

Extraction and Degradation of Timothy Pollen Allergen during Simulated *in vivo* Conditions

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Extraction and degradation of timothy pollen in saline has been compared with extraction in nasal secretion and gastric fluid. By measuring the absolute amount of one important allergenic substance by quantitative immunoelectrophoresis and the total allergenic activity by RAST as a function of time it was shown that the allergens were extracted extremely fast and that both the total allergenic activity and the concentration of one allergen reached maximum after about 20 min. Compared with saline, extraction under the simulated *in vivo* conditions gave a lower maximum level and a faster degradation of allergens.

Key words: allergen; degradation; extraction; *in vivo*; timothy.

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Timothy pollen which is one of the most common causes of allergic airway disease contains a series of allergens. *In vitro* extraction in saline and subsequent crossed immunoelectrophoresis have been used to identify the allergens, and their recent isolation has made an absolute quantification possible (3). Some allergens are extracted extremely fast *in vitro* (1, 4) and the fact that rhinitis symptoms are provoked 30 sec after allergen challenge (6) indicates a similar *in vivo* extraction. However, our knowledge of *in vivo* extraction and degradation of pollen allergens is insufficient.

When pollens are inhaled during normal respiration they are trapped in the nose, and due to their size (25-30 μ m) only an insignificant number will reach the bronchi (8). From the nasal cavity they are conveyed to the pharynx and swallowed. With a mean mucus flow rate of 0.5 cm/min the maximum stay in

the airways is about 30 min at 30-32°C. After that they are carried to the stomach.

It has been the aim of the study to compare allergen extraction and degradation during ordinary *in vitro* conditions with extraction and degradation in a set-up, imitating the *in vivo* circumstances. We have measured total allergenic activity as well as the concentration of a single well-defined allergen (Ag 19), characterized by a fast *in vitro* extraction (4).

MATERIAL AND METHODS

In a series of experiments timothy pollen grains in a concentration of 10 mg/ml were extracted for various time periods in one of the following solutions: (a) 0.15 M sodium chloride; (b) 0.15 M sodium chloride containing 0.2 mg/ml lysozyme (Sigma); (c) a mixture of nasal secretions, passed through a

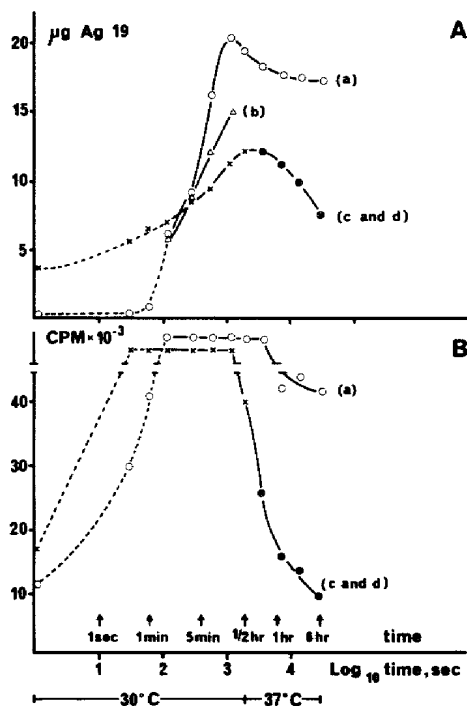


Fig. 1. Extractability of timothy pollen allergens in buffer and secretions as a function of extraction time. In A the absolute amount of extracted antigen 19 measured by quantitative immunoelectrophoresis and in B the total allergenic activity measured by means of RAST is given. Extraction solutions used: (a) 0.15 M sodium chloride ($\text{---}\bigcirc\text{---}\bigcirc\text{---}$); (b) 0.15 M sodium chloride and 0.2 mg ml^{-1} lysozyme ($\text{---}\triangle\text{---}\triangle\text{---}$); (c) 50% (v/v) nasal secretion ($\text{---}\times\text{---}\times\text{---}$) for the first 30 min followed by (d) gastric secretion ($\text{---}\odot\text{---}\odot\text{---}$). The horizontal part of the curves in B indicate that maximum counting of the paper discs used in RAST was reached. The curves for the first minute of extraction were dashed to indicate the uncertainty in exact time control.

Millipore filter, and 0.15 M sodium chloride 50% (v/v); nasal secretions were collected from six subjects by a method described elsewhere (7); (d) gastric secretion (pH 2.5) from one fasting subject.

The extractions were performed in thermostats at 30°C for the first 30 min using solutions (a), (b) and (c), and at 37°C for a time exceeding 30 min using solutions (a) and (d). The pollen grains extracted with

A solution (c) were transferred to solution (d) after isolation by centrifugation for 5 min at 10,000 g , 0°C. After the extraction period all samples were freed from pollen grains by filtration through a 0.65 μm sterile filter and subsequently stored at -20°C until analysis. The extracts were analysed for total allergenic activity by means of RAST (2) and for content of Ag 19 by means of quantitative immunoelectrophoresis (4). Each extraction was performed at least twice.

RESULTS AND DISCUSSION

The results of pollen extraction during a certain period of time are given both in terms of μg Ag 19 and of total allergenic activity (counts per min) (Fig. 1A and B). In saline (solution a) the concentration of Ag 19 reached maximum after 20 min and that of total allergenic activity after extraction of 2-45 min. Both measured parameters decreased moderately after conversion to 37°C. Addition of lysozyme to saline (solution b) gave somewhat lower concentrations of Ag 19 compared to extraction with saline. In nasal secretions the Ag 19 level reached maximum after 30 min and total allergenic activity after 0.5-20 min. Both parameters decreased drastically by conversion to gastric acid (solution d) at 37°C.

By extracting pollen grains first in nasal secretions at 30°C for 30 min, and second in gastric fluid at 37°C we have tried to imitate the conditions *in vivo*. Compared with ordinary *in vitro* extraction in saline this gives a lower maximum concentration and a faster degradation of the allergens. These differences are certainly due to enzymatic activity in nasal secretions, as indicated by extraction in the lysozyme-containing solution. It seems likely as proposed by Norman (5) that lysozyme in nasal secretions facilitates allergen extraction by dissolving the pollen grains, but on the other hand, a lower allergen level is reached in airway secretion than in saline; certainly due to enzymatic degradation of proteins. However,

the different physiochemical composition of buffer and secretion may also influence the extraction rate.

While the properties of airway secretion apparently may both facilitate and inhibit allergic symptoms, it is quite evident from the experiments that gastric fluid possesses a marked protecting effect by quick degradation of allergenic substances. The results indicate also that pollen after a normal transfer period in the stomach still can release allergenic substances in the intestine. Although the amounts seem small they can probably, after absorption in the blood, provoke symptoms in very sensitive subjects.

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