

Cockroach-allergen study: Allergen patterns of three common cockroach species probed by allergic sera collected in two cities

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Antigens/allergens of three common cockroach extracts, crude whole body extract of the American cockroach (CRa-A), crude whole body extract of the German cockroach (CRa-G), and crude whole body extract of the Oriental cockroach (CRa-O), were studied with crossed immunoelectrophoresis, crossed radioimmunoelectrophoresis, and Western blot analysis. Sera of cockroach-allergic patients with asthma, 10 from Chicago, Ill. (C group) and six patients from Lexington, Ky. (L group), were used; results were then compared with sera of control subjects with asthma. Qualitative differences in protein bands were noted among CRa-A, CRa-G, and CRa-O by crossed immunoelectrophoresis and sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Allergen bands on Western blot were analyzed for distribution by molecular weight (MW) with relative intensity scores. Results were compared by species and by geography. Two to 12 allergenic bands of variable MW (14 kd to >116 kd) were identified by 13 of 16 individual sera from cockroach-allergic patients from all three extracts. CRa-A demonstrated 55 bands with an intensity score of 125; CRa-G, 58 bands with an intensity score of 100; and CRa-O, 51 bands with an intensity score of 108. Allergenic bands of CRa-A were identified by six sera of the C group and one sera of the L group, whereas bands of both CRa-G and CRa-O were noted by nine sera of the C group and four sera of the L group. All three species had an allergen band in MW range of 40 to 45 kd that reacted to most sera from cockroach-allergic patients with asthma. (J ALLERGY CLIN IMMUNOL 1991;87:1073-80.)

Several studies have supported the clinical importance of cockroach hypersensitivity among inner-city populations with allergic respiratory diseases.¹⁻⁴ Development of cockroach hypersensitivity is closely associated with the rate of cockroach infestations in human habitats.^{5, 6} In North America, the roach species most closely associated with human environment are *Blattella germanica* (German cockroach, CRa-G), *Periplaneta americana* (American cockroach, CRa-A), and *Blatta orientalis* (Oriental cock-

Abbreviations used

WBE:	Whole body extract
AST CR:	Allergy skin test for cockroach
FAST CR:	Fluorescence allergosorbent test for cockroach
Ag:	Antigen
Ab:	Antibody
GM:	Geometric mean
MW:	Molecular weight
CIE:	Crossed immunoelectrophoresis
CRIE:	Crossed radioimmunoelectrophoresis
SDS-PAGE:	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
TBLB:	Tris-barbital-lactate buffer
CRa-A:	Crude whole body extract of the American cockroach
CRa-G:	Crude whole body extract of the German cockroach
CRa-O:	Crude whole body extract of the Oriental cockroach
CRa-M:	Mixture of equal parts of CRa-A, CRa-G, and CRa-O

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TABLE I. Clinical characteristics of subjects whose serum were studied

Name	Age (yr)	Sex	Asthma (yr)	Total IgE (IU/ml)	AST CR	FAST CR	Western blot
Chicago							
G.	18	M	1	873	4	4	4
B.	12	M	6	335	4	4	2
W.	47	F	17	1298	4	4	3
L.	30	F	30	1501	4	3	4
C. W.	25	F	24	771	4	0	0
L. W.	36	F	10	2418	4	4	2
A.	30	M	3	399	4	2	3
T.	27	F	11	475	4	2	3
Bl.	33	F	19	144	4	1	3
H.	20	F	20	677	4	4	2
Mean	27.8 ± 9.9		14.1 ± 9.5	GM 675			
Sm.*	55	F	24	7	0	0	0
Lexington							
S.	22	F	22	92	4	2	1
E.	37	F	37	810	4	2	1
A.	20	F	1	82	4	0	3
W.	34	F	34	216	4	1	3
T.	20	M	19	39	4	0	0
Sn.	37	F	37	172	4	0	0
Mean ± SD	28.3 ± 8.5		20.5 ± 13.8	GM 144			
K.*	66		5	124	0	0	0

*Noncockroach-sensitive subject.

