antigenic species. Gel filtration on Sephadex G-75 of the retained portions of both American and German

cockroach extracts resulted in elution patterns similar to that shown in Fig. 1. Skin test reactivity was routinely limited to the early small peak, the Cr-III allergen having been removed by dialysis. Sephadex fractions yielding positive skin tests were pooled, concentrated, and applied to DEAE-cellulose in a starting buffer of 0.005 M phosphate, pH 7.4. The protein was eluted with a salt gradient (limit buffer 0.005 M phosphate, 0.5 M NaCl, pH 7.4), and antigenic activity appeared in late fractions (Fig. 2). This step was eliminated in the preparation of larger batches to avoid excessive loss of antigen.

Final purification was achieved by preparative agarose electrophoresis of either the DEAE-cellulose purified material or (as noted above) a pool of fractions yielding positive skin tests from Sephadex G-75. Protein was eluted from 2-mm strips of the agarose and used for skin testing. Two major allergenic species were separated by this procedure. The first (Cr-I) migrated rapidly, well ahead of prealbumin, using normal human serum as a marker (Fig. 3). This antigen elicited positive scratch tests in approximately 70% of individuals with positive reactions to crude extracts. A second antigen (Cr-II) migrated in the β -globulin region. This antigen elicited positive scratch tests in a similar percentage (approximately 70%) of individuals. However, there was not complete concordance between patients having positive skin tests to each of these purified antigens. Similar results were obtained for isolation of these allergens from crude extracts of both American and German cockroach.

Physicochemical characteristics of Cr-I and Cr-II

Molecular weight determinations. An estimate of the molecular weight of purified Cr-I was obtained in 5% SDS polyacrylamide gels under reducing conditions (mercaptoethanol and urea). A standard mixture of proteins of known molecular weight, purified Cr-I, and crude American cockroach were run concurrently. Cr-I migrated as a single band with an estimated molecular weight of 25,500 (Fig. 4). Two closely approximated bands were visible with the Cr-II antigen on SDS gels and had apparent molecular weights of 63,000 and 65,000. The migration of Cr-II was unchanged under reducing conditions (mercaptoethanol).

Temperature stability and acetic acid hydrolysis. Cr-I and Cr-II from both American and German cockroach extracts were tested for resistance to boiling both in 4 N acetic acid and in saline. Acetic acid hydrolysis completely abolished the antigenic activity of both Cr-I and Cr-II when tested in four individuals

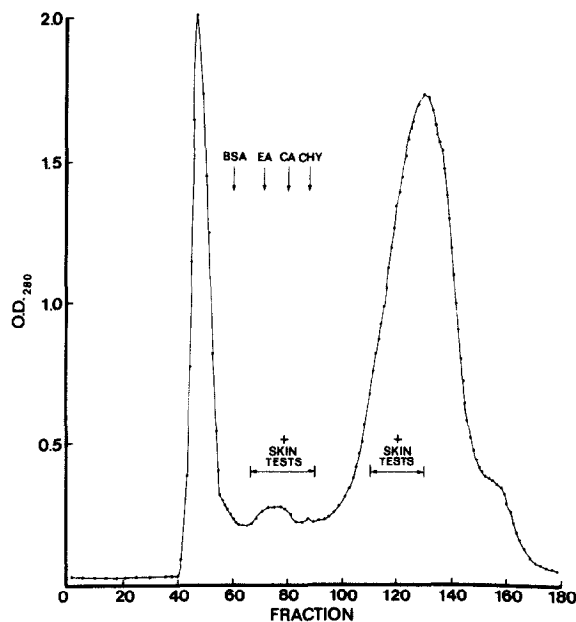


FIG. 1. Sephadex G-75 elution pattern of crude American cockroach extract. Antigenic activity as assessed by skin testing was limited to areas so marked.

