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Occupational allergy to locusts: An investigation of the sources of the allergen

R. D. Tee, PhD, D. J. Gordon, MSc, E. R. Hawkins, SRN, A. J. Nunn, MSc,*
J. Lacey, PhD,** K. M. Venables, MD, MSc, MRCP, R. J. Cooter, PhD,***
A. R. McCaffery, PhD,*** and A. J. Newman Taylor, MSc, FRCP, MFOM
London and Herts, England

Allergic symptoms occur commonly in subjects working closely with locusts and are associated with specific IgE antibody. Extracts of intact locusts (Schistocerca gregaria and Locusta migratoria) were used to identify specific IgE antibody, to define the major allergens of the locust and their sources, and to estimate aeroallergen concentration in the working environment. With questionnaire, skin prick tests, and specific IgE measurements, 35 individuals, working in a research center, were surveyed. Of the 15 currently exposed individuals, contact with locusts provoked asthma, rhinitis, and contact urticaria in five, rhinitis and urticaria in three, and rhinitis alone in one individual. Symptoms provoked by locusts and skin test reactions to locust extracts were associated with specific IgE antibody in the serum. The "immunoblot" technique demonstrated the presence of multiple allergens in the locust extracts of approximately 68, 66, 54, 43, 37, 29, and 18 K daltons molecular weight. Locust antigen was identified in the atmosphere by means of an immunochemical method involving elution of high-volume air-sampler filters exposed in the locust breeding room and analysis of eluate allergen content by RAST-inhibition assays. Logit transformation of RAST-inhibition lines demonstrated that the filter extract shared a common slope with the locust extract and with an extract of locust gut. This gut extract also shared a common slope with extracts of locust feces and peritrophic membrane. The major source of allergen appears to be the peritrophic membrane that is present in the gut and is excreted surrounding the feces. (J ALLERGY CLIN IMMUNOL 1988;81:517-25.)

Insect dust is a major source of allergens in occupational inhalant allergies. Orthopterans, which include the locust and the cockroach, are an important occupational hazard because of the potency of the

Abbreviation used

SDS-PAGE: Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

From the Department of Occupational Medicine, Cardiothoracic Institute and *Medical Research Council Tuberculosis and Chest Diseases Unit, Brompton Hospital, London, **Plant Pathology Department, Rothamsted Experimental Station, Harpenden, Herts, England, and ***Tropical Development and Research Institute, London, England.

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Reprint requests: R. D. Tee, PhD, Department of Occupational Medicine, Cardiothoracic Institute, Brompton Hospital, Fulham Road, London SW3 6HP, England.

allergens from this order and the severity of symptoms that they cause.¹ Very high sensitivities to locust extracts were reported by Fuchs and Gronemeyer² with positive skin responses observed at dilutions of 10⁻⁹. Bellas³ in his bibliography, *Insects as a Cause of Inhalant Allergies*, cites studies of allergy to locusts from the United Kingdom,^{4,6} Germany,⁷ France,^{8,9}

Denmark,⁴ Pakistan,¹⁰ and from the United States.¹¹ Frankland⁴ was the first to describe the importance of locust allergy when he reported that, of 34 laboratory workers, some developed an irritation of the skin and four developed allergic rhinitis and asthma when they were in close contact with *S. gregaria* and *L. migratoria*, two species that we have studied. Skin tests suggested feces to be a potent source of locust allergen.

Burge et al.⁶ made a prevalence study of 117 research workers at a center breeding locusts, and of workers handling the locusts, one fourth had work-related wheeze or breathlessness, and one third had work-related rhinitis and urticaria. Atopic workers who handled the locusts appeared to develop occupational asthma more frequently and more rapidly than similarly exposed nonatopic workers. Specific locust IgG and IgE antibodies of the workers were measured by ELISA, but whereas both types of antibody were found in workers with work-related asthma, only specific IgG antibodies were raised in workers with work-related rhinitis. Edge and Burge,¹² using immunofluorescent staining, provided evidence suggesting the source of the antigen might be the peritrophic membrane that lines the gut and surrounds feces.

We have had the opportunity to restudy some of the same work force investigated by Burge et al.⁶ and have been able to overcome the lack of specificity of the immunologic tests found previously. Of prime importance is the preparation of the locust antigen extracts. In order to produce a more specific antigen, the locust parts were not homogenized before extraction. This resulted in less cross-reactivity being found with house dust mite (*Dermatophagoides pteronyssinus*) and cockroach (*Blattella germanica*) antigens and allowed the development of a reliable method for measuring specific IgE antibodies to locusts.