roach, CRa-O).⁷⁻⁹ These cockroaches may infest dwellings predominantly by one species or mixtures of all three species, depending on the geography of the dwellings. Yet, there are no reports on local specific cockroach allergies nor species-specific superiority among the common roach hypersensitivities studied. Empirically, however, single cockroach extract, CRa-G or CRa-A, has been used for diagnostic and research purposes.¹⁰ Some investigators have used mixtures of three common cockroach extracts (CRa-M) for diagnostic and therapeutic purposes with good effect.^{4, 5, 11, 12}

Therefore, this study was undertaken to determine a presence of unique and/or shared allergen(s) in the proteins among three common domiciliary cockroaches. We also intended to detect any geographically distinctive species in allergen pattern probed by patients' sera from two distant cities, Chicago, Ill., and Lexington, Ky.

MATERIAL AND METHODS

Crude cockroach extracts

Cockroach WBEs of CRa-A, CRa-G, and CRa-O, were supplied in a lyophilized form (lots R8768, R8767, and R8766) by the Hollister-Stier Laboratories, (Spokane,

Wash.). CRa-A, CRa-G, and CRa-O were prepared and aliquoted (1 mg/ml [wt/vol]), of which protein contents were 144, 89.5, and 30 µg/ml, respectively.

Serum from cockroach-allergic patients

Sera were collected from 10 cockroach-allergic patients with asthma from Chicago, Ill. (C group) and six patients from Lexington, Ky. (L group) and stored at -20° C until use. Cockroach sensitivity of the patients was defined as having positive AST CR with corresponding clinical symptoms. The method and criteria of these tests were described earlier.^{2, 4, 5} Control sera were obtained from two patients with asthma, one from each city, who revealed negative skin testing to cockroach Ag.

Clinical characteristics of individual subjects with asthma who donated sera are summarized in Table I. Cockroach hypersensitivities tested by AST CR, FAST CR, and Western-blot techniques against cockroach Ag (CRa-M) are also summarized in Table I. Both groups had chronic bronchial asthma of similar intensity and duration. The average age of both groups was 28 years. Patients with asthma from Chicago demonstrated markedly elevated total IgE levels (GM, 675 IU/ml), whereas patients from Lexington were elevated but lower than the levels of patients from Chicago (GM, 144 IU/ml). Eight subjects of Chicago exhibited a FAST CR rating ≥ 2, whereas two of six Lexington subjects exhibited FAST CR ratings of this intensity. Two

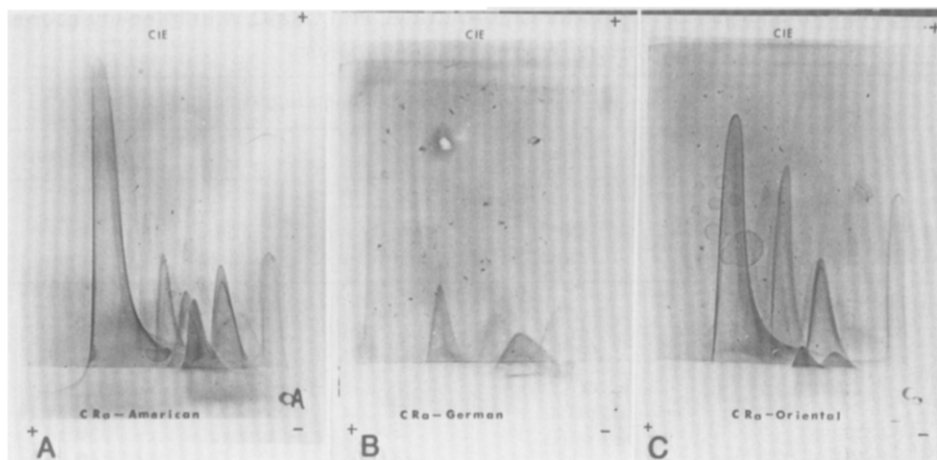


FIG. 1. CIE patterns of the WBE of the three common cockroaches. Gel containing 5 μ l of extract (6 mg/ml wt/vol) reacted with 2.0 ml of anti-CRa-M rabbit antiserum (titer, 1:16). **A**, American. **B**, German. **C**, Oriental.