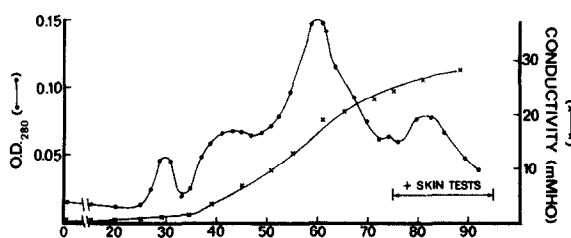


FIG. 2. DEAE-cellulose chromatography of a pool from the early skin test-positive peak eluted from Sephadex G-75. Starting buffer was 0.005 M phosphate pH 7.4 and elution was carried out using a linear salt gradient (limit buffer: 0.005 M phosphate 0.5 M NaCl pH 7.4).

who at the same time demonstrated skin test sensitivity to untreated as well as heated (100° C, 4 hr) preparations.

Ultraviolet radiation of antigens. Ultraviolet treatment of both Cr-I and Cr-II for 1 hr, as described by Berrens, Henocq, and Radermecker,¹⁸ did not alter the ability of the antigen to elicit positive skin reactions in the four individuals tested with these preparations.

Biologic activity of Cr-I and Cr-II

Skin testing. Cr-I and Cr-II both were capable of eliciting positive skin tests by the scratch method at concentrations of approximately 3 μ g/ml.

Histamine release. Significant histamine release was obtained from leukocytes of 11 skin test-positive patients when crude American cockroach was employed at a dilution of 1:12,500. Cr-I elicited histamine release in seven separate leukocyte prepara-

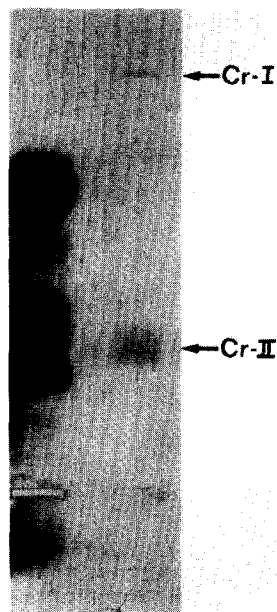


FIG. 3. Agarose electrophoresis of normal human serum marker (on left) and a pool (on right) prepared from the large molecular weight skin test-positive peak from Sephadex G-75. Skin test-positive fractions eluted from the agarose corresponded to the areas labeled Cr-I and Cr-II.

tions obtained from patients with skin test reactivity. It was capable of inducing >50% histamine release at a concentration as low as 0.05 ng/ml. Lower concentrations were not tested. Crude extract in a concentration as high as 1:1,000 and Cr-I tested to a concentration of 1,000 ng did not release histamine from a skin test-negative individual.

DISCUSSION

Cockroach sensitivity has been implicated as a cause of perennial allergic rhinitis and asthma.⁷⁻¹⁴ The frequency of sensitivity appears to be related to degree of exposure. Bernton and Brown⁷ demonstrated a significantly greater frequency of skin test sensitivity in those ethnic groups exposed to environments heavily contaminated with cockroaches than in those with lesser exposure. Our data support this conclusion, as frequency of skin reactions was significantly more common in patients with positive history of exposure than in apparently nonexposed individuals.

Bronchial provocation testing has not been undertaken with the purified antigens. There is evidence that cockroach antigens are capable of inducing clinically significant bronchospasm. Bernton, McMahon, and Brown⁹ obtained significant changes in forced expiratory volume in one second (FEV₁) and vital capacity in 3 of 10 patients with presumed cockroach

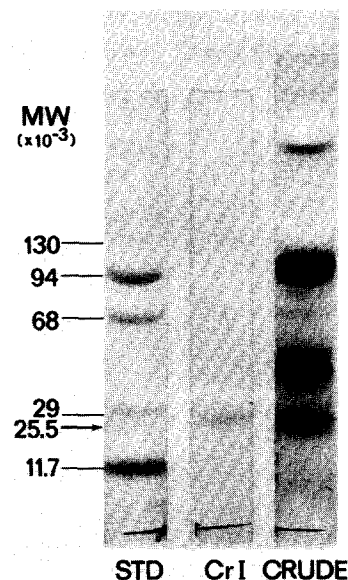


FIG. 4. SDS-polyacrylamide gel of a standard mixture of proteins of known molecular weight, purified Cr-I, and crude cockroach extract. Apparent molecular weight of Cr-I was calculated to be 25,500 daltons.

sensitivity. More recently, Kang¹³ reported positive bronchial challenge with crude extract in 11 of 12 cockroach-sensitive individuals. This reaction could be partially blocked by administration of disodium cromoglycate 30 min before challenge. Five of the twelve patients developed peripheral eosinophilia following provocation.