MATERIAL AND METHODS

Study group

Thirty-five research center workers were studied by questionnaire and specific locust skin prick tests and IgE antibody measurements. Questionnaire-derived diagnoses were based on the development of allergic symptoms (asthma, rhinitis, and urticaria) associated with exposure to locusts, which occurred after a latent period and improved at periods away from work. The mean exposure duration of the 15 currently exposed workers was 8.2 years. Of the 20 not currently exposed workers, duration was 3.8 years. Locust contact among those "currently exposed" ranged from working near the locusts, through experimental work with locusts, to general care of them. Nine workers, all currently exposed, had locust-related symptoms. Five workers had bronchial asthma, allergic rhinitis, and urticaria. An additional three workers had rhinitis and urticaria, and one worker had rhi-

nititis alone. These questionnaire categories were confirmed by clinical interview. No subject who was not currently exposed had locust-related allergic symptoms.

Antigens

Antigens used were (1) adult *S. gregaria*-intact (non-homogenized) bodies, (2) adult *L. migratoria*-intact (non-homogenized) bodies, (3) adult *S. gregaria* gut, (4) adult *L. migratoria* gut, (5) *S. gregaria* peritrophic membrane from the midgut dissected out and washed in diethyl ether, (6) *S. gregaria* and *L. migratoria* mixed feces, (7) *D. pteronyssinus* lyophilized, dialyzed whole culture mite extract (Dome Hollister-Stier Miles Laboratories, Bridgend, U.K.), and (8) Chitin (poly-*N*-acetylglucosamine) (Sigma Chemical Co., C3641, Poole, Dorset, U.K.).

Preparation of extracts

To prepare "intact" locust body antigens, locusts were killed by freezing them at -80°C . No dissection or homogenization was performed. All locust antigens were defatted in three changes of ether during 48 hours, allowed to dry in the air, and were extracted for 16 hours in 10 vol of Coca's solution (5 gm of sodium chloride, 2.75 gm of sodium bicarbonate, and 5 gm of phenol made up to 1 L with distilled water). The extracts were filtered through Whatman (Whatman Ltd., Maidstone, England) paper No. 1, followed by Sartorius (Sartorius, Gottingen, West Germany) glass fiber prefilters, concentrated and ultrafiltered in an Amicon CH2A (Amicon Ltd., Gloucestershire, U.K.) hollow fiber system, and lyophilized.

Skin prick testing

Skin prick test solutions of the six lyophilized locust antigens were prepared in 50% Coca's solution and 50% glycerol at 1 mg/ml concentration and passed through a ≥ 0.22 mm Millex (Millipore [U.K.] Ltd., Harrow, Middlesex, U.K.) GS filter. This diluent and histamine dihydrogen chloride at 1 mg/ml were used as negative and positive control solutions, respectively. All 35 subjects had skin prick tests with the six locust extracts and also with commercial extracts (Bencard, Brentford, Middlesex, U.K.) of B2 grass pollens (4100), *D. pteronyssinus* (2801), and cat fur (3204). We defined atopy as the presence of a positive skin prick test (>2 mm wheal) to one or more of these three commercial extracts.

Human sera

All 35 subjects in the study were bled for antibody studies.

Environmental monitoring

A Staplex high-volume air sampler (Staplex Co., Brooklyn, N.Y.) was used to sample the atmosphere of the locust rearing room for particulate allergens during 72 hours. This sampler maintains an airflow of 1.4 m³/min and is estimated to retain 95% of particles >0.3 μm on a fiberglass filter sheet, 22.7 by 17.7 cm². Air was similarly sampled in a laboratory, free of locusts, as a control filter sample.

TABLE I. Relationship between locust skin prick test response, exposure, and symptoms in the 35 research center workers

		Skin prick test		Total
		+	-	
Currently exposed	+	9	0	9
	-	1	5	6
Currently unexposed	+	0	0	0
	-	2	18	20
Total		12	23	35

Elution of filter sheet

Soluble material was eluted from each filter sheet by descending chromatography with a borate buffer (sodium citrate 0.034 mol/L, and sodium borate, 0.0325 mol/L in 0.16 mol/L of sodium chloride/distilled water, pH 8.2). The eluates collected after 8 hours were dialyzed against distilled water and lyophilized.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

A resolving 15% SDS-PAGE gel with a 3% acrylamide upper stacking gel was cast according to the method of Laemmli,¹³ in a vertical Protean slab cell (Bio-Rad, Laboratories, Watford, Herts, U.K.). Lyophilized samples of different locust extracts were solubilized in 0.5 mol/L of Tris HCl buffer, pH 6.8, containing 3% SDS at 60° C for 30 minutes. The prepared samples and standard molecular weight protein solutions (Pharmacia, Uppsala, Sweden), solubilized at 100° C for 5 minutes, were electrophoresed in duplicate at 18 mA for 1 hour, followed by 4 hours at 30 mA. The gel was cut in half lengthwise. One half was processed by the immunoblot technique, and the duplicate half was fixed in 25% propan-2-ol:10% acetic acid for 1 hour, followed by staining in 0.1% Coomassie blue R250 for 16 hours, and then destaining in 8% acetic acid.