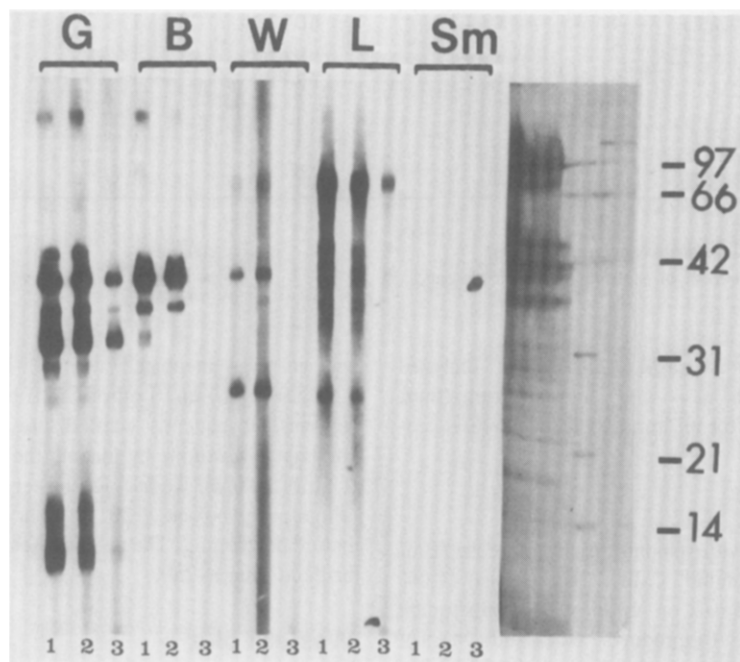


FIG. 2. Western blot of CRA-A probed with sera of subjects with asthma from Chicago. G., B., W., and L. were cockroach-sensitive subjects, and Sm. was a noncockroach-sensitive subject. 1, 2, and 3 of each serum represent serum diluted to 1:5, 1:25, and 1:50, respectively.

sera of control subjects with asthma (one from each city) did not demonstrate any CR-specific Ab by all three testings.

Measurement of total IgE and cockroach-specific IgE assays

FAST CR was used to determine total IgE and cockroach-specific IgE by use of a commercial kit (3M Diagnostic

Systems, Mountain View, Calif.). Cockroach Ag of the FAST CR was WBE of CRA-G.

Antisera

ABs (Rb-anti-CRa-M) were raised in rabbits against equal mixtures of three cockroach Ags as previously described.¹¹ Antisera were pooled and precipitated with 18% and followed by 14% sodium sulfate precipitations. The

TABLE II. Distribution of allergen bands of the CRa-A, Cra-G, and CRa-O with intensity scores depicted by 16 sera from cockroach-allergic subjects by Western-blot analysis

Estimated MW kd (SDS-PAGE)	American cockroach allergen					
	Chicago			Lexington		
	Pos* subject	No. band	Sum† intensity band	Pos subject	No. band	Sum† intensity band
120 ≤ MW		0			0	
105 ≤ MW < 120		0			0	
95 ≤ MW < 105		0			0	
85 ≤ MW < 95		0			0	
75 ≤ MW < 85	2	2	2		0	
70 ≤ MW < 75	5	5	14		0	
65 ≤ MW < 70		0			0	
60 ≤ MW < 65	5	5	13		0	
55 ≤ MW < 60	1	1	1		0	
50 ≤ MW < 55	4	4	8		0	
45 ≤ MW < 50	3	3	5	1	1	1
40 ≤ MW < 45	7	8	19	1	3	6
35 ≤ MW < 40	4	4	10	1	3	7
30 ≤ MW < 35	2	2	6	1	3	6
25 ≤ MW < 30	2	2	3	1	4	9
20 ≤ MW < 25		0			0	
15 ≤ MW < 20	1	1	3	1	1	3
MW < 15	2	2	6	1	1	3

Pos, Positive.

*Number of subjects revealing positive allergen band(s).

