

Time Dependent Release of Allergens from Some Xerophilic Fungi

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Particles smaller than 10 μm , e.g. diaspores of species of the genera *Aspergillus* and *Wallemia*, may penetrate into the human airways in spite of the presence of mucous membranes. Within 1 h the inhaled material is returned to the pharynx by the aid of these mucous membranes (12). Therefore, substances responsible for allergic reactions must be released within 1 h. Fast release of allergens from pollen has been reported before (4, 5, 6, 7).

A number of investigators have tried in vain to isolate one single substance responsible for the allergic reaction from several kinds of materials (1, 3, 9, 10, 11). The opinion has formed that more than one allergic factor must be involved in allergy. According to Berrens (1) there are two mechanisms: 1) the specific reaction between antigen and antibody; 2) the non-specific reaction between lysine-sugar compounds and the human complement.

In this short communication we will study the effect of extraction time on recovery of the two types of allergic factors and hypothesise on their relevance to the allergic patient.

MATERIAL AND METHODS

The experiments were carried out with the fungi *Aspergillus penicilloides* (Spegazzini), *A.*

repens (de Bary) and *Wallemia sebi* ((Fr.) v. Arx).

The *Aspergillus* species were grown on malt extract agar with 64% sucrose (composition: 25 g malt extract agar (Oxoid), 320 g sucrose and 155 g aqua dest.), *Wallemia sebi* was cultured on a fluid malt medium with 64% sucrose (composition: 36 g malt extract (from a brewery) and 64 g sucrose).

Before the extraction, the fungal material was taken from the medium, lyophilised for 48 h with a Virtis lyophilisator, and homogenized in a mortar with Sephadex G-10. 500 mg (*Aspergillus* species) or 1,000 mg (*Wallemia*) was placed on a Sephadex G-10 column and extracted by leading 90 ml phosphate buffered saline (PBS, pH=7.4) per hour through the column (by means of a LKB pump, type 2120 varioperpex II). Extinction at 280 nm was measured continuously during the extraction process. The extract was sampled in fractions of 2 min flow during 40 min.

The corrected coefficient E_{305}/E_{280} (E_{280} corrected = $E_{280} - E_{305} \times 0.82$) is a rough measure for the concentration of lysine-sugar conjugates in the extract (1). The coefficient was calculated for the combined fractions of 0–10 and 10–20 minutes. The extinction was measured with a Beckman photospectrometer Model 25.