Immunoblot

Proteins from the duplicate half SDS gel were electrophoretically transferred onto a nitrocellulose sheet as described by Towbin et al.¹⁴ A Trans-Blot apparatus (Bio-Rad) was used, and the transfer was carried out at 0.1 A overnight in 25 mmol/L of Tris, 192 mmol/L of glycine buffer, pH 8.3, containing 20% wt/vol of methanol. Any additional protein binding sites were blocked by immersing the nitrocellulose sheet in 2% bovine serum albumin in phosphate-buffered saline for 1 hour at 39° C. After washing, the sheet was processed for autoradiography.

Autoradiography

A 1:5 dilution of pooled sera from five patients hypersensitive to locusts was allowed to react over the nitrocellulose sheet for 16 hours. After washing, this was followed by a further 16-hour incubation with the tracer ¹²⁵I-labeled anti-IgE (Pharmacia) (100 µl contained approximately

10,000 cpm). The washed and dried sheet was exposed to X-OMAT L Kodak (Kodak Ltd., Hemel Hempstead, Herts, U.K.) film with Dupont (Dupont Ltd., Stevenage, Herts, England) Quanta III intensifying screens at -70° C for 30 hours and was then developed. A negative control sheet was processed in parallel where sera from nonsensitive individuals to locusts replaced sera from allergic subjects.

Isoelectric focusing

LKB (LKB Instruments, Croydon, Surrey, U.K.) polyacrylamide gel plates, pH 3.5 to 9.5, were used according to the instructions. Twenty microliters of locust samples at 10 mg/ml were focused in parallel with 10 µl of low isoelectric point protein markers (pH 2.5 to 6.5, Pharmacia), and the plate was stained for protein with 0.1% Coomassie blue R250.

Serum antibody studies

A mixture of intact *S. gregaria* and *L. migratoria* extracts was used in the RAST studies since (1) exposure was to both these locust species, and (2) evidence from the skin prick testing demonstrated that most sensitized subjects reacted to all six locust antigens. A mixture of intact *S. gregaria* and *L. migratoria* was therefore representative of the hypersensitivity response.

RAST: Preparation of allergen polymer complex

Ten milligrams of lyophilized extracts of intact locust (*S. gregaria* and *L. migratoria* in equal quantities) was coupled per gram of CNBr-activated Sepharose 4B (Pharmacia).

Assay

Optimum conditions for the assay were established with two 16-hour incubations with 50 µl of serum and 100 µl of allergen polymer complex (6.25% concentration) in the first and 50 µl ¹²⁵I-labeled anti-IgE (Pharmacia) in the second incubation. To investigate the specificity of the IgE antibody response to locusts, 10 sera from patients hypersensitive to locusts were additionally assayed by RAST to the house dust mite, *D. pteronyssinus* (lyophilized extract coupled to CNBr-activated paper disks), and to the cockroach *Blattella germanica* (Pharmacia disks). Cord blood sera with no de-

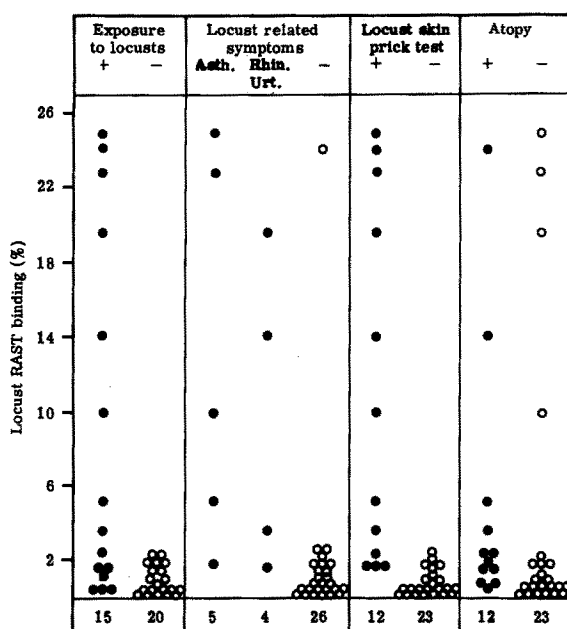


FIG. 1. Intact locust RAST ^{125} I-labeled anti-IgE binding in sera from 35 research center workers in relation to their exposure, symptoms, specific skin prick test response, and atopic status.

monstrable IgE (total) were used in all assays as negative controls. The Phadebas RAST birch reference system (Pharmacia Diagnostics AB) was included in the assays to equate the RAST binding in the disk assays with other centers and to enable an estimate in the locust Sepharose assay (see Table II for results).

