

Occupational asthma in a research centre breeding locusts

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Summary

The prevalence of work-related asthma, rhinitis and urticaria was measured in a scientific establishment working with locusts. Twenty-six percent of those handling the locust in the research centre had work-related wheeze or breathlessness, and one-third had work-related rhinitis and urticaria. Work-related symptoms were uncommon in scientists exposed to locusts in the field, and in other employees at the centre. Antigens were prepared from the locusts, *Schistocerca gregaria* and *Locusta migratoria*, as well as the moth, *Chilo partellus*, which was also bred at the centre. Skin prick testing with the locust antigens showed positive reactions in 55% of the atopic workers at the centre, but were also present in 43% of unexposed atopic workers. In this latter group there was a correlation between positive reaction to locust and dermatophagoides antigens. Atopic workers handling the locusts developed occupational asthma more often and more quickly than similarly exposed non-atopic workers. IgG and IgE antibodies to the locust antigens were found to correlate significantly with both the degree of exposure and the presence of disease.

Introduction

In 1713 Ramazzini wrote 'small fragments of dead silkworms as well as brukes (locust larvae) and caterpillars possess some sort of noxious and corrosive acrimony injurious to the lungs'. Since then insect antigens have become well recognized in domestic and occupational asthma. Laboratory workers breeding insects are at particular risk, and some have been sensitized to the grain weevil, *Sitophilus granarius* (Frankland & Lunn, 1965), bee moth larvae (*Galleria mellonella*) used for fish bait (Stevenson & Mathews, 1967) and locusts (Frankland, 1953). Farmers, millers and bakers may develop asthma due to mites and insects in the grain, e.g. *Pediculoides ventricosus*, *Pymotes ventricosus*

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.(Ancona, 1923). *S. granarius* (Lunn, 1966), *Ephestia kuehniella* (Cabanieu et al., 1971). *Acarus siro*, *Glycophagus destructor* and *Tyrophagus putrescentiae* (Cuthbert, 1978).

Occupational asthma has also been described in bean sorters with sensitization to the Mexican bean weevil *Zabrotes subfasciatus* boh (Wittich, 1940) and in sewage workers, with sensitization to sewage filter flies, *Psychoda* (Ordman, 1946).

Frankland (1953) studied selected workers breeding locusts in two research establishments. He found work related asthma in 4/34 workers he studied, but positive skin tests in 17/34. He also found positive skin tests to locust antigens in 13% of hay fever sufferers and 26% of atopic asthmatics not exposed to locusts. The majority of these non-exposed patients also had positive skin tests to cockroach antigens, which he thought was cross reacting with the locust antigens. The antigen was found in the faeces and bodies of the locusts.

In 1962 the research establishment 'B' in Frankland's report moved to new buildings where some attempt was made to confine the locust antigens. The two sensitized workers from this establishment described in his paper are still working there. We have restudied this research establishment to try to answer the following questions.

- (1) What is the prevalence of work related asthma in the employees at the centre?
- (2) What predisposing factors for sensitization can be found?
- (3) Is there evidence of reduced lung function in the sensitized workers?

Materials and methods

The staff employed by the research centre on 16 December 1977 were divided into four exposure groups by a member of its staff. The groups were designed to be of decreasing exposure to locusts. Group one consisted of the forty-three workers who handled locusts as part of their work at the centre. This group included those workers who cleaned the locust breeding rooms as well as the laboratory scientists. Group 2 were field workers who were occasionally heavily exposed to locusts in the field, but whose exposure was very intermittent. Only the eighteen of the twenty-seven who were in England during the 3 months of the survey were seen. Those who returned to write up their work in this period are thought to be an unbiased sample of this group. Group 3 were thirty laboratory scientists who did not work with insects. Many had had intermittent locust exposure in their training. They were employed as biogeographers, information scientists, scientific editors, scientific librarians and meteorologists. Group 4 were the twenty-eight administrative, secretarial and domestic staff including telephonists, messengers and security guards. None had a scientific training.

This represents a prevalence study of the defined population. All but one biogeographer in group 3 were seen. An enlarged MRC respiratory questionnaire was completed by one of three trained interviewers. The questionnaire included details of present and past insect exposure at home and work. The question 'does your wheeze/breathlessness improve at the weekend away from work?' was added after the MRC questions on wheeze and breathlessness. A positive answer to either was classified as work related asthma. Rhinitis or urticaria which improved at the weekend was also classified as work related.

