

described (5), whereas no inhibition could be observed with *R. Nigricans* or *P. Notatum* extracts. Precipitating antibodies and specific IgG measured by ELISA (3) against the EE, AFE, *M. faeni*, *T. vulgaris*, hay, and pigeon droppings extracts (Lab. Aristegui, Bilbao, Spain) were not found in the patient's serum.

The patient underwent a bronchial provocation test (BPT) with EE, as previously described (4), resulting in a dual asthmatic reaction. Bronchial challenge performed with AFE showed an immediate response, but not a late reaction (Fig. 1). Four atopic unexposed control individuals and four asymptomatic esparto-exposed stucco makers did not exhibit reaction with any of the tests listed above.

Esparto fibers are widely used in the Iberian Peninsula and other Mediterranean countries for the manufacture of rope, sandals, rush mats, and baskets; it is also used in decorative stucco plates for walls and ceilings and other decorative work in the building industry. Esparto fiber processing includes the manual removal of blades from the plant, followed by "soaking" in water pools for 15–20 days to make it resistant. During this stage, the plant may be contaminated with certain microorganisms such as molds and thermophilic *Actinomyces*, because of favorable moisture and temperature conditions. The mass obtained is then dried, crushed, and combed.

Recently, esparto fibers have been described as a cause of hypersensitivity pneumonitis in stucco makers (1), and *A. fumigatus* was shown to be the causative agent (6). Bronchial asthma due to this fiber has not been previously reported.

An IgE-mediated immunologic mechanism appeared to operate in the patient's asthmatic responses to esparto grass fibers. Skin test and BPT performed on the patient with both EE and AFE gave rise to an immediate response. A late asthmatic reaction was seen after BPT performed with EE, but not AFE. The presence of specific IgE against these two extracts in the patient's serum was demonstrated by ELISA, while a group of unexposed or exposed nonsymptomatic subjects did not react to such tests. We also

found a marked inhibition of the esparto activity by AFE with IgE ELISA inhibition.

In conclusion, we showed that the dust derived from esparto fibers can induce occupational asthma in an exposed subject, and *A. fumigatus* appears to be the causative agent.

Esparto supports a large industry. Further studies are necessary to determine the prevalence of esparto-induced occupational asthma in exposed workers.

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A new apple extract

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Key words: apple; food allergy; oral allergy syndrome; skin testing.

● APPLE is the most frequently reported offending food in birch-pollen-sensitive patients with oral allergy syndrome (OAS) (1) due to homology between the birch-pollen allergen (Bet v 1) and the major apple allergen Mal d 1 (2). Allergens responsible for OAS are mostly unstable and easily denatured by extraction procedures (3); as a consequence, most commercial extracts for skin prick tests (SPT) show very poor sensitivity and are less reliable than fresh fruit for the detection of sensitive patients (4, 5). In this study, we compared a new apple extract for SPT with four conventional apple extracts and with SPT with fresh apple in 35 birch-pollen-sensitive adults (mean 33 years; M/F 11/24) with OAS after eating

apples. Patients underwent SPT with four commercially available apple

The peel extract was useful in SPT diagnosis of pollen-related OAS.

extracts (Lofarma, Milan, Italy; Dome/Hollister-Stier, Spokane, WA, USA; Stallergènes, Fresnes, France; and Allergopharma, Reinbeck, Germany), with fresh Golden Delicious apple (by prick-prick technique), and with a new apple extract (Lofarma: see below). SPT were performed by standard methods; wheals equal to or greater than 2+ were considered positive (6).

Both commercial and new Lofarma apple extracts were prepared from Granny Smith strain apples, using fresh pulp plus peel, or fresh peel only, respectively, which were homogenized at 10% w/v in 0.1 M potassium phosphate buffer, pH 7.0, containing 2% PVPP, 2 mM Na₂EDTA, 7 mM diethyldithiocarbamate (DIECA), and 2.6 mM NaN₃. Homogenate was centrifuged at 18 000 g for 30 min at 4°C. The supernatant of peel extract was dialyzed against NH₄HCO₃ 5 mM at 4°C for 20 h, lyophilized, and resuspended (×10 concentrated) in saline plus phenol 0.4%. The supernatant of the pulp plus peel extract was directly dialyzed against saline plus phenol, since the high content in carbohydrates and pectin did not allow lyophilization and concentration of the

extract. Extracts were diluted 1:2 in glycerol to prepare SPT solutions, or directly biotinylated (0.6 mg of activated biotin/1 mg of extract) for *in vitro* testing.