RAST inhibition with sera from patients hypersensitive to locusts

Intact locust RAST-inhibition studies were performed by preincubating 100 μ l of the optimum dilution of a positive serum pool (six sera) for 16 hours with 100 μ l of the following concentrations of seven unfractionated antigens:

1. Intact *S. gregaria* and *L. migratoria* extract 0.001, 0.01, 0.1, 1.0, 5.0, and 10.0 mg/ml
2. *S. gregaria* and *L. migratoria* gut extract 0.01, 0.1, 1.0, 2.5, and 5.0 mg/ml
3. Peritrophic membrane 0.5, 1.0, 2.5, 3.0, and 4.0 mg/ml
4. *S. gregaria* and *L. migratoria* feces 0.1, 1.0, 5.0, 10.0, and 20.0 mg/ml
5. High-volume air-sampler filter extract 0.05, 0.195, 0.78, 3.125, 6.25, 12.5, 25, 50, and 100 mg/ml
6. *D. pteronyssinus* 0.1, 5.0, 8.0, and 10.0 mg/ml
7. Chitin 0.1, 1.0, 10.0, 25.0, and 50.0 mg/ml

The mixed-species, intact-locust RAST was performed, and percentage binding of the ^{125} I-labeled anti-IgE was compared with that of the unabsorbed serum pool.

RAST-inhibition analysis

A logit transformation $\log_e(p/[1-p])$, of the percentage inhibition of the locust RAST assays was plotted against log concentration of inhibitor, the regression lines obtained were analyzed for parallelism, and a common slope was fitted where it was possible, as described previously.¹⁵

Locust-gut RAST and RAST-inhibition assays

To elucidate further the main source of the locust allergens, a gut RAST was established (equal quantities of lyophilized *S. gregaria* and *L. migratoria* gut extracts were coupled to activated Sepharose 4B), and inhibition studies were similarly performed with the unfractionated antigen inhibitors, 1 to 4, previously listed, that is, intact locust, gut, peritrophic membrane, and feces.

RESULTS

Skin prick testing

Immediate skin prick test response to at least one of the six locust extracts was found in all nine workers with allergic symptoms (all of whom were exposed) but in only three of the 26 without symptoms ($p < 0.001$, Fisher's exact test) (Table I). Of these 12 workers with positive locust skin prick tests, six were positive to all six locust antigens, and four workers were positive to all but intact *L. migratoria* extract. Three workers were not tested with the feces extract; two of these workers were positive to the other five antigens, and the third worker was one of the four workers not reacting to intact *L. migratoria* extract. There was a significant association between the immediate skin prick test response to locust extract and exposure ($p < 0.002$), but not between a positive locust skin prick test and atopy, as defined by skin tests with common aeroallergens ($p < 0.1$). In this population, the sensitivity of the skin tests in relation (1) to symptoms is 100% and (2) to current exposure is 67%, with specificities of 88% and 90%, respectively.

All 10 locust-exposed subjects with a positive skin prick test response to intact locust also had a positive skin prick test to gut, peritrophic membrane, and fecal extracts. Ten (28.6%) of the 35 study subjects had a positive skin prick test to *D. pteronyssinus* and eight (22.9%), to grass pollen and cat fur. Six subjects with a positive skin prick test response to locust extracts had a negative skin prick test to *D. pteronyssinus*, and five with a positive skin prick test to *D. pteronyssinus* had a negative skin test to locusts.

RAST

There was good differentiation between levels of specific intact locust IgE antibody (Fig. 1). Workers with a positive skin prick test response to one or more of the six locust extracts are defined as locust skin prick test positive. None of the workers not currently

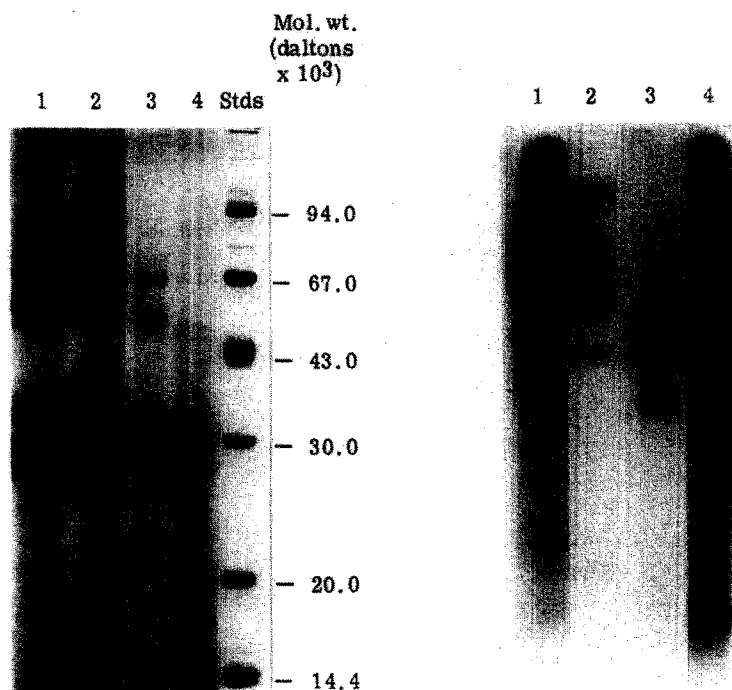


FIG. 2. Fifteen percent acrylamide SDS-PAGE gel stained for protein and respective autoradiograph of immunoblot with ^{125}I -labeled anti-IgE. 1, locust feces; 2, peritrophic membrane; 3, locust gut; and 4, intact locust.

