

Allergy to castor bean in The Sudan: measurement of serum IgE and specific IgE antibodies

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Summary

Allergy to castor bean was diagnosed in thirty-nine dockworkers from Port Sudan, on the basis of rhinitis and/or asthma provoked by castor bean dust, together with a positive prick test to a high dilution of castor bean extract. These were compared to twelve non-allergic dockworkers from Port Sudan and forty-three other Port Sudan residents. The castor bean allergic group had significantly higher IgE levels (mean 902 iu/ml) than the non-allergic dock workers (mean 245 iu/ml) or the Port Sudan residents (mean 404 iu/ml) $P < 0.01$. IgE antibody to castor bean was detected in all the castor bean-allergic group, and also in 25-35% of the Port Sudan resident group, but at a much lower level.

No evidence was found to support the view that parasitic infestation (evidenced by IgE antibodies to *Ascaris lumbricoides*) prevented sensitization to castor bean or any other allergen. There was a higher incidence of IgE antibodies to *Dermatophagoides pteronyssinus* in the castor bean-allergic dockworkers (18/36) than in the non-allergic Port Sudan residents (5/43), although we were unable to show that sensitivity to other inhalant allergens pre-disposed to castor bean allergy.

Introduction

Allergy to the castor bean *Ricinus communis* is common among people working in, or living in the vicinity of, castor oil mills (Panzani, 1957; Ordmann, 1955; J.E. Salvaggio, 1979, personal communication). The castor bean is a potent sensitizer capable to evoking severe allergic symptoms in sensitive individuals. There is a report of a woman suffering anaphylaxis due to an Indian necklace made of dried castor beans (Lockey & Dunkelberger 1968). It can affect people who are not allergy-prone; such 'normal' individuals can produce IgE antibody to castor bean allergen and develop symptoms of local urticaria, conjunctivitis, rhinitis and asthma on exposure to castor bean dust.

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In this study of castor bean-allergic dock workers from Port Sudan, total serum IgE and specific IgE antibodies to two varieties of castor bean (Cimmaron and Pacific) were measured and also to *Ascaris lumbricoides*, *Dermatophagoides pteronyssinus*, Nimitti fly (*Cladotanytarsus lewisi*) and Neem pollen (*Azadirachia indica*). These measurements were also made in dock workers who were not allergic to castor bean, residents of Port Sudan who were not closely exposed to castor bean, and in non-allergic British subjects.

Materials and methods

Total serum IgE

Serum IgE levels were measured by the double antibody assay (Nye *et al.*, 1975) using the Pharmacia kit. Sera were assayed in duplicate at a dilution where the counts per minute bound (ct/min) fell on the straight portion of the standard curve, and are expressed as international units per ml (iu/ml).

IgE antibodies

IgE antibodies were measured by the radioallergosorbent test (RAST) (Ceska, Eriksson & Varga, 1972) with some modifications (Kemeny, Lessof & Trull, 1980). Allergen was coupled to cyanogen bromide activated filter paper discs (Whatman 541), 5.5 mm diameter. Unbound allergen was washed away and vacant sites were blocked by washing with ethanolamine 0.1 M and sodium acetate pH 4.0. For castor bean RAST, 20 ml of extract containing 100 000 noon units (100 mg/ml) were coupled to 2 g of discs. *Ascaris lumbricoides* RAST discs were prepared by coupling the crude extract of a 5 g worm (Ambler *et al.*, 1972) to 2 g discs. For *D. pteronyssinus*, Nimitti fly, and Neem pollen RAST, 20 mg of each allergen were coupled to 2 g of activated discs. The test was performed by incubating 100 μ l of a 1/10 dilution of test serum with the allergen disc overnight, at room temperature. The test sample was diluted in normal human serum (NHS) for castor bean RAST and in horse serum containing 0.5% Tween 20 for the other assays. Unbound IgE was washed off using 1–2 litres of phosphate buffered saline (PBS) containing 0.5% Tween 20 using the constant flow washing device of Kemeny, West & Smith (Kemeny *et al.*, 1980). IgE antibodies bound to the allergen discs were detected by incubation overnight with 50 μ l ^{125} I anti IgE (50 000 ct/min) diluted in horse-serum (HS) + 0.5% Tween 20 for all assays. Excess labelled anti-IgE was washed off and measurements of the residual ct/min carried out in a LKB ultragamma counter. All samples were assayed in duplicate and were repeated where there was a variation of greater than 10%. Results are expressed as percentage ct/min bound, except for the castor bean RAST where they are given as units/ml of a known positive serum. Samples were reassayed at a higher dilution when results did not fall on the straight portion of the standard curve. A positive is given when the result is higher than the mean of nine negative sera plus 3 standard deviations.

