

Occupational asthma in fruit salad processing

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Summary

Background Three subjects employed in the preparation of fruit for fruit salads reported work-related respiratory symptoms. Their work entailed removing the peel from citrus fruits, primarily oranges, following soaking of the fruits in a bath of enzymes including fungal derived pectinase and glucanase.

Objectives To investigate the respiratory symptoms reported by these workers and determine their causes.

Methods The three workers were investigated by a respiratory physician, including spirometry and serial peak flow measurements. Blood was taken for the measurement of IgE and IgG antibody responses against the enzyme solution.

Results Predominant symptoms in these workers were shortness of breath, chest tightness and wheezing which were all alleviated at weekends and holidays only to occur when returning to work. Serial peak flow measurements showed a clear work-related pattern. All three had strong IgE responses to the enzyme solution used at the workplace and showed distinct patterns of binding in immunoblots. All three improved immensely following withdrawal from the workplace environment.

Conclusion Enzymes appear to be widely used in the preparation of fruit and although they are used in liquid form, exposure can occur to induce immunological sensitization and asthma.

Keywords: occupational asthma, pectinase, glucanase, enzymes

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Introduction

Occupational asthma is a disease characterized by variable air flow limitations and/or airway hyperresponsiveness due to causes and conditions attributable to a particular occupational environment and not to stimuli encountered outside the workplace [1]. Occupation is important both as a direct cause of asthma and for providing an environment, namely the workplace, where sensitization to 'novel allergens' [2], such as enzymes, can also take place. The latest analysis of surveillance of work-related and occupational respiratory disease in the UK (SWORD) [3] would suggest that cases attributable to such agents, as enzymes, are increasing.

Asthma in the food industry has been discussed (and reported) by several study groups in relation to various food agents including eggs, tea and coffee, spices [4,5] seafoods [6], and enzymes [7]. Montanaro [8], in his review of

medical literature, found that occupational asthma due to inhalation of proteolytic enzymes is a rare but well documented event in many occupational settings including the food and beverage industries.

Pectolytic enzymes (pectinases) from *Aspergillus niger* are used in fresh fruit processing. The enzyme is used for clarifying fruit juices. During the making of juices, particularly citrus, many of the flavouring constituents (high insoluble solids) can directly effect the flavour. Pectinase is used to treat the pulp of soft fruits prior to de-pithing; it also acts by dissolving protopectin and degrading the natural pectin thereby facilitating filtration and preventing the subsequent precipitation of the pectin.

Occupational asthma following exposure to pectinase and glucanase but in powdered form and at the enzyme production stage has been previously reported. Two cases of occupational asthma caused by exposure to dust of the pectolytic enzyme pectinase were reported by Hartmann *et al.* in 1983 [9]. (They recommended the use of granular or

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liquid preparations of the enzyme.) An investigation of 42 chemical plant workers handling various types of enzymes, all reporting work-related symptoms, which included bronchial challenge tests and skin-prick and enzyme allergosorbent tests, showed enzyme allergosorbent test to be 62% sensitive and 96% specific [10].

We report on a workplace outbreak of asthma, in three fruit salad processors, associated with an enzyme solution. The enzyme solution used by the workers described in this report was a blend of pectinase and glucanase derived from *Aspergillus* and *Trichoderma* species. All three workers were clinically diagnosed as having occupational asthma; sensitization was demonstrated by RAST testing using a sample of enzyme solution from the workplace, and all had specific IgG antibodies reflecting their exposure (though not necessarily the disease).

Workplace and process descriptions

The Company produces various fruit salads for its customers. A solution of pectinase and glucanase is used in the de-pithing and preparing of citrus fruits, mainly oranges. To assist the segmentation process further, a vacuum is applied to the fruit submersed in solution. On release of the vacuum, the oranges and enzyme solution are tipped onto a stainless steel bench where the fruit is peeled and segmented by hand, operators spending roughly equal time in each activity. They wear overalls and rubber boots. After each batch processed, the whole area, including walls and floor, is cleaned with hosed water and brushes.