FEV₁ and FVC were measured on a dry wedge spirometer (Vitalograph Ltd., Buckingham, England). Recordings were continued until there were satisfactory traces with the best two FEV₁ and FVC readings within 0.2 litres of each other. The best

readings were recorded and expressed as a percentage predicted for age, sex and height (Cotes, 1975) with 10% subtracted from the predicted values for Asians and Negroes. FEV₁ and FVC were repeated 10–15 min after inhaling 200 µg salbutamol.

Test antigens

Antigens of adult male and female *Locusta migratoria*, *Schistocerca gregaria*, and the moth, *Chilo partellus*, were prepared from whole, frozen specimens (–70°C). Insects were defatted in ether for 4 days, then homogenized in Coca's solution using an MSE 'Atomix' blender. After standing at room temperature for 4 days, the homogenates were filtered through Whatman no. 1 filter paper and the filtrates then Seitz filtered, dialysed against running tap water for 24 h and freeze dried.

Antibody tests—ELISA

Specific IgG and IgE antibody levels to the locust antigens were determined semiquantitatively using a microplate enzyme-linked immunosorbent assay (ELISA) (Voller *et al.*, 1974).

Rigid polystyrene, U-bottom plates (Dynatech) were used. Wells were coated with 200 µl volumes of the reagents. Optimum antigen and serum concentrations were selected by checkerboard titration of three sera, one from a worker who was skin test and symptom negative, one skin test positive and symptom negative and one skin test and symptom positive. Antigen coating was carried out overnight at 4°C, using the antigens at 50 µg/ml in carbonate buffer, pH 9·6.

Specific IgG antibody measurements were carried out in duplicate on each of the four antigen preparations. After washing with phosphate buffered saline pH 7·4, containing 0·05% Tween 20, the diluted serum samples were added. Sera were diluted 1 in 50 in PBS-Tween 20 solution and the plates were incubated at room temperature in a humidity chamber for 4 h. After further washing the conjugate, goat anti-human IgG-alkaline phosphatase (1 in 2000 in PBS-Tween 20) (Miles Yeda Lot S432) was added and incubated for 4 h at room temperature. After further washing p-nitrophenyl phosphate (Sigma 104 substrate) was added and the results read photometrically at 405 nm.

For specific IgE antibody estimations, equal portions of the male and female locust antigens of each species were combined, and measurement again carried out on duplicate serum samples using a double antibody technique (Sepulveda, Longbottom & Pepys, 1979). Sera were diluted 1 in 2 with PBS-Tween 20 and incubated overnight at 4°C. After washing, rabbit anti-human IgE (1 in 500) (Behring Lot no. 1203F) was added and incubated for 4 hr at room temperature. After further washing the conjugate, goat anti-rabbit IgG-alkaline phosphatase (1 in 2000) (Miles Yeda Lot S483) was added, and the plates incubated overnight at 4°C. After final washing the substrate was added and results read photometrically.

Skin tests

The locust and *C. partellus* antigens were skin prick tested at 0·1, 1·0 and 10 mg/ml by the same person. Workers were also prick tested with the following Bencard antigens: *Dermatophagoides pteronyssinus*, cockroach, grass pollen, horse hair, cat dander, feathers and *Aspergillus fumigatus*. Weals were measured 10–15 min after testing. A 2 mm weal was called positive in the presence of a completely negative

control. An atopic worker was defined as one having one or more positive reactions to a common environmental antigen (the Bencard antigens).

Results

Table 1 shows details of the participants in the survey. Those exposed to insects at work for one or more years showed a clear gradient of exposure from 91% in Group 1 to 0 in Group 4. The mean duration of exposure to insects was similar in Groups 1 and 2, the differences being that the exposure in Group 1 was in the laboratory and Group 2 in the field. Groups 3 and 4 had a greater proportion of females who are more likely to be breathless as defined by the MRC questionnaire, than men (Waller, Brooks & Adler, 1974; Burge *et al.*, 1979). The proportion of current smokers was similar in the three scientific groups, but greater in the administrators. Table 2 shows the results of the respiratory questionnaire in the four groups of workers. Work-related respiratory disease was almost confined to those handling insects in the laboratory where 26% had work-related wheeze or breathlessness, 35% work-related rhinitis and 33% work-related urticaria. Work-related rhinitis and urticaria were present but uncommon in the other groups.