Interference of heterologous IgE

To ensure that the high IgE levels, observed in the sera of many of the subjects studied, did not give false positives we measured the binding of IgE from a patient with Hodgkin's disease (who had an IgE level of 100 000 iu/ml) in our RAST system. We found that at IgE levels as low as 20 iu/ml the level of binding was high (Fig. 1). By

diluting the IgE-containing serum in horse serum + 0.5% Tween 20 this was reduced substantially. Using NHS as the diluent this binding was reduced still further. There was, however, no significant effect on antibody binding (Fig. 2). Using NHS to dilute the ^{125}I anti IgE did lower detection of antibody/antigen binding (Fig. 3). Interactions between Ricinus agglutinin and human IgM and IgG3 have been demonstrated (Saltvedt *et al.*, 1975) and it seems possible that this agglutinin may also bind human IgE. The IgG3 bound to Ricinus agglutinin could be eluted with 0.2 M lactose (Saltvedt *et al.*, 1975) and the use of this sugar in the assay diluent is being investigated. The high level of non-specific binding observed may also be due to the high concentration of allergen coupled to the discs. When 20 mg of extract is used non-specific binding is still observed but to a much lesser extent (R. Davis, 1980, personal communication).

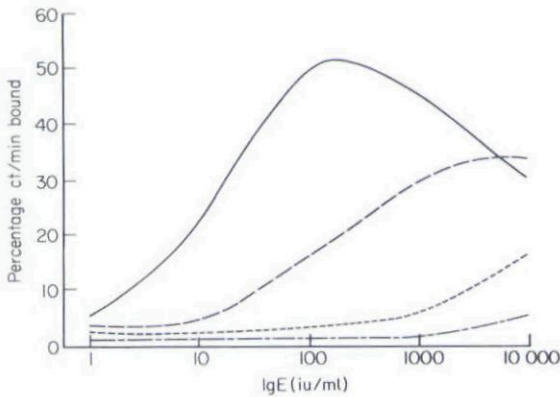


Fig. 1. The effect of the diluent used in the castor bean RAST on the non-immunological binding of IgE. —, PBS + 0.2% BSA; ---, HS + 0.5% Tween 20; ····, NHS; -.-.-, NHS for both incubations.

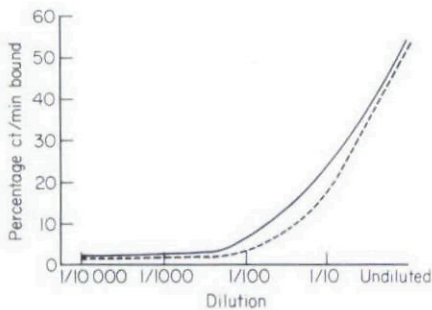


Fig. 2. The effect of the diluent used in the first incubation of castor bean RAST on IgE antibody binding. —, PBS + 0.2% BSA; ····, NHS.

Statistical analysis

Comparison of serum IgE levels was made using the one way analysis of variance test. Comparison of IgE antibodies to castor bean and other allergies was made using the χ^2 test after grouping results into positives and negatives according to criteria already defined here.