†Sum of intensity scores, 1 to 4, of each positive allergen band.

partially purified Rb-anti-CRa-M IgG Ab was dialyzed against TBLB (pH, 8.6, at 4° C). This antiserum (titer, 1:16 to 1:32) was used for all immunoelectrophoresis procedures.

CIE

CIE was performed with standard low-M_r agarose (Bio-Rad Laboratories, Richmond, Calif.). Agarose gel (1.0% in TBLB, pH, 8.6) was cast on an 85 by 100 mm square of gel-bond film (FMC Bioproducts, Rockland, Mass.). Wells were punched and filled with 5 µl of the respective cockroach extracts. Electrophoresis was performed with TBLB for 1 to 2 hours at 10 V/cm. The first-dimension gel was cut from the plate and transferred individually to an 85 by 100 mm gel-bond film. Ten milliliters of 1.0% agarose containing 1.5 ml of Rb-anti-CRa-M was poured onto the plate. The second-dimension electrophoresis was performed with TBLB at 2 V/cm for 18 hours at 4° C.

CRIE

The unstained CIE gel plate was placed in a plastic box. Two milliliters of pooled human serum (1.4 ml of human serum in 4 ml of phosphate-buffered saline) was added and incubated overnight at room temperature. After washings,

the gel was incubated with ¹²⁵I-labeled antihuman IgE (200,000 cpm) in 10 ml of CRIE buffer for 24 hours. The gel was subsequently washed, pressed, and dried. Autoradiography was done by placing the plate on Kodak XRLP-1 film (Eastman Kodak, Rochester, N.Y.) and storing it in a lightproof box at -20° C for a period of 5 and 7 days before developing. The gel was then stained with Coomassie brilliant blue R-250.

Western blot

Proteins were electrophoretically separated on 14% denaturing SDS-PAGE slab gels and transferred to Immobilon (Millipore Corp., New Bedford, Mass.) with a buffer system according to the method described by Bowens et al.¹³ After transfer, SDS-PAGE was stained with Coomassie brilliant blue R-250 to monitor the efficiency of polypeptide removal. The Ag-bound IgE Abs were detected by autoradiography with ¹²⁵I-labeled antihuman IgE Ab.

Statistical analysis

Arithmetic means ± SD was used except for serum total IgE Ab, for which GM was used. Chi-square test was used to compare Western-blot analysis.

German cockroach allergen						Oriental cockroach allergen					
Chicago			Lexington			Chicago			Lexington		
Pos subject	No. band	Sum† intensity band	Pos subject	No. band	Sum† intensity band	Pos subject	No. band	Sum† intensity band	Pos subject	No. band	Sum† intensity band
	0			0			0			0	
	0			0		3	3	5	1	1	1
2	2	2	1	1	1	4	4	8	2	2	5
5	5	11	2	2	2		0			0	
	0			0		1	1	3		0	
	0			0		1	1	3	1	1	2
1	1	2	1	1	2		0			0	
2	2	2	2	2	4	1	1	2		0	
3	3	5	2	2	3	2	2	3	2	2	3
2	2	2	3	4	4		0			0	
2	2	4	1	1	2		0		2	2	2
3	3	8	3	3	6	8	9	21	3	5	12
	0		2	3	5	3	4	4	2	4	4
4	5	10	1	2	2	2	5	9	2	4	4
5	6	13	1	2	4	3	4	9	2	2	3
	0			0			0			0	
2	2	4	1	1	1	2	2	3	1	2	2
1	1	1		0			0			0	

RESULTS

Specificity among three extracts by CIE and SDS-PAGE

Precipitin patterns on the CIE plates were different among CRa-A, CRa-G, and CRa-O (Fig. 1). CRa-A contained at least 16 to 19 clear arcs; CRa-G, 9 to 14 arcs; and CRa-O, 14 to 18 arcs, documented by CIE. CRIE with pooled serum from three cockroach-allergic subjects also demonstrated different allergen patterns as well among the three species (data not presented). SDS-PAGE revealed that the three extracts contained proteins of variable MW, diffusely scattered, ranging from <14 kd to 220 kd, in addition to gross differences in amounts. Equal weight per volume (1 mg/ml) of CRa-A, CRa-G, and CRa-O were used that contained 144, 89.5, and 30 µg of proteins, respectively.