exposed to locusts or with a negative skin prick test had a RAST binding of $>2.5\%$, and only one exposed individual, without symptoms, had a high specific IgE level. All nine of the symptomatic patients had a Phadebas (Pharmacia Diagnostics, Uppsala, Sweden.) RAST class of one or higher. Cord blood sera, with total IgE antibody content of 0.5 IU, had a locust RAST binding of 0.4%. A gradation of antibody levels was found in the study group, lowest in workers currently unexposed with a negative skin prick test (0.77% mean RAST binding) and slightly higher in workers currently exposed with a negative skin test (0.97% mean binding). Workers currently unexposed who had a positive skin prick test to locust extracts had higher RAST binding (2.1% mean binding), whereas workers currently exposed with a positive skin prick test had the highest mean binding (12.7%).

RAST-binding values were significantly different (Wilcoxon rank-sum test) between (1) exposed versus unexposed ($p < 0.01$), (2) symptomatic versus asymptomatic ($p < 0.05$), (3) skin prick test positive versus negative ($p < 0.001$), and (4) atopic versus nonatopic ($p < 0.05$). There was no significant relationship between symptoms and atopy.

The sensitivity of the RAST in relation (1) to symptoms is 100% and (2) to current exposure is 73%, with specificities of 73% and 50%, respectively.

Antigens

The SDS-PAGE gel stained with Coomassie blue and the immunoblot autoradiograph from the duplicate half of the gel are illustrated in Fig. 2. Multiple protein bands were observed in all four of the nonreduced locust extracts. The main common bands occurred at 68, 66, 56, 37, 33, 29, and 18 K daltons molecular weight. By referring to the known molecular weight standards in the stained half of the gel, the size of the strongest allergen bands of the locust extracts in the autoradiograph were found to be 68, 66, 54, 43, 37, 29, and 18 K daltons molecular weight. Negligible uptake of the isotope occurred in the negative control blot, where sera from individuals from the study not sensitive to locusts replaced the allergic sera.

Isoelectric focusing

Both the intact locust and gut extracts elicited seven protein-stained bands, ranging between isoelectric point 3.7 and 6.1, namely, acidic proteins.

Comparison of intact locust RAST (*S. gregaria* and *L. migratoria*) with those of house dust mite (*D. pteronyssinus*) and cockroach (*B. germanica*)

The RAST assay of 10 locust RAST positive sera to *D. pteronyssinus* and *B. germanica* are presented

TABLE II. Comparison of RAST (percent isotope binding and Phadebas class) to *S. gregaria*/*L. migratoria*, *D. pteronyssinus*, and *B. germanica* in 10 patients hypersensitive to locusts

Serum	RAST					
	<i>S. gregaria</i> / <i>L. migratoria</i>		<i>D. pteronyssinus</i>		<i>B. germanica</i>	
	% Binding	Phadebas class	% Binding	Phadebas class	% Binding	Phadebas class
a	21.1	3	6.7	2	8.5	2
b	2.1	1	21.0	3	0.7	0
c	27.5	3	0.4	0	1.4	0
d	11.1	3	5.9	2	0.9	0
e	8.8	2	0.4	0	1.6	0
f	18.6	3	0.5	0	0.6	0
g	18.2	3	0.6	0	0.8	0
h	3.8	2	28.9	4	1.0	0
i	10.0	2	0.5	0	2.6	1
j	3.2	1	37.0	4	1.1	0
Cord blood	0.4	0	0.4	0	0.4	0

Phadebas RAST class: 4, 3, 2, 1, and 0 = level of specific IgE antibody: very high, high, moderate, low, and absent or undetectable, respectively. Cord blood RAST indicates background percentage isotope binding in all three assays.

in Table II. Sera, c, f, and g, having a very strong locust RAST, had negative (one low positive) RAST binding to both house dust mite and cockroach. Furthermore, sera b, h, and j had very high *D. pteronyssinus* RAST binding but very much lower locust RAST binding (2.1%, 3.8%, and 3.2%) and low cockroach RAST binding (0.7%, 1.0%, and 1.1%). Absence of specific IgE antibody to *D. pteronyssinus* in five and to *B. germanica* in eight of the 10 sera containing specific IgE to locust suggests that RAST binding to locust is not a reflection of an immunologic response to antigens present in *D. pteronyssinus* and *B. germanica*. Specificity of the locust RAST in relation to *D. pteronyssinus* was confirmed by the low inhibition of *D. pteronyssinus* (22% at 10 mg/ml).