The *in vitro* allergenic activity of the two Lofarma extracts was compared by Realtest (Lofarma) on sera from 11 patients. A volume of 50 µl of standard IgE (0, 0.5, 1.0, 5.0, 10, and 25 kU/l) or patient sera was added to wells coated with anti-IgE antibodies. After washings, 100 µl of biotinylated anti-IgE (for the reference curve) or biotinylated apple extracts (for sample and control sera) were incubated for 1 h. The use of peroxidase-conjugated streptavidin followed by the addition of the enzyme chromogen and substrate gave rise to a colorimetric reaction. Optical density, read at 405 nm, was expressed as kU/l by interpolation with the reference curve.

All patients were positive on SPT with fresh apple; reactions of ++, +++, and ++++ were observed in 5, 19, and 10 patients, respectively. No reactivity was observed on SPT with DHS, Stallergènes, or Allergopharma apple extracts (sensitivity 0%). Five of 35 patients were positive on SPT with Lofarma standard apple extract (sensitivity 14%); skin reactions were ++ and +++ in four

cases and one case, respectively. Thirty-two of 35 patients were positive on SPT with the new (apple peel) extract (sensitivity 91%); skin reactions were ++, +++, and ++++ in 7, 20, and four patients, respectively. Fresh apple and new extract induced equivalent skin reactions in 21 patients; three and 11 patients showed a stronger skin reactivity to new extract or to fresh apple, respectively.

Serologic analyses confirmed the superior allergenic activity of peel extract in 10/11 cases ($P < 0.01$, Mann-Whitney U-test; Table 1). One of 11 (9%) vs 6/11 (55%) sera did not show reactivity to the new and standard extracts as allergens, respectively.

A slight modification in extraction procedures (the use of peel only rather than pulp plus peel) produced the marked increase in diagnostic sensitivity of the new apple extract tested here. This fact might be due to a higher expression of Mal d 1 in apple peel (7). Alternatively, the extraction of Mal d 1 from pulp might be hampered by the presence of pectin, or differences in biologic activity might simply reflect differences in protein content.

While SPT with fresh material still remains the reference method to diagnose apple allergy, this new extract represents a first step toward the production of reliable commercial diagnostic means of *in vivo* testing.

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Combined immediate- and delayed-type hypersensitivity to metamizole

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Key words: acetylsalicylic acid; drug allergy; metamizole; oral challenge; patch tests.

● NONSTEROIDAL anti-inflammatory agents (NSAIDs) are known to cause adverse drug reactions characterized by symptoms such as asthma, urticaria-angioedema, or anaphylactic shock. Patients who experienced these symptoms may be further subdivided into two groups: those who suffer from acetylsalicylic acid (ASA)-induced asthma, the probable pathogenesis of which is nonimmunologic or pseudoallergic, and those with hypersensitivity reactions to pyrazolone agents (such as noramidopyrine or aminophenazone) in which IgE-mediated reactions have been implicated (1, 2). The presence of an IgE-mediated allergy to such drugs can be confirmed by skin prick tests and intradermal (ID) testing (1, 3). However, cases with positive delayed responses to patch tests have been described (4), especially with late reactions to the drug.

Table 1. Serologic findings

Patient no.	New extract (apple peel)	Standard extract (apple pulp plus peel)
1	2.5	0
2	1.3	0
3	12.4	7.4
4	0.7	0
5	12.1	3.3
6	1.1	0
7	4.7	1.9
8	2.6	0
9	20.7	5.2
10	3.0	2.9
11	0	0

IgE levels are expressed as kU/l. Sera showed significantly stronger reactivity to apple-peel extract ($P < 0.01$).