

Asthma in merchant seamen and laboratory workers caused by allergy to castor beans: analysis of allergens

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Summary

Three merchant seamen and two laboratory workers who developed allergic symptoms following exposure to castor beans have been investigated. Bronchial-provocation testing with castor beans in the merchant seamen demonstrated a late reaction in two. Specific IgE against whole castor-bean extract and ricin, ricinus agglutinin and dericinated extracts of castor bean were found in the patients' sera using radioallergo-sorbent tests (RAST). RAST inhibition, toxocological and haemagglutination tests suggest that the ricin and dericinated extracts contain distinct allergens.

Introduction

Allergy associated with castor beans (*Ricinus communis*) was initially recognized in laboratory workers (Alilaire, 1914; Robbins, 1923) and later in industrial workers (Figley & Elrod, 1928). The toxic properties of castor beans, which have been recognized for many years (Osborne, Mendel & Harris, 1905; Arnold, 1924), have complicated the handling and investigation of the allergens in castor beans. The toxin, ricin, is exceptionally potent (Osborne, Medel & Harris, 1905), but its instability to heat has led to the preparation of heat-treated detoxified castor-bean extracts (Spies & Coulson, 1943), and multiple allergens have been identified in these extracts (Layton, Moss & De Eds, 1961). Ricin has never been adequately investigated and is often assumed to be non-allergenic (Layton, Moss & De Eds, 1961).

We report three merchant seamen and two research workers who developed allergic symptoms following exposure to castor beans. The conditions on board ship, which increased the risks of the seamen becoming sensitized, are discussed. Ricin, ricinus agglutinin and a dericinated extract of castor bean were prepared by chemical methods not requiring heat treatment. Studies were undertaken to establish whether separate allergens could be identified in these extracts using toxicology, haemagglutination and radioallergosorbent tests (RAST), and RAST inhibition analysis.

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Materials and methods

Preparation of allergens

Whole castor-bean extract. An aqueous extract was prepared from crushed castor beans obtained from the ship.

Ricin and ricinus agglutinin extracts. It is known that ricin is composed of a number of isotoxins which are lectins (Nicolson & Blaustein, 1972; Lin and Li, 1980). These have an affinity for galactose-like residues and a molecular weight of approximately 60 000 (Nicolson & Blaustein, 1972). Castor beans also contain a major haemagglutinin, ricinus agglutinin. This is also a lectin with an affinity for galactose-like residues and a molecular weight of approximately 120 000 (Nicolson & Blaustein, 1972). Ricin and ricinus agglutinin extracts were prepared following the principles of Nicolson and Blaustein (1972). Pooled castor-bean pomace was defatted with petroleum ether. The dried material was extracted with 10 volumes (v/w) phosphate-buffered saline (PBS), pH 7.4, overnight at 4°C and the supernatant clarified by centrifugation. This crude extract was fractionated first by ammonium sulphate precipitation, the fraction between 40–60% saturation being found to contain 90% of the haemagglutinating and toxic activity. After dissolving and dialysis against PBS this material was applied to an affinity column of acid-treated Sepharose 4B and eluted in PBS. After a peak of non-absorbed material had emerged from the column the agglutinin and toxin were simultaneously displaced with 0.1 M D-galactose in PBS. The agglutinin and toxin were then resolved by gel filtration on Sephacryl S-200.

Dericated castor bean extract. This (free of toxic and haemagglutinating activity) was prepared by a smaller-scale version of the affinity chromatographic step described above. Five millilitres of the extract of pooled beans was clarified by centrifugation and passed immediately down a column (45 × 1.5 cm) of acid-treated Sepharose 4B. The non-adsorbed protein peak was collected (total volume 16.5 ml) and the adsorbed proteins then displaced with 0.1 M galactose. After dialysing away galactose the displaced agglutinin/toxin peak was also adjusted in volume to 16.5 ml, as was a further 5 ml aliquot of the original extract.