Specificity in allergens by Western-blot analysis

Western-blot analyses generally revealed multiple allergen bands diffusely scattered through a wide range of MWs, which was determined best at a serum dilution of 1:25 (Fig. 2), and also demonstrated that

individual allergic sera identified significant allergen bands located at different MWs of CRa-A (Fig. 2, *G.* and *B.* versus *L.*). One of strong allergen bands of CRa-A (MW, 33 kd), which demonstrated intense radioactivity by serum from *G.* (Fig. 3), was recognized by only two of 16 sera (Table II). Furthermore, numbers of allergenic bands as well as intensity of each band from different extracts also varied widely.

In analyzing Western blot, we counted numbers of allergic sera identifying positive bands in the MW range of 5 kd. We also arbitrarily designated an intensity score, 1 to 4, for each allergen band to identify significant allergen band(s) and compare its relative intensity. The distributions of allergenic proteins of the three cockroach extracts identified by 16 individual sera are summarized in Table II. In CRa-A, a total of 55 bands were detected in 10 of 16 sera tested with a total intensity score of 125. A major common allergen of CRa-A, found in eight of 16 sera tested, was a protein band in MW range of 40 to 45 kd. This allergen band was noted from CRa-G (6/16) and from CRa-O (11/16).

CRa-G exhibited more diffusely scattered allergens. A total of 58 allergen bands were recorded in 13 of

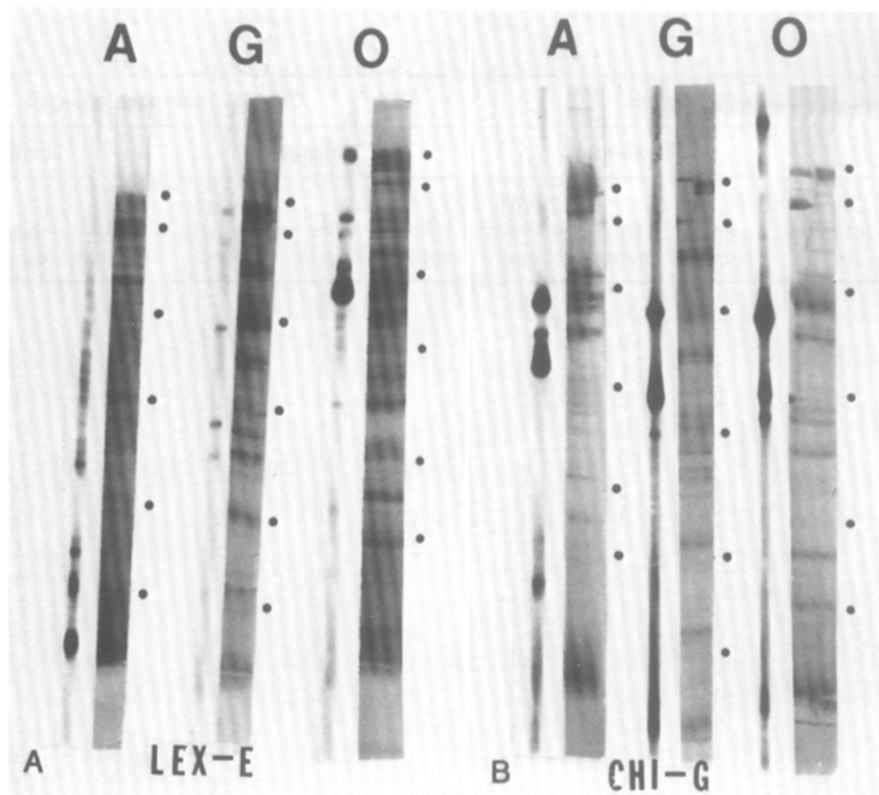


FIG. 3. **A**, Western-blot analysis probed on three cockroach extracts with a cockroach-sensitive serum (*E*) from Lexington, Ky. *A*, *G*, and *O* indicate respective pairs of a strip of Western blot (*left*) alongside protein separation on SDS-PAGE (*right*) of CRa-A, CRa-G, and CRa-O, respectively. Black dots on each column are molecular markers used, starting from top to bottom: 97, 66, 42, 31, and 14 kd, respectively. **B**, Western blot analysis probed on three cockroach extracts with a serum (*G*) (*B*, case 1) from Chicago, Ill. Arrangement of data is the same as in **A**.