We have identified three allergenic components in crude cockroach extracts. It was possible to isolate these antigens from crude extracts of both American cockroach and German cockroach with a sequential purification procedure employing Sephadex G-75, DEAE-cellulose, and finally agarose gel electrophoresis. The major allergens, Cr-I and Cr-II, with molecular weights of approximately 25,500 and 65,000 daltons, respectively, elicited positive scratch tests in 70% of patients sensitive to the crude extract. A low molecular weight material, Cr-III, MW, <10,000, elicits positive skin tests in approximately 30% of patients sensitive to the crude extract. Further characterization of this allergen is currently underway. There was not complete concordance among individuals having positive skin tests with these antigens, suggesting both that sensitization to each of the antigens was independent and that the purified proteins did not merely represent complexes of smaller subunits. These results are similar to those found with ragweed hypersensitivity where multiple allergens and varying patterns of sensitivity are seen.

Both the elution from DEAE at high salt concentrations and anodal migration in agarose gel elec-

trophoresis suggest that the Cr-I antigen is highly acidic. This is a characteristic shared with many other allergens, including cat and dog danders,^{20, 21} pelt antigens (rat, mouse, guinea pig, and rabbit),²² and "house dust."²³ Acid hydrolysis under conditions shown to cleave sugars bound in an Amadori configuration¹⁷ completely abolished allergenic activity. Berrens²³ has proposed that allergenicity of "house dust" is related to sugar residues bound in an Amadori configuration to lysine side chains within the carrier molecule. Our results are consistent with this theoretical structure though further studies will be required to define the structure of Cr-I. The Cr-II allergen is less anodal in its migration in agarose, but it shares the characteristics of heat stability and sensitivity to acid hydrolysis with Cr-I.

Molecular weight estimates on SDS polyacrylamide gels were approximately 25,500 daltons for Cr-I and 65,000 daltons for Cr-II. This size is within the general range of other allergens. These include ragweed antigen E, MW, 37,800²⁴; rye grass antigen I, MW, 32,000²⁵; birch pollen, MW, 17,800²⁶; pelt antigen, MW, 10,000 to 28,000²², as well as others.

The antigenic relationship between the Cr-I and Cr-II allergens isolated from crude American and German cockroach extracts remains incompletely studied. The good concordance of skin test reactivity to crude American and German cockroach extracts can be explained either on the basis of simultaneous environmental exposure or on the basis of cross-reacting antigens. Physicochemical characteristics suggest that the antigens are similar.

Similarities between these allergens and other purified allergens indicate that some common properties may contribute to their allergenicity. Further characterization of these allergens may reveal homologous structural sites essential for triggering the allergic response. The possibility of synthesizing allergoids or synthetic allergens for use in immunotherapy makes it imperative that a better understanding of the structures of common allergens be pursued.

The results obtained with house dust extracts underline the necessity for characterization of the major allergens in all crude extracts. Our results demonstrated a lack of correlation between the skin test sensitivity and historical data, suggesting that either the skin test was not an effective discriminator of clinically significant house dust sensitivity, that the historical data were not reliable, or both. Identification of the major allergens present in house dust, which differs both quantitatively and qualitatively depending upon the source, could aid in correcting the poor correlation presently shown between history and skin test results. Furthermore, standardization of

crude extracts of any source requires the use of purified, standard reference allergens.