The dericated extract was assessed for whole animal toxicity in 25-g CBA mice and for haemagglutinating activity against neuraminidase-treated human Group-O erythrocytes. No deaths were caused following the injection of 200 µg of protein from the breakthrough peak in each of five mice whereas the LD₅₀ of the toxin/agglutinin mixture was 80 ng. Similarly, no haemagglutinating activity (HA) was detectable in the breakthrough peak, whereas both the diluted initial extract and the toxin/agglutinin mixture had HA titres of 1:8000. These observations demonstrate that the affinity chromatographic step (which removes galactose binding proteins) reduces the toxicity of the castor-bean extract by at least 2500-fold and the haemagglutinating activity by 8000-fold.

Radioallergosorbent test (RAST)

The allergens, a whole castor-bean extract, ricin, ricin agglutinin and the dericinus extract of castor bean were coupled to CN-Br activated paper disc. The RAST tests were performed using a standard technique (Phadebas RAST, Pharmacia Diagnostics) and the results expressed as the percent binding of the total radioactivity (counts per minute added). Cord serum and sera from two individuals with raised total IgE levels, who had not been exposed to castor beans, were used as controls.

RAST inhibition

This was performed in two steps using the principles of Gleich *et al.* (1974). The first competitive step was performed by incubating the allergen-coated disc, the patients' serum and a test allergen, the latter being the putative inhibitor of the RAST. The second step was identical to that in the standard RAST. The results have been expressed as the percentage inhibition of the RAST and plotted on semi-log paper. In order to determine the allergenic relationship between the ricin and the dericinated extract the principle of cross-RAST inhibition was used (Karr *et al.*, 1978). Ricin was used as the putative inhibitor of the dericinated extract RAST, and then as a positive control ricin was used to inhibit the ricin RAST. Likewise the dericinated extract was used as the putative inhibitor of the ricin RAST, and as a positive control the dericinated extract was used to inhibit the dericinated extract RAST.

Bronchial provocation tests

These were performed using standard techniques (Davies, Hendrick & Pepys, 1974). Serial forced expiratory volumes in one second (FEV₁) throughout the preceding 3 days and on the morning of the test were stable. Whole castor beans and castor-bean fragments from the ship were shaken between trays by the patients for 15 min.

Patients*Merchant seamen who were admitted to hospital*

Three Sudanese merchant seamen became wheezy approximately 1 h after the hold of their ship was opened for unloading. Their voyage had started in Sudan and lasted 17 days. The cargo was castor beans, cattle feed and animal skins. Their symptoms continued until they were admitted to hospital 5 days later. They had continued to live aboard ship whilst in port. They thought that their illness was due to castor beans because many sailors and dockers, who had previous exposure and illness, had reported sick when the beans had arrived for loading in Port Sudan. All three denied

Table 1. Total IgE and RAST counts to castor-bean extracts in the five patients and three controls

	Total IgE iu/ml	RAST: % total counts min ⁻¹ added			
		Whole castor bean	Ricin	Ricinus agglutinin	Dericinus
Patient 1	270	53.2*	47.5*	52.9*	51.9*
Patient 2	3500	59.9*	57.8*	58.3*	55.6*
Patient 3	250	55.1*	53.1*	53.9*	44.1*
Patient 4	31	7.9*	7.0	9.6	13.2*
Patient 5	560	28.4*	26.0*	27.0	18.0
Control 1 (cord serum)		2.7	3.8	4.1	5.5
Control 2	280	3.5	5.0	12.2	6.1
Control 3	4500	6.0	7.8	22.4	14.4

* Indicates a positive RAST.

previous contact with castor beans. There was no personal or family history of asthma. None had a peripheral blood leucocytosis or eosinophilia and all had normal chest X-rays. The total serum IgE values are shown in Table 1.

Patient 1

This 31-year-old engine-room worker was extremely short of breath at rest on admission. He was a non-smoker. He had a pulse rate of 120 min^{-1} , bilateral expiratory wheezes and a peak flow of 110 l min^{-1} . Skin-prick testing showed immediate reactions to the control, mixed grasses, *Dermatophagoides pteronyssinus* and mixed feathers.