Methods

Clinical assessments

Within a 12 month period a Chest physician was referred three patients working in the same factory in whom a possible diagnosis of occupational asthma had been made by different General Practitioners. All had detailed history taken with examination. Serial peak flow measurements (PEFR) were made both on and off work. Follow up continued even after their employment had ceased.

Immunoassays

Specific IgE antibody measurements were carried out by radioallergosorbent test (RAST) using a sample of the enzyme solution obtained from the workplace [11]. The results were expressed as a ratio of the binding of a negative human serum (RAST score). RAST scores >2 were considered positive.

Specific IgG antibody measurements were carried out by enzyme-linked immunosorbent assays (ELISA) following

coupling of enzyme solution to plastic ELISA plates. A strongly positive serum was given an arbitrary value of 10 000 μmL and a standard curve fitted from which the values of tests were calculated from the average of the results at two serum dilutions.

Samples of the enzyme solution were separated by reducing sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) and either stained with coomassie blue [12] or semi-dry blotted onto nitro-cellulose for immunoblotting with workers' sera in which bound IgE antibodies were visualised using an anti-human-IgE monoclonal antibody and enhanced chemiluminescent detection.

Results

Patient characteristics

Table 1 shows the patients' characteristics. Two were male, one female. Age range was between 19 and 45 years. Two patients had had asthmatic symptoms prior to their employment at the food processing plant. One had been on oral inhaled steroids for 1 month following some wheeze after an upper respiratory tract infection, 12 months previously. The other patient was on low dose inhaled steroids (400 $\mu\text{g/day}$) plus an inhaled bronchodilator. Smoking history is as shown in the table. All three developed severe asthmatic symptoms within 6 months of the first exposure to enzymes in the workplace. Predominant symptoms given were shortness of breath, chest tightness and wheezing which were all alleviated at weekends and holidays only to recur on returning to work. Serial peak flow measurements performed during work and days off showed a clear work-related asthmatic pattern with a progressive decrease in the peak flow measurements during the working week only to improve on days off (Fig. 1). Immunological tests confirmed elevated RAST scores for either mixed grass pollens, cat fur or *D. pteronyssinus*, and all three had strongly positive RAST scores to the enzyme solution used at the workplace. All three remained in the working exposure for between 7 and 16 months after developing asthma; their symptoms disappeared within several months after removal from work and lung functions were found to have improved during follow up (Fig. 1). Immunoblot results for enzyme solution and all three cases is shown in Fig. 2.

Discussion

All three patients described here were from an exposed group of 11 workers. Although two out of the three had pre-existing asthma they had been symptom-free for approximately 12 months. All three developed asthmatic symptoms within a short time of exposure to the food processing which would suggest an occupational cause. Their subjective

Table 1. Summary of clinical details and immunology of three fruit-salad processors exposed to enzymes

Case number	1	2	3
Age when first seen	28	20	46
Sex	Male	Female	Male
Previous asthma (before starting work)	Yes (3 months before)	Yes (12 months before)	No
Smoker	No	No	Yes
Asthma treatment when starting work	None	None	None
Time interval to develop first symptoms	5 months	3 months	4 months
Associated rash	No	No	No
Eye irritation	Yes	Yes	Yes
Sneezing	No	Yes	Yes
Time interval of working with symptoms	7 months	6 months	12 months
Recovery of full health after leaving work	1 week	Still on therapy	3–4 months
RAST score - mixed environmental allergens	93.7	84	11.9
RAST score - enzyme solution	20.8	7.3	10.2
IgG - enzyme solution (μmL)	10 000	589	1030

work-related symptoms correlated well with their objective measurements of PEFR and they improved significantly when their exposure to the food processing ceased. We therefore believe that the three patients had developed occupational asthma. All 3 were exposed to a pectolytic enzyme solution. Antibodies to the solution were detected in highly significant amounts in all three at the time of initial presentation. It is therefore thought that their occupational asthma was due to their exposure to the enzymes pectinase and glucanase.