Figure 1 shows the incidence of occupational asthma in the highest exposure group. There is a latent interval of 3 months before the first worker became symptomatic. Symptoms were more frequent and developed after a shorter period of exposure in the atopic than in the non-atopic workers ($P < 0.025$, log rank test).

Skin prick tests

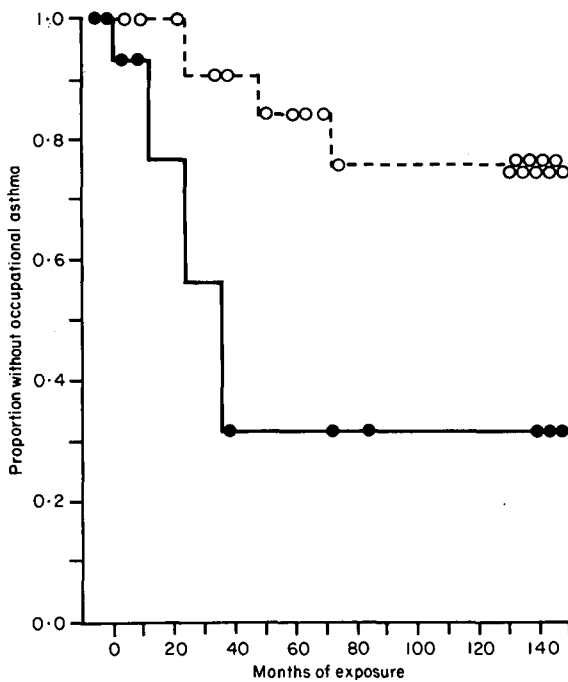
Table 3 shows the results of skin prick tests to one or more of the locust and Chilo antigens, and the Bencard cockroach antigen. Positive prick tests to locust antigens were present in 43% of Group 1 workers, 33% of Group 2 workers and 19–27% in the other groups (differences not significant). However 7/25 non-atopic workers in Group 1 had a positive prick test to a locust antigen compared with 2/39 non-atopics in the other groups ($P = 0.0145$, Fisher's exact test). Only four workers had positive prick tests to the cockroach antigen, all were atopic.

Table 1. Details of the four exposure groups in the survey

	Laboratory insect handlers	Field workers	Other laboratory scientists	Administrators, etc.
Total	43	18	30	28
Participated in survey	43	18	29	28
Age (mean $\pm$ s.e.m.)	36.8 (2.0)	40.7 (2.8)	34.8 (2.1)	40.6 (2.8)
% male	70	83	40	36
% female	30	17	60	64
% exposed to insects for 1 year or more	91	78	23	0
Insect exposure years (mean $\pm$ s.e.m.)	11.1 (1.5)	10.3 (2.6)	2.5 (1.2)	0
Current smokers (%)	35	34	35	53
Ex-smokers (%)	16	22	3	11
Non-smokers (%)	49	44	62	36

Table 2. Proportions of work-related respiratory disease, rhinitis and urticaria in the four exposure groups

	Laboratory insect handlers	Field workers	Other laboratory scientists	Administrators, etc.
Total	43	18	29	28
Work-related respiratory disease	26%	0	7%	0
Work-related rhinitis	35%	6%	13%	7%
Work-related urticaria	33%	6%	7%	0

**Fig. 1.** Scientists handling locusts in laboratory. o --- o, Non-atopic ($n=23$); ● — ●, atopic ($n=15$). $P<0.025$ **Table 3.** Skin prick tests results to locust, *Chilo partellus* and cockroach antigens in the four exposure groups

	Laboratory insect handlers		Field workers		Other laboratory scientists		Administrators, etc.	
	Non- Atopic	atopic	Atopic	Non- atopic	Atopic	Non- atopic	Atopic	Non- atopic
Number	17	25	10	8	9	18	13	13
Locust antigens % positive	65	28	60	0	44	6	46	8
<i>Chilo partellus</i> % positive	12	12	40	0	22	0	23	0
Cockroach % positive	18	0	0	0	0	0	8	0

Table 4. Prick tests to locust antigens in cases

		Asthma	Rhinitis/ urticaria
Positive at	0.1 mg/ml	5	1
	1 mg/ml	3	0
	10 mg/ml	3	4
Negative		1	5
Not tested		1	
Total		13	10

Table 4 shows the concentration at which positive prick tests were elicited in the cases. Eleven of twelve workers with work related asthma had positive prick tests to locust antigens, in five at 0.1 mg/ml (one worker on chronic haemodialysis was not tested at his request). Only five of the ten rhinitis patients had positive prick tests to locust antigens, all of whom were in exposure Group 1.