16 of the sera with a total intensity score of 100. Two common CRa-G allergens were found: one in MW range of 85 to 95 kd in seven sera, and another CRa-G allergen in MW range of 30 to 35 kd in six of the 16 sera tested.

Allergenic proteins in CRa-O totaled 51 bands recorded in 13 of 16 sera with 108 intensity scores. One major common allergen band in CRa-O was a band located in MW range of 40 to 45 kd detected in 11 of 16 sera tested. A second strong CRa-O allergen band was located in MW range of 95 to 105 kd noted in six of 16 sera.

An allergenic band of CRa-A (MW, 70 to 75 kd) demonstrated a score of 14, but was invisible in CRa-G, and scored only 5 in CRa-O. An allergenic protein band of CRa-G (MW, 85 to 90 kd) demonstrated a score of 13 but was not visible in CRa-A. Similarly, a strong allergen band noted on CRa-O, with an intensity score of 13, was in a protein MW 105 to 120 kd, but 0 and 3 in CRa-A and CRa-G, respectively.

The strongest allergenic band, the most intensive binding of antihuman IgE depicted, also varied among species (Fig. 3, *A* and *B*). Serum *E*, case 2 of the L group, identified a strong allergen band (MW, <14 kd) plus several weak allergen bands of variable intensity from CRa-A. This serum, however, depicted several weak bands from CRa-G and a strong allergen band of MW 39 kd from CRa-O plus an allergen band MW, 97 kd (Fig. 3, *A*).

Geographic influence on the allergens of CRa-A, CRa-G, and CRa-O

Incidence of cockroach hypersensitivity was low among patients with asthma from Lexington, and generally the reactivity was not intense (Table I). Although cockroach sensitivity was recognized by positive AST CR, FAST CR, and Western blot, sera of the L group revealed less reactivity, if not negative, than those of the C group.

Intensity and numbers of allergen bands on Western blot were greater with sera of the C group in all three

extracts (Fig. 3, A versus B). This finding appeared to be related to elevated IgE levels and FAST CR ratings for the patients.

Western blot of CRa-A identified a total of 39 bands by sera from the C group with an intensity score of 90, whereas sera from the L group depicted 16 allergen bands with an intensity score of 35 from CRa-A ($p < 0.001$). Seven individual sera of the C group depicted strong and multiple allergens in CRa-A scattered through MW range from <14 to 85 kd level, whereas one of six sera of the L group demonstrated several weak allergens of low MW (<14 to 50 kd) (Table II). Sera of the C group recognized 34 bands with an intensity score of 64 from CRa-G, whereas sera of the L group depicted 24 bands with an intensity score of 36. Sera of the C group identified 36 bands with a total score of 70 from CRa-O, whereas sera of the L group identified 25 bands with an intensity score of 38. Nine of 10 sera from the C group recognized two to many allergen bands from CRa-G, whereas four of six sera from the L group identified allergens.

DISCUSSION

This study analyzed and compared Ags and allergens of the three most common cockroaches. Differences were noted in the contents of protein, Ags, and allergens among CRa-A, CRa-G, and CRa-O, detected by SDS-PAGE, CIE, and by CRIE. CIE of the CRa-G in this study revealed up to 14 precipitins with the use of hyperimmune Rb-anti-CRa-M, of which CRIE demonstrated 15 allergen arcs. Stankus and O'Neal¹⁴ reported up to 56 precipitin bands in CRa-G using hyperimmune rabbit serum immunized solely against CRa-G. Yet, they found a few allergen arcs by CRIE in their study. Jones et al.¹¹ detected up to 22 precipitin bands using Rb-anti-CRa-M. Although the significance of the number of precipitin bands in WBE of cockroaches is not clearly understood, yet, it is in agreement with the fact that a few of these selected precipitins were binding human IgE Abs. This study further verified the fact that allergens of the cockroach extracts were not necessarily the fraction of high-quantity proteins of any of three cockroach extracts, as previously reported.¹¹