Intact locust RAST inhibition

Preincubation of a locust RAST positive serum pool with increasing concentrations of lyophilized intact locust, gut, peritrophic membrane, and fecal extracts inhibited the mixed species intact locust RAST in a dose-dependent fashion (Fig. 3). An extract from the high-volume air-sampler filter exposed in the locust breeding room similarly inhibited this RAST, but a filter extract from a laboratory free of locusts elicited zero inhibition. There was limited inhibition by the *D. pteronyssinus* extract (22% inhibition at 10 mg/ml) but no inhibition with chitin, a possible allergen source, even at 50 mg/ml.

RAST-inhibition analysis

Logit transformation of the RAST-inhibition curves of the four locust antigens and antigens of the filter extract and *D. pteronyssinus* are illustrated in Fig. 4. To aid clarity, only two points per line are illustrated. The slopes obtained with these regression lines were 1.11, peritrophic membrane; 1.01, feces; 0.73, gut; 0.61, intact locust; 0.48, air filter; and 0.33, *D. pteronyssinus*.

A common slope could be fitted to the lines for intact locust, filter extract, and mixed gut extract (Fig. 5), implying that the filter extract contained allergens with immunologic identity with the intact locust and gut extracts. The concentration of inhibitor required to obtain 50% RAST inhibition was 120 μ g/ml for the intact locust, 170 μ g/ml for the gut extract, and 4.4 mg/ml for the filter extract. Thus, the potency of the intact locust extract was only 1.4 times that of the gut extract, but nearly fortyfold more potent than the air-filter extract.

The slope of the line for gut extract was also not significantly different from the line for feces and peritrophic membrane extracts (Fig. 6). Here, the concentration of inhibitor required to obtain 50% inhibition of intact locust RAST was 180 μ g/ml of gut extract, 840 μ g/ml of peritrophic membrane extract, and 900 μ g/ml of fecal extract. The potency of the peritrophic membrane and fecal extracts was therefore very similar and approximately one fifth of the gut extract.

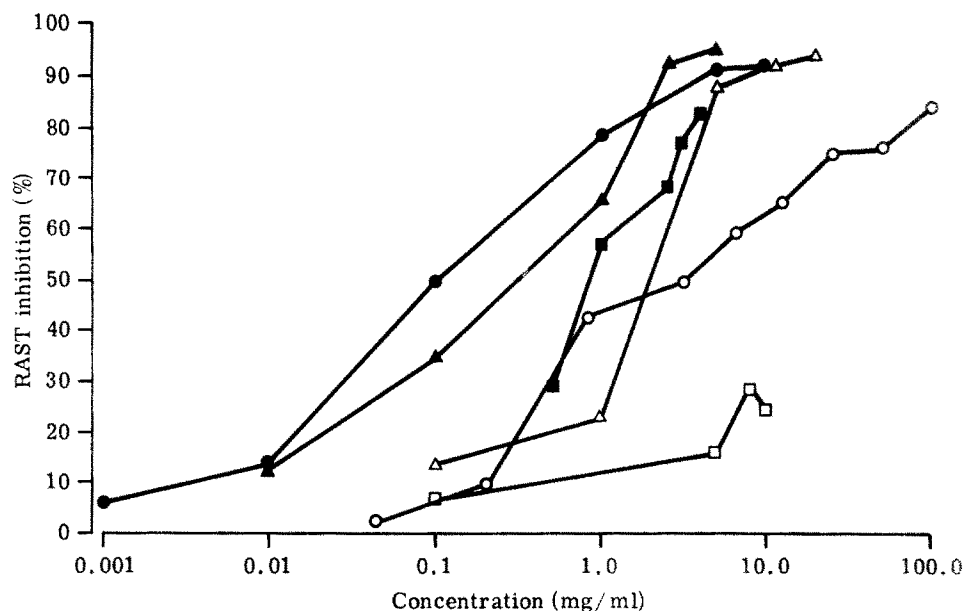


FIG. 3. Inhibition of intact locust RAST with serum pool from six patients hypersensitive to locusts with increasing concentrations of intact locust (●), gut (▲), peritrophic membrane (■), feces (△), locust room filter extract (○), and *D. pteronyssinus* (□).

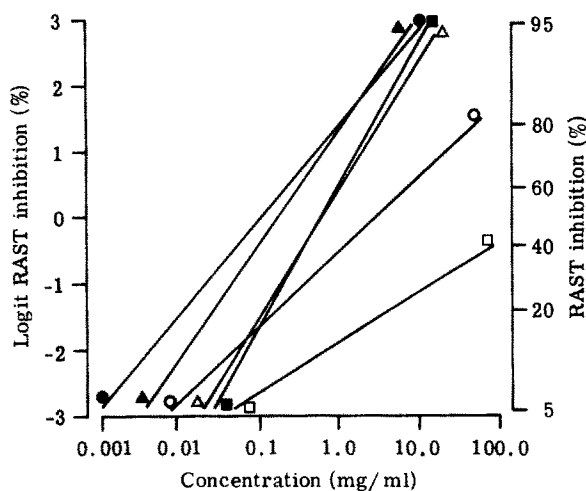


FIG. 4. Logit transformation of intact locust RAST inhibition by extracts of intact locust (●), gut (▲), peritrophic membrane (■), feces (△), locust room filter (○), and *D. pteronyssinus* (□), without common slopes.