Skin prick tests in industrial workers not exposed to locust at work

The *S. gregaria* antigen was used for skin prick testing in 110 industrial workers selected only for their job in factories with trivial problems with occupational asthma from other causes (shoe and solder manufacturers). The results are shown in Table 5. Fifty-seven percent were non-atopic. Of these only one West African had a positive prick test to *D. pteronyssinus* than in the other atopic workers ($P < 0.025$). Thus a positive prick test to the locust antigen correlated significantly with atopy in general and with *D. pteronyssinus* sensitivity in particular.

Specific IgG antibody

The results of mean specific IgG levels to the four locust antigens are shown in Fig. 2. This shows that there is a significant correlation with exposure group ($P < 0.025$ Kruskal Wallace H statistic) and disease group ($P < 0.001$) although the asthma and rhinitis groups had similar levels. The results of the mean specific IgE levels to the two locust antigens tested are shown in Fig. 3. This again shows a significant correlation

Table 5. Numbers of non-exposed industrial workers with positive prick tests to *S. gregaria* (10 mg/ml)

	<i>S. gregaria</i> positive	<i>S. gregaria</i> negative
Non-atopic	1	63
Atopic— <i>D. pteronyssinus</i> positive	16	11
Atopic— <i>D. pteronyssinus</i> negative	4	15
All atopic	20	26
Total	21	89

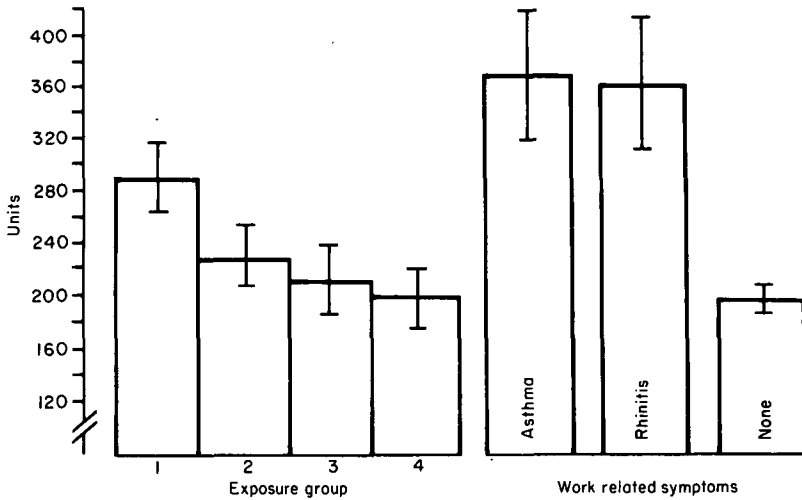


Fig. 2. Pooled antilocus IgG (mean $\pm$ s.e.mean).

with exposure ($P < 0.05$ —one way analysis of variance on log transformed data) and with disease group ($P < 0.01$) although in this case the rhinitis group are similar to the unaffected workers.

Pulmonary function

Table 6 shows the mean FEV₁ and FVC expressed as a percentage of predicted values for the normal workers, and those with work related asthma and rhinitis. The asthma group had a reduced mean FEV₁ but this did not quite reach statistical significance. There was no improvement in the mean results after 200 μ g of inhaled salbutamol.

Discussion

The two scientists identified by Frankland in 1953 as having asthma due to locust

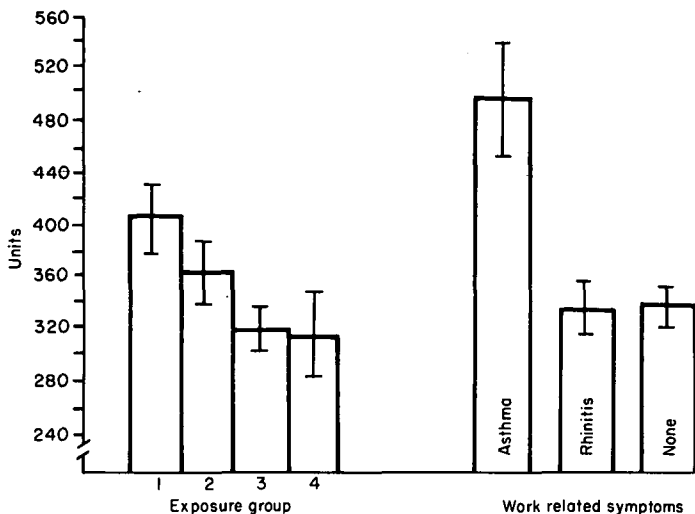


Fig. 3. Pooled antilocus IgE (geometric means $\pm$ s.e.mean).

Table 6. Lung function in workers with occupational asthma, rhinitis and unaffected workers

	Wheeze	Rhinitis/ urticaria	Normal
Total	13	10	91
FEV ₁ % predicted (mean $\pm$ s.e.m.)	93 (6.0)	104 (4.7)	102 (1.6)
FVC % predicted (mean $\pm$ s.e.m.)	95 (3.1)	97.5 (4.0)	100 (1.5)
FEV ₁ /FVC (mean $\pm$ s.e.m.)	80 (2.9)	86 (2.1)	84 (0.9)
After 200 μ g salbutamol			
FEV ₁ % predicted (mean $\pm$ s.e.m.)	93 (6.2)	102 (5.4)	104 (1.7)
FVC % predicted (mean $\pm$ s.e.m.)	94 (3.9)	95 (4.6)	101 (1.6)
FEV ₁ /FVC (mean $\pm$ s.e.m.)	79 (3.2)	87 (2.1)	86 (1.0)

sensitivity are still working at the research centre studied here. Sensitized workers have been moved to other jobs resulting in a very stable workforce. The results of this prevalence study are therefore likely to give a reasonable estimate of the incidence of locust sensitivity. The response rate to the survey was over 99%, removing bias from this source.

The prevalence of chronic chest disease has a marked social class gradient (OPCS, 1978) with increased disease in the lower social classes. This almost certainly leads to more responses to the breathlessness and wheeze question in the MRC questionnaire. The workforce studied was particularly interesting as groups 1–3 were almost exclusively scientists, removing possible bias from a social class effect.

Work related respiratory disease was defined as breathlessness or wheeze which improved at the weekend away from work. We have previously found this to be a reasonably good discriminating question separating work-related symptoms from those due to smoking and other causes (Burge *et al.*, 1979). It is likely to underestimate the prevalence of work-related symptoms in workers with asthma taking several days to recover after exposure. Those with work-related respiratory disease mostly had wheeze which improved at the weekend but breathlessness which took longer to recover. Twenty-six percent of workers handling insects at the centre had work-related respiratory disease which was present in only two other workers. The place of work was the main determinant of disease. There was no measurable effect of smoking. Similarly exposed atopic workers were about twice as likely to have work-related asthma or rhinitis as non-atopic workers. The group with work-related asthma had decreased mean values of FEV₁, the mean values were close to predicted in the rhinitis and normal groups.

Positive prick tests to locust antigens were found in over 55% of atopic workers in the research centre, and in 43% of atopic non-exposed industrial workers. The positive reactions in the non-exposed appeared to correlate specifically with *D. pteronyssinus* reactivity. This suggests that some of this antibody may be cross reacting with *D. pteronyssinus* or that the more highly atopic workers are more likely to additionally react to the locust antigens. Positive prick tests to locust antigens in the non-atopic workers was largely confined to the scientists handling the insects in the centre where 14% had positive reactions compared with 1.5% in the non-exposed workers. Positive prick tests to the Bencard cockroach antigens was very infrequent. The Bencard

material is prepared from *Periplaneta americana* which is in the same genus (Orthoptera) as the locusts. These results do not suggest that the cockroach antigens tested cross reacted with locusts, but the antigens tested may have been different from the one used by Frankland (1953).

Workers with work related asthma had significantly elevated specific IgG and IgE levels to locust antigens as measured by the ELISA technique. Those with work related rhinitis had elevated specific IgG antibodies but not IgE antibodies, which is an interesting finding, according with the low rate of skin test reactions in the rhinitis group.

This survey has shown that antigens derived from locusts are a source of clinical sensitization in workers exposed to them in the laboratory. Locusts are no longer confined to endemic areas and research laboratories and are widely used in schools giving exposure to many children and teachers. The possibility of cross reaction antigen suggests that specific sensitization resulting from laboratory exposure may be perpetuated after work exposure has ceased.

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