Patient 2

This 30-year-old engine-room worker was very short of breath on admission. He smoked 20 cigarettes per day. He had a pulse rate of 120 min^{-1} , bilateral wheezes and a peak flow of 120 l min^{-1} . Skin-prick testing showed immediate reactions of 3 mm to mixed grasses, 2 mm to mixed feathers and 1 mm to *D. pteronyssinus*; there was no reaction to the control.

Patient 3

This 26-year-old steward was short of breath on admission. He smoked 10 cigarettes per day. He had bilateral wheezes and a peak flow of 350 l min^{-1} . Skin-prick tests showed an immediate reaction of 2 mm to *D. pteronyssinus* and 1 mm to mixed feathers.

All three patients recovered rapidly after treatment with bronchodilators and corticosteroids and later bronchial provocation tests were performed.

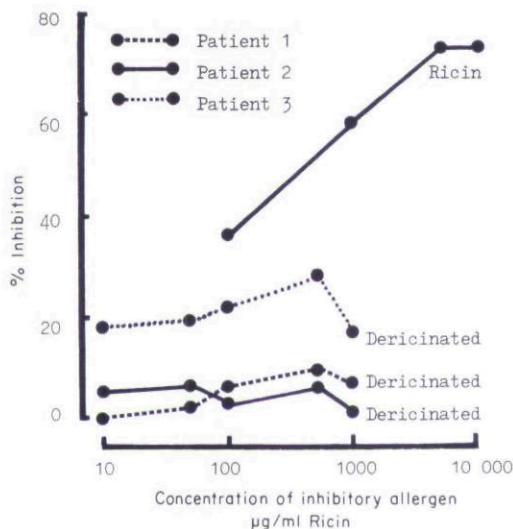


Fig. 1. RAST inhibition tests on serum from the three seamen. The effect of increasing concentrations of ricin (the inhibitory allergen) on RAST using the patients' serum and discs coated with dericated extract (lines labelled 'dericated'). Positive control: the inhibitory effect of ricin on RAST using a patient's serum and discs coated with ricin (lines labelled 'ricin').

The ship

The three seamen admitted to hospital claimed that their ship was extremely dusty and it was therefore inspected. The cargo had been discharged 7 days previously and no new cargo had been loaded but there were still a large number of castor-bean fragments and whole castor beans scattered around the ship. The beans had been carried in hessian sacks many of which had broken during loading and unloading.

Three further seamen had developed allergic symptoms out of the total crew of forty-five. One of these had previously been exposed to castor beans and he developed urticaria, which he demonstrated, on contact with the beans. Another seaman had developed wheezing and urticaria, for the first time, following entry into the hold after unloading. The wheezing had resolved but he still had severe urticaria over his face. The third seaman had developed, for the first time, wheezing during the voyage but this had resolved since being in port.

The ship had four holds, the first was towards the bow. The castor beans had been carried in the second and fourth holds. The inlet and exhaust ventilators for the second hold were just forward of the inlet and exhaust ventilators for the engine room and crews' quarters. The crew reported that the odour of any aromatic cargo would be carried from the holds into the crew quarters and the engine room.

Patient 4

This 36-year-old laboratory worker had developed wheezing and chest tightness on each of the three occasions that he had prepared a defatted extract of castor bean. These symptoms persisted into the evening and had become worse with each preparation. He had no previous history of asthma.

Patient 5

This 44-year-old laboratory worker had not been actively involved in the preparation of the defatted extract but worked in the same laboratory. During the third preparation he had also developed wheezing with chest tightness. He has a history of mild seasonal asthma since childhood.

Results*Radioallergosorbent tests (RAST)*

The results for the five patients and three controls are shown in Table 1. The control values using serum with varying total IgE levels show that non-specific binding occurred with all the allergen preparations and increased as the serum level of IgE rose. The RAST test has been considered as positive for a particular allergen if the test binding count was twice the control value, where the control serum had a similar total IgE level to the test serum. The counts in patients 1, 3 and 4 have therefore been compared with control 2, and the counts in patients 2 and 5 with control 3. Specific IgE were therefore found against whole castor bean, ricin, ricinus agglutinin and the dericinated extracts in the merchant seamen admitted to hospital, and against whole castor bean in both, and against ricin in one, and dericinated extract in the other of the laboratory workers.