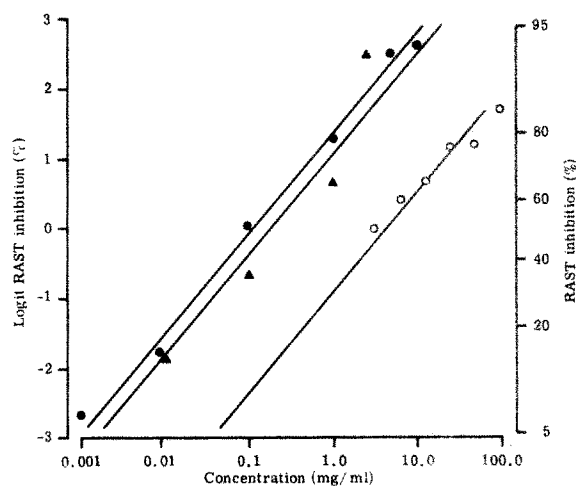


FIG. 5. Logit transformation of intact locust RAST inhibition by extracts of intact locust (●), gut (▲), and locust room filter (○), with common slopes.

The slope of the regression line for *D. pteronyssinus* was significantly different from the slopes of the four locust extracts, and therefore, immunologic identity was not found between the two insects. A common slope was found for the air-filter extract and *D. pteronyssinus*. However, although 1.88 mg/ml of the filter extract was required for 50% locust RAST inhibition,

146.87 mg/ml concentration of *D. pteronyssinus* was required to achieve this, nearly an eightyfold difference in potency.

Locust gut RAST inhibition

Because there were not common slopes between the regression lines of all four of the locust antigens

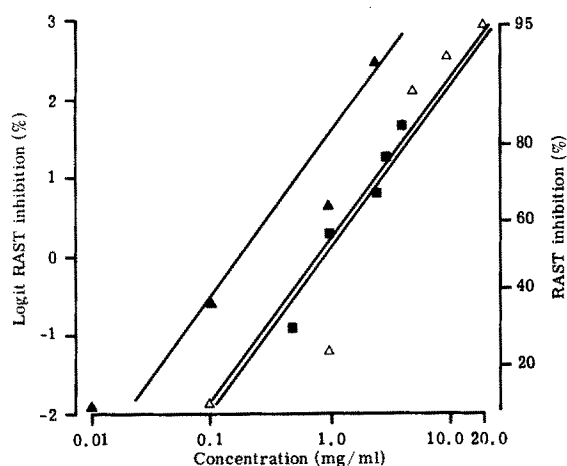


FIG. 6. Logit transformation of intact locust RAST inhibition by extracts of gut (▲), feces (△), and peritrophic membrane (■), with common slopes.

in the intact locust RAST inhibitions and because the regression line for the gut extract did have common slopes with all these other three locust extracts, a locust gut RAST was established, and similar inhibition studies were done (Fig. 7). A common slope was found between all four locust extracts; 50% RAST inhibition was achieved by 50 μ g/ml of the gut extract, 76 μ g/ml of the feces extract, 88 μ g/ml of the peritrophic membrane extract, and 1.29 mg/ml of intact locust extract.

DISCUSSION

Locusts are an important cause of insect-inhalant allergy, causing sensitization and the development of allergic symptoms in a high proportion of exposed persons. The preparation of relevant locust extracts of appropriate specificity has enabled us to identify specific IgE antibody in individuals with allergic symptoms, to define the major sources of locust allergens, and to estimate aeroallergen concentration in the working environment.

In the study of 35 research center workers, a positive immediate skin prick test response and specific IgE antibody to locust extracts were associated with exposure and symptoms, but only with RAST was there a relationship with atopy. Burge et al.⁶ using an extract of homogenized locust, found that 43% of industrial workers not exposed to locusts had an immediate skin prick test response to this extract and that this correlated both with atopy and a positive *D. pteronyssinus* skin prick test. We found considerable discordance, both in specific IgE antibody and skin prick test, between antigens of intact locust and house dust mite. Nineteen of the study patients had a positive skin prick test to one or more of these