Bronchial provocation tests to the enzymes were not performed while the three were working because they had all decided to leave employment given their obvious work-related symptoms. Once they had left employment such tests were of academic interest only and could have been harmful to the patients. Also, assessments of airborne concentrations (personal and static monitoring) of enzymes

were not carried out because, to the best of our knowledge and belief, reliable and valid methods for doing so with these enzymes do not exist at present.

Occupational asthma induced by enzymes has been known since the late 1960s when the first reports of enzyme sensitivity were described in workers handling *B subtilis*. The only previous case reports of pectinase-induced asthma had involved exposure to enzyme dust. (Using the enzyme in a liquid form had been recommended as a means of avoiding sensitization.) Although a recent New Zealand study [13] has found food processors among the highest-risk occupations for the development of occupational asthma, fruit-salad processing has not previously been associated with such a risk.

We believe that the patients became sensitized to the enzymes by three possible routes:

- The inhalation of powdered enzyme produced as a result of the drying out of work clothes previously soaked in enzyme solution and which left 'tide marks' on sleeves of overalls (as reported by those involved in the tipping out and peeling of the fruits), a mechanism similar to that producing airborne rat urinary allergen in the laboratory [14].
- Aerosolization of enzymes during the cleaning of work surfaces, floors and walls with hosed water [15]. Enzyme aerosols may also have been produced during the tipping out process mentioned in a).
- Occupational and non-occupational ingestion of fruit and fruit juices 'contaminated' with the enzymes pectinase and gluconase giving rise to immunological sensitization analogous to the 'egg-bird syndrome' [16] associated with egg allergy, where the initial sensitivity is due to ingested egg protein, followed by subsequent inhalatory sensitivity to bird antigens (feathers, droppings etc.). Also of interest with

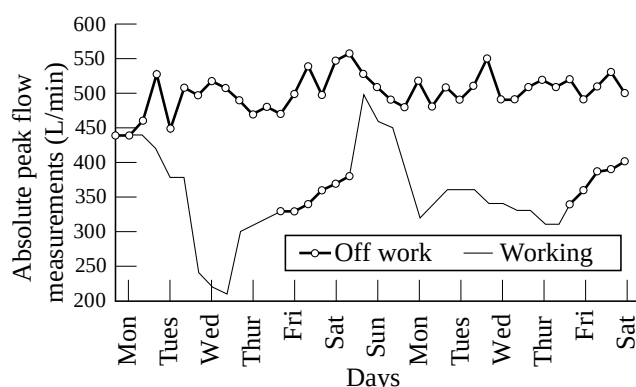


Fig. 1. Showing absolute peak flow measurements from patient 1 whilst at work and after leaving work.

this group is the fact that associated asthma is more prevalent, total IgE is usually higher and egg white sensitization is more persistent.

Finally, we believe that all workers in the fruit-salad

processing industry exposed to enzymes, dry or in liquid form, require pre-employment and periodic health surveillance. Symptoms and signs of occupational asthma should be sought coupled with serial spirometric measurements and