Radioallergosorbent-test (RAST) inhibition

Ricin inhibited the ricin RAST and the level of inhibition continued to rise as the

concentration of ricin increased, and had reached 73% with a ricin concentration of $10\,000\ \mu\text{g ml}^{-1}$ (Fig. 1). On the other hand, the level of inhibition of the dericated extract RAST with ricin did not exceed 10% in two patients; in the other patient the inhibition was approximately 20% but did not rise with increasing concentration of ricin (Fig. 1).

The dericated extract inhibited the dericated extract RAST and the level of inhibition continued to rise as the concentration of dericated extract increased, and had reached 49% at a concentration of $700\ \mu\text{g ml}^{-1}$ (Fig. 2). The inhibition of the dericated extract RAST with ricin was much less and the highest level in the three patients was 28%, and there was little change in the level of inhibition as the concentration of the dericated extract increased (Fig. 2).

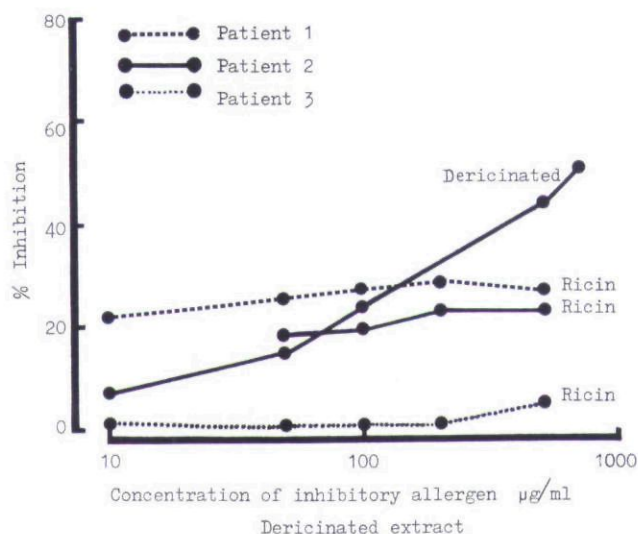


Fig. 2. RAST inhibition tests on serum from the three seamen. The effect of increasing concentrations of dericated extract (the inhibitory allergen) on RAST using the patients' serum and discs coated with ricin (lines labelled 'ricin'). Positive control: the inhibitory effect of the dericated extract on RAST using a patient's serum and discs coated with the dericated extract (lines labelled 'dericated').

Bronchial provocation tests

No patient had an immediate reaction. The first patient developed a 49% reduction in his FEV_1 after 9.5 h, and the second patient a 40% reduction at 11.5 h. The third patient did not develop a significant fall in his FEV_1 (Fig. 3).

All three seamen complained of skin irritations within an hour of the provocation. The first and second patients also developed rhinitis and conjunctivitis. There was pressure for the seamen's immediate discharge and time did not allow for a more formal study.

Discussion

Castor oil has mainly been used in the past as a cathartic, though now it is principally used industrially in the production of cosmetics, nylon, explosives, paints and inks. The recent oil crises have led to an increase in demand and production of this non-petroleum-derived oil. Caster oil is neither allergenic nor toxic and it is extracted

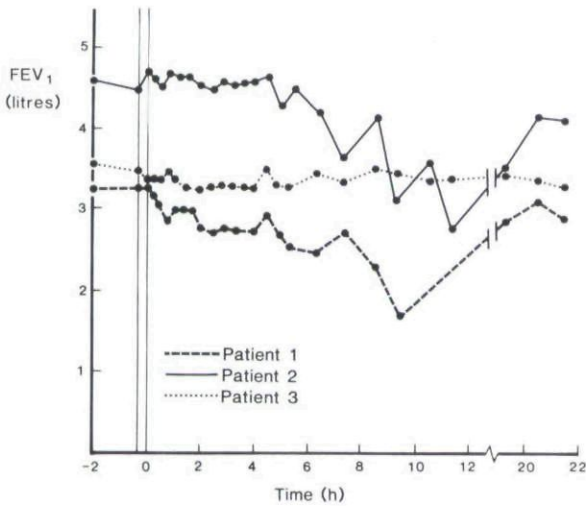


Fig. 3. Bronchial provocation testing with castor beans in patients 1–3.