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REFERENCES

1. Berrens, L.: The allergens in house dust, *Prog. Allergy* **14**:259, 1970.
2. Von Brunswijk, J. E. M. H., and Sinka, R. M.: Pyoglyphid mites (Acari) and house dust allergy, *J. ALLERGY* **47**:31, 1971.
3. Wagner, H. C., and Rackeman, F. M.: Kapok: Its importance in clinical allergy, *J. ALLERGY* **7**:224, 1966.
4. Halpin, L. J., and Prince, H. F.: Clinical studies with feather dust, *Ann. Allergy* **21**:577, 1963.
5. Kantor, S. Z., and Wasserman, L.: Studies on the antigenicity of molds and house dust, *Isr. J. Med. Sci.* **2**:534, 1966.
6. Cohen, M. B., Cohen, S., and Hanker, K.: Further observations on the nature of the house dust antigen, *J. ALLERGY* **10**:561, 1939.
7. Bernton, H. S., and Brown, H.: Cockroach allergy. II. The relation of infestation to sensitization, *South. Med. J.* **60**:852, 1967.
8. Bernton, H. S., and Brown, H.: Insect allergy: The allergenic potentials of the cockroach, *South. Med. J.* **62**:1207, 1969.
9. Bernton, H. S., McMahon, T. F., and Brown, H.: Cockroach asthma, *Br. J. Dis. Chest* **66**:61, 1972.
10. Mendoza, J., and Synder, R. D.: Cockroach sensitivity in children with bronchial asthma, *Ann. Allergy* **28**:159, 1970.
11. Schulaner, F. A.: Sensitivity to the cockroach in three groups of allergic children, *Pediatrics* **45**:475, 1970.
12. Coovivathanavanich, P.: Insect allergy: Antigenicity of cockroach and its excrement, *J. Med. Assoc. Thai.* **57**:237, 1974.
13. Kang, F.: Study on cockroach antigens as a probable causative agent in bronchial asthma, *J. ALLERGY CLIN. IMMUNOL.* **58**:357, 1976.
14. Picone, F. J., Strunk, R. C., and Colten, H. R.: Hypersensitivity to cockroach: Purification of the antigens, *J. ALLERGY CLIN. IMMUNOL.* **55**:107, 1975. (Abst.)
15. Cockroaches—Leaflet no. 430, Washington, D. C., U. S. Department of Agriculture, 1965.
16. Weber, K., and Osborn, M.: Reliability of molecular weight determinations by dodecyl sulfate polyacrylamide gel electrophoresis, *J. Biol. Chem.* **244**:4406, 1969.
17. Bunn, H. F., Haney, D. N., Gabbay, K. H., and Gallop, P. M.: Further identification of the nature and linkage of the carbohydrates in hemoglobin A_{1c}, *Biochem. Biophys. Res. Commun.* **67**:103, 1975.
18. Berrens, L., Henocq, E., and Radermecker, M.: Photo-inactivated allergens. I. Preparation, physicochemical, and biological properties, *Clin. Allergy* **3**:449, 1973.
19. May, C. D., Lyman, M., Alberto, R., and Cheng, R.: Procedures for immunochemical study of histamine release from human leukocytes with small volume of blood, *J. ALLERGY* **46**:12, 1970.
20. Varga, J. M., and Ceska, M.: Characterization of allergen extracts by polyacrylamide gel, isoelectro-focusing, and radio-immunosorbent allergen assay. II. Dog and cat allergen, *Int. Arch. Allergy App. Immunol.* **42**:438, 1972.
21. Ohman, J. L., Lowell, F. C., and Bloch, K. J.: Allergens of

- mammalian origin: Characterization of allergen extracted from cat pelts, *J. ALLERGY CLIN. IMMUNOL.* **52**:231, 1972.
22. Ohman, J. L., Lowell, F. C., and Bloch, K. J.: Allergens of mammalian origin. II. Characterization of allergens extracted from rat, mouse, guinea pig, and rabbit pelts, *J. ALLERGY CLIN. IMMUNOL.* **55**:16, 1975.
23. Berrens, L.: Inhalant allergens in human atopic disease: Their chemistry and modes of action, *Ann. N. Y. Acad. Sci.* **221**:183, 1974.
24. King, T. P., and Norman, P. S.: Isolation studies of allergens from ragweed pollen, *Biochemistry* **1**:709, 1962.
25. Johnson, P., and Marsh, D. G.: The isolation and characterization of allergens from the pollen of rye grass (*Lolium perenne*), *Eur. Polymer J.* **1**:63, 1965.
26. Ceska, M., and Brandt, R.: Ultracentrifugation patterns of allergens in sucrose gradients and assessment of their activities by paper disc radioallergosorbent method, *Int. Arch. Allergy Appl. Immunol.* **45**:808, 1973.