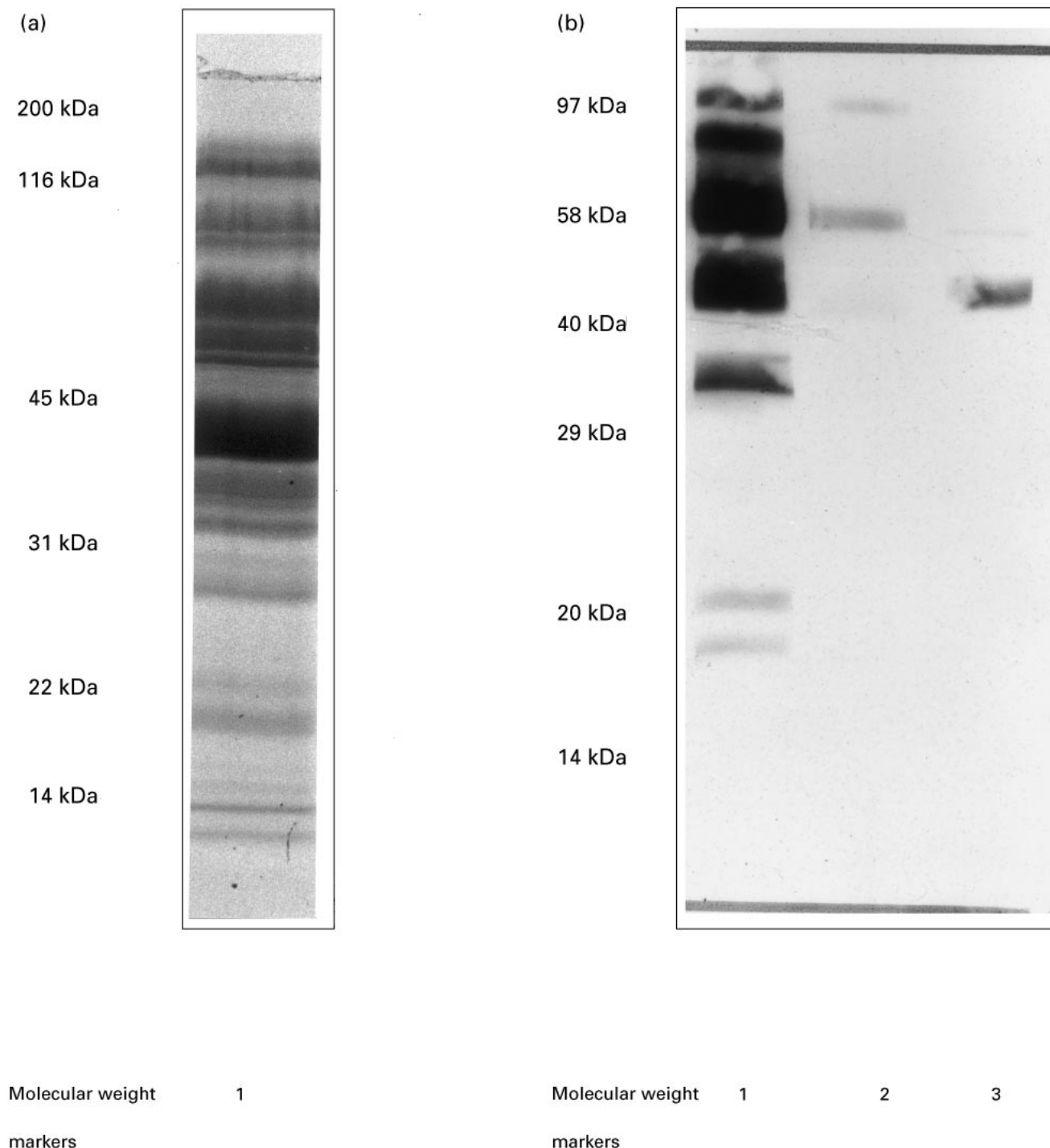


Fig. 2. (a) Coomassie blue stained SDS-PAGE. Lane 1, enzyme solution. (b) Immunoblot of cases' IgE antibodies binding to enzyme solution proteins separated by SDS-PAGE. Lane 1 (LHS), case 1; lane 2, case 2; lane 3, case 3.

serological testing for antibodies to enzymes present in the workplace. Although further workplace-based research is needed to determine the relationship between exposure and response and effect of enhancing factors such as smoking and atopy (all three patients were atopic), medical surveillance of the kind described may reduce the number of cases of occupational asthma.

The views expressed in this paper are those of the authors and are not necessarily those of the Health and Safety Executive.

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