mechanically by crushing castor beans. The yield of castor oil can be increased by the use of an organic solvent such as hexane. The pomace is the residue left after the extraction. It has a high nitrogen content and is used as a fertilizer. Following solvent extraction the pomace is dry and powdery and most of the allergic problems have usually been associated with the pomace rather than with whole castor beans. Allergy has been reported in, amongst others, oil-mill workers, populations around oil-mills (Figley & Elrod, 1923), farmers (Panzani & Layton, 1963), and following contact with hessian bags which previously contained pomace (Figley & Rawling, 1950).

Asthma is the usual allergic symptom which has been associated with castor-bean exposure, although rhinitis, conjunctivitis and urticaria also occur (Panzani & Layton, 1963). The predominant symptom was also asthma in the cases reported here, but urticaria was present in two of the seamen seen on board ship. Specific IgE in the serum against an aqueous extract of whole castor bean in the five patients and the significant falls in FEV₁ following bronchial provocation in two of the seamen, support the clinical evidence that the castor bean was responsible for the asthma. The third seaman had no response following bronchial provocation but he had the mildest symptoms on admission and it is possible that the provocation was insufficient to provoke an asthmatic reaction.

In order to characterize the allergens in the castor-bean preparations of ricin, ricinus agglutinin and dericinated extracts were made. Very high specific IgE levels were found using RAST against all three extracts in the three seamen who were admitted (patients 1–3). Specific IgE were also found against the dericinated extract in the fourth patient and the ricin extract in the fifth patient. The haemagglutination and toxicological tests indicate that the haemagglutinating and toxic properties of the castor bean had effectively been removed from the breakthrough peak and that this peak represents a 'dericinated extract'. If there had been common allergens contained in the ricin and dericinated extracts it would be expected that the inhibition of the ricin RAST by the dericinated extract, and the inhibition of the dericinated extract RAST by the ricin would have been greater and similar to the positive controls with increasing

concentrations of putative inhibitor. The low level of inhibition which did occur in some test samples appears to be non-specific as it did not follow the characteristics of the positive controls. These studies suggest that there was no substantial contamination of the ricin extract with the dericinated extract or vice versa, and that there are separate and distinct allergens in the ricin and dericinated extracts of castor bean.

The methods of preparation of the allergenic extracts of castor bean described here have advantages over previously prepared extracts (Spies & Coulson, 1943; Layton, Moss & De Eds, 1961). The ricin and ricinus agglutinin and a detoxified preparation (dericinated extract) were prepared by chemical methods and without heat treatment. This avoided denaturing any allergens which were unstable to heat and maintained the full allergenic potency of the extracts.

One of the major problems in cancer chemotherapy has been the lack of selectivity of anticancer agents. Attempts are now being made to purify ricin and link ricin or subunits of ricin to tumour specific antibodies and to produce an immunotoxin which is toxic and highly selective (Olsnes, 1981). Isoelectric focusing of our preparation of ricin has shown the presence of a number of components of different isoelectric points. Some of these components have been isolated by ion-exchange chromatography and all have similar whole animal toxicities (J. A. Forrester, unpublished observations). It is not known if all these are both toxic and allergenic.

There had been no prior known contact by the merchant seamen with castor beans and sensitization to the various castor-bean allergens probably occurred after a comparatively short exposure. Three major factors were identified which increased the risks of sensitization. Firstly, the beans were carried in hessian sacks which would have acted as sieves and allowed fine particles to escape. Secondly, there was considerable spillage of beans during loading and unloading. This could have been due to either poor-quality sacks or poor loading and unloading techniques. Thirdly, the ventilation system may have allowed dust to pass from the hold containing the castor beans to the engine room and crew quarters. Asthma developing at sea could have serious consequences because of the unavoidable antigen challenge. There are no specific regulations in the U.K. governing the carriage of castor beans or pomace but the risks of sensitization would be considerably reduced if they were carried in strong airtight bags.

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