The present study revealed clearly that allergenic fractions in cockroach extracts are numerous and are distributed throughout a wide range of MWs. Furthermore, the depicted fractions vary by individual sera as well as by the probed WBE of the roach species. A similar result was noted in a study of CRa-G by Baldo and Panzani.¹⁵ Yet, a comparison of three species has not been reported in the literature. We have not clarified that an autoradiographic band noted in Western blot is in fact a defined allergen as yet.

However, our recent work on CRIE of cockroach extract (CRa-M) suggested that the allergenic band(s) may probably be the allergenic protein(s).¹⁶

This study identified many discrete allergens from the three WBE by a parallel Western blot. However, there was one allergen band (MW, 40 to 45 kd) that was recognized by most individual sera, regardless of their locale. This allergen can be considered as a probable shared allergen among the three extracts. This allergen may be the allergenic fraction described in fraction 2 of American roach WBE and German roach WBE previously reported.¹⁴ This allergen band also appears to be in close proximity of MW to one of the allergens detected in CR-M2 of CRa-M.¹⁶ Further study is necessary to determine if this is indeed a shared allergen, which could be a major allergen that can be used for standardization of commercial cockroach extracts.

Our study also indicates that there may be one or two single extract-specific allergens that include bands in CRa-A of MW 35 to 40 kd and MW 70 to 75 kd, a band in CRa-G of MW 85 to 90 kd, and a band in CRa-O of MW 105 to 120 kd. The CRa-G specific allergen of MW 36 kd, described by Helm et al.,¹⁷ might be one of the two highly active bands that we described in the CRa-G (Table II).

Geography of two cities, Chicago, Ill., and Lexington, Ky., apparently influenced cockroach allergies in two aspects, as reported by Yun et al.¹⁸: (1) Subjects from Lexington with asthma had a lower incidence of cockroach allergy than subjects with asthma from Chicago. (2) Sera of the L group demonstrated significantly lower CRa-A allergy compared to CRa-G or CRa-O. The explanation(s) of both aspects, however, is not clear at present. According to an entomologist consulted, both cities are probably infested with all three cockroaches.

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Effective site of bronchodilation by antiasthma drugs in subjects with asthma

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*We studied the effective sites of airway response to atropine and fenoterol aerosols and to the intravenous injection of aminophylline in patients with stable and spontaneous asthma, by the simultaneous assessment of respiratory resistance (Rrs) and anatomic dead space (V_D). Central airway response was determined by V_D, and overall response was determined by Rrs. Peripheral airway response was inferred from Rrs when the change in V_D was slight. Atropine (4 mg/ml) or fenoterol (0.4 mg/ml) was continuously inhaled during tidal breathing for 5 minutes. Inhalation of both atropine and fenoterol increased Grs (reciprocal of Rrs) ($p < 0.01$) with a simultaneous increase in V_D ($p < 0.01$) in the patients with stable and spontaneous asthma. Fenoterol increased Grs more than did atropine at an equivalent increase in V_D in patients with spontaneous asthma ($p < 0.05$). Intravenous injection of aminophylline (250 mg) had no effect on either Grs or V_D in patients with stable asthma, but it significantly increased Grs ($p < 0.01$) without change in V_D in patients with spontaneous asthma. These results suggest that the predominant sites of bronchodilation induced by inhaled atropine are the central airways, that those sites induced by intravenous injection of aminophylline are the peripheral airways, and that inhaled fenoterol dilates both the central and peripheral airways in subjects with asthma. Differences among clinically used bronchodilators on the effective sites may be considered in the treatment of bronchial asthma. (*J ALLERGY CLIN IMMUNOL* 1991;87:1080-7.)*

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Abbreviations used

- Rrs: Respiratory resistance
- Grs: Respiratory conductance
- V_D: Anatomic dead space
- VC: Vital capacity
- FRC: Functional residual capacity
- V_T: Tidal volume
- f: Respiratory frequency