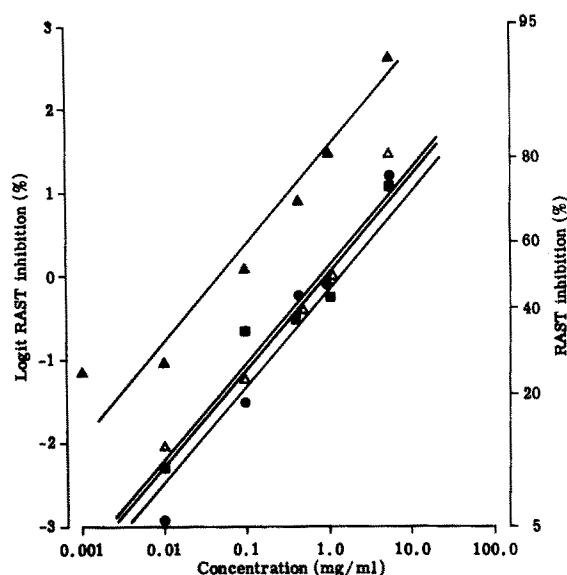


FIG. 7. Logit transformation of locust gut RAST inhibition by extracts of gut (▲), intact locust (●), feces (△), and peritrophic membrane (■), with common slopes.

antigens, but six of these had a positive locust and negative house dust mite skin test, and five of these patients had a negative locust and positive house dust mite skin test. These results were confirmed by RAST assays (Table II).

Frankland⁴ believed there must be "antigenic similarity" between locust and cockroach antigens, since he found that four of seven subjects with asthma with a positive skin prick test to homogenized locust extract also had a positive skin test to cockroach. Using commercial cockroach allergen disks (Pharmacia) and our nonhomogenized locust allergen in RAST assays, we have not found evidence of such cross-reactivity (Table II).

The antigen derived from intact (nonhomogenized) locusts demonstrated specificity in binding to serum IgE, and RAST binding correlated with a positive skin prick test response to locust extract. All nine symptomatic patients had a raised RAST measurement, including those with rhinitis but without asthma. Burge et al.⁶ had found that the specific IgE antibody levels in such patients, measured by ELISA to homogenized extract, were not different from antibody levels of his control patients.

In the present study, we did not find atopy to be a prerequisite for symptoms. This is again contrary to what Burge et al.⁶ found, and although this may in part be due to our observing a "survivor population" in which the worst affected may have left the workplace, it appears more likely to be the reflection of the greater specificity of our antigen that cross-reacts less with *D. pteronyssinus*.

RAST inhibition has been used to determine the major source of locust allergen. In view of the previous suggestion that midgut and peritrophic membrane might be important antigen sources,¹² great care was taken in the dissection of these parts before extraction; the peritrophic membrane was washed out with diethyl ether, rather than aqueous buffer, to prevent any contaminating allergens being eluted with those of the membrane.

Peritrophic membrane is secreted by the midgut epithelium where it forms a delicate lining layer consisting of a network of chitin fibrils (3% to 13% dry weight of membrane) set in a protein-carbohydrate matrix (protein 20% to 55%).¹⁶ The membrane encloses the food and protects the midgut cells from abrasion by food particles, thus performing a function analogous to mucus in vertebrates. The membrane exhibits some selective permeability, permitting enzymes to pass in from outside, but not allowing the outward movement of polysaccharides or proteins from the gut lumen. The intestine first separates fecal pellets from the midgut contents by a constriction and then, by bending, breaks the peritrophic membrane between them. Thus, peritrophic membrane encloses the food throughout its passage in the intestine and surrounds the feces when it is excreted.

Extracts of gut, peritrophic membrane, and feces, all inhibited the intact locust RAST in a dose-dependent fashion. The gut had a common regression slope with intact locust and also with peritrophic membrane and feces. Although we have not demonstrated immunologic identity between intact locust, peritrophic membrane, and feces in the RAST-inhibition assay of intact locust extract, the immunologic relationship is close because in the gut RAST-inhibition assay, all four locust extracts have a common slope, implying antigenic identity.

Aeroallergen concentrations in the locust rearing room were investigated by high-volume air sampling, elution of soluble material from the filter sheet, and analysis of eluate allergen content by intact locust RAST-inhibition assays. Locust antigen was identified in the air with immunologic identity found between the extracts of filter eluate, intact locust, and gut (4.4 mg/ml of filter eluate extract was required to obtain 50% RAST inhibition).

Chitin was used as an inhibitor in the locust RAST because it is a component of peritrophic membrane. However, it failed, even at a concentration of 50 mg/ml, to inhibit the locust RAST, eliminating it as a possible allergen in locust allergy.

Although *D. pteronyssinus* had a common regression slope with the filter eluate extract, it did not have a common slope with any of the four locust allergens,

and thus, significant immunologic identity was not found between locust and *D. pteronyssinus*.

By not homogenizing the locust before extraction, we obtained a more specific antigen, which is more representative of material that can become airborne and be inhaled. The demonstration of gut and peritrophic membrane as important inhibitors of the locust RAST and as strong allergens in vivo, strongly suggests that they are major sources of locust allergen. In the United Kingdom, the locust is replacing the cockroach for dissection in schools and colleges, increasing considerably the population at risk of developing allergy to these insects.

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