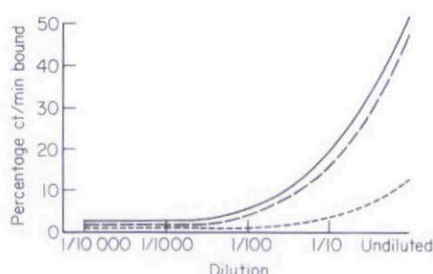


Fig. 3. The effect of the diluent used in the second incubation of castor bean RAST on the detection of bound IgE antibody. —, PBS + 0.2% BSA; ---, HS + 0.5% Tween 20; ·····, NHS.

Subjects studied

Four groups of people were studied. Group 1 was taken from forty-four male dock workers from Port Sudan who were exposed to castor bean dust through their work and visited the occupational health unit in Port Sudan with symptoms of rhinitis, asthma or both. All had positive skin prick tests to a high dilution of castor bean extract. Four of these who were Australia antigen-positive and a fifth who had insufficient serum were excluded from the study, leaving thirty-nine in this castor bean allergic group. Group 2 was taken from male dock workers who were exposed to castor bean extract but did not complain of symptoms when exposed. One sample was Australia antigen-positive and was therefore excluded, leaving twelve in this group. Group 3 was taken from forty-three males and females resident in Port Sudan who were not directly exposed to castor bean through their work, and who were prick test-negative to castor bean extract. Group 4 were thirty-six non-allergic U.K. subjects.

Results

Total serum IgE

The level of serum IgE was found to be higher in the castor bean allergic group 1 (mean 902 iu/ml) than in group 2 (245 iu/ml) or group 3 (404 iu/ml). All were higher than the U.K. population (Nye *et al.*, 1975) but were significantly different in each group $P < 0.05$ (see Fig. 4).

IgE antibodies to castor bean

All of the castor bean-allergic group 1 had a positive RAST to castor bean Pacific and 98% were positive to Cimmaron.

The levels of IgE antibody were very high, approximately tenfold higher than those seen in grass pollen- or bee venom-allergic patients (Kemeny, 1980) and was significantly higher than groups 2 and 3 (Fig. 5) $P < 0.01$. It should be noted that in those without symptoms but living in Port Sudan (group 3) there was a high incidence of positive RAST (35% positive to castor bean Pacific and 26% positive to Cimmaron). There was no significant difference in the response to the two varieties of castor bean.

There was a good correlation between the level of IgE antibody to castor bean and total serum IgE in the allergic group 1 (Fig. 6) $r = 0.68$, $P < 0.001$. this was not found in group 3, which comprised Port Sudan residents who were not dock workers. This suggests that high serum IgE levels do not result in false positive IgE antibody results, since some of the sera without IgE antibodies to castor bean had high IgE levels.

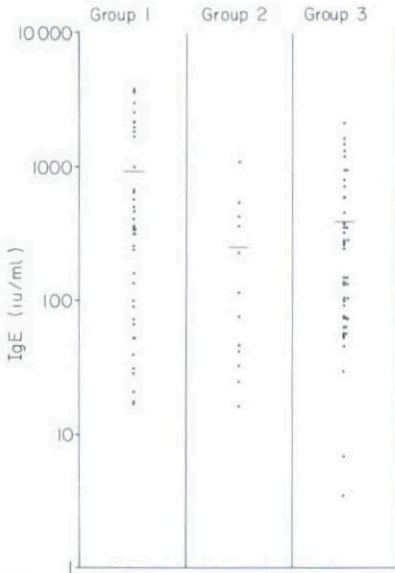


Fig. 4. Total serum IgE levels were higher in the castor bean-allergic dock worker (group 1) than in the non-allergic dock workers (group 2) and the Port Sudan residents (group 3).

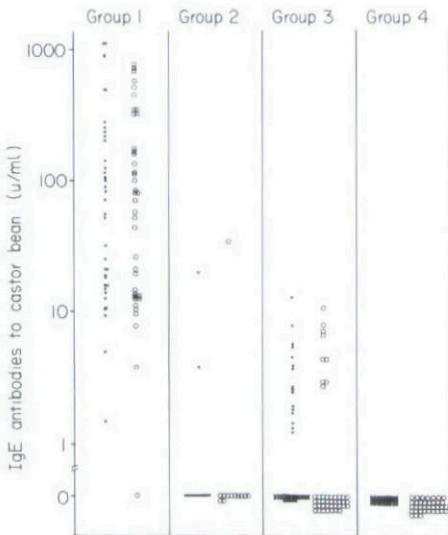


Fig. 5. IgE antibodies to castor bean Pacific (●) and Cimmaron (○) were found in 100 and in 98% of group 1, in 16 and 8% of group 2, and in 32 and 26% of group 3. None of group 4 gave a positive result.

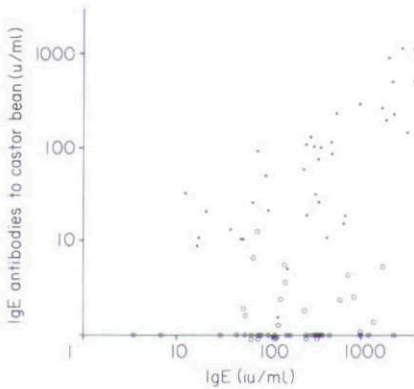


Fig. 6. The correlation between IgE antibody to castor bean Pacific and total serum IgE was good in the castor bean allergic group (1) (●) but there was no correlation in the non-allergic group (3) (○).

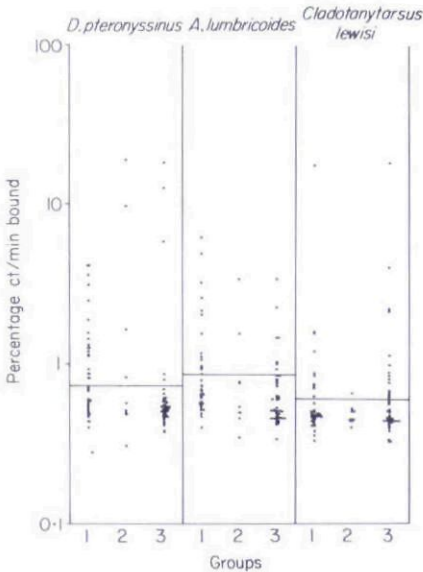


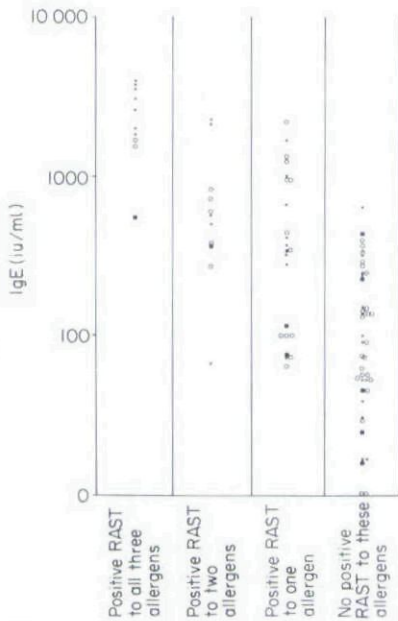
Fig. 7. IgE antibodies to *D. pteronyssinus*, *A. lumbricoides* and *C. Lewisii*. —, mean + 3s.d., the cut off used in our RAST.

IgE antibody to other allergens

The levels of IgE antibody to *D. pteronyssinus*, *A. lumbricoides* and Nimitti fly are shown in Fig. 7 and expressed as percentages ct/min bound. Results above the solid line were taken as positives. There were no positives to neem pollen which causes seasonal allergic symptoms in Khartoum but does not grow much at Port Sudan. The frequencies of positive RAST to these allergens are given in Table 1. There was no significant difference between the groups except for *D. pteronyssinus* RAST where there was a higher incidence of a positive in the two dock worker groups than in group 3, χ^2 , $P < 0.001$. There was a good correlation between total IgE levels $r = 0.74$ $P < 0.001$ and the number of positive RAST results (Fig. 8).

Table 1. The frequency of a positive RAST to *D. pteronyssinus*, *A. lumbricoides* and *C. lewisi* in groups 1-3

Group	Positive RAST to <i>D. pteronyssinus</i>		Positive RAST to <i>A. lumbricoides</i>		Positive RAST to <i>C. lewisi</i>	
	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%
1	18/36	50	14/37	38	9/37	24
2	4/10	40	2/10	20	1/10	10
3	5/43	12	8/43	19	13/43	30

**Fig. 8.** The comparison between IgE levels and the number of positive RAST to *D. pteronyssinus*, *A. lumbricoides*, and *C. lewisi* in a Port Sudan population. ●, Group 1; ■, group 2; ○, group 3.

Discussion

In this study the radioallergosorbent test was used to measure IgE antibodies to castor bean extract in Port Sudan dock workers who were allergic to castor bean dust. Almost all clinically sensitive patients gave a strongly positive RAST to castor bean extract. There was also a high incidence of IgE antibodies to castor bean in non-allergic Port Sudan residents, who were not exposed to castor bean through their work, though at a lower level than that found in the allergic group. The significance of this may lie in the finding that people living in the vicinity of castor oil mills can become sensitized to castor bean dust (Ordman, 1955; Panzani, 1957; Salvaggio, 1979, personal communication). Sub-clinical sensitization may thus have begun to develop in this population, although castor bean has only been grown in the Sudan for a few years. It seems possible that castor bean-induced asthma may subsequently be found among the residents of Port Sudan and appropriate tests may therefore be advisable in new, unexplained cases of asthma in this community.

The levels of total serum IgE found in the Port Sudan sera were notably higher than found in those of Europeans (Nye, 1975), but lower than found in the sera Tanzanian children (Carswell *et al.*, 1977) or Ethiopian children (Johansson, Mellbin & Vahlquist, 1968). It has been suggested that the lower incidence of asthma found in bush dwelling people (Van Niekerk *et al.*, 1979) is caused by large amounts of IgE antibodies to helminths which could theoretically saturate the mast cell IgE-binding sites, so displacing IgE antibodies to other allergens. In the Southern Maldives, asthma was found to be common, despite high levels of IgE antibodies to *A. lumbricoides* (Wolstenholme, 1979). In our study, individuals with the highest IgE levels were more likely to have positive RAST scores both to castor bean and to other allergens. Our results are consistent with the finding that parasitic infection in man may potentiate the production of IgE antibodies to unrelated allergens (Turner, Feddema & Quinn 1980), as it is known to do in animals.

We were interested to see whether there was a general relationship between allergy to inhalant allergens, such as *D. pteronyssinus*, and sensitization, as has been reported between inhalant allergens and bee venom (Miyachi *et al.*, 1979). There was a lower incidence (5/43) of positive RAST for *D. pteronyssinus* in the group of Port Sudan residents who were not allergic to castor bean as compared with the castor bean-allergic dock workers (18/36). However, four out of ten of the dock workers who were not allergic to castor bean showed a positive RAST to *D. pteronyssinus*, suggesting the possibility that the higher incidence of positive RAST to *D. pteronyssinus* might be due to a common factor at their place of work or in their homes. Since the number of non-asthmatic dock workers was small, a further prospective study is in hand, in order to examine the susceptibility of different populations to castor bean allergy and its relationship to other types of inhalant allergy and parasitic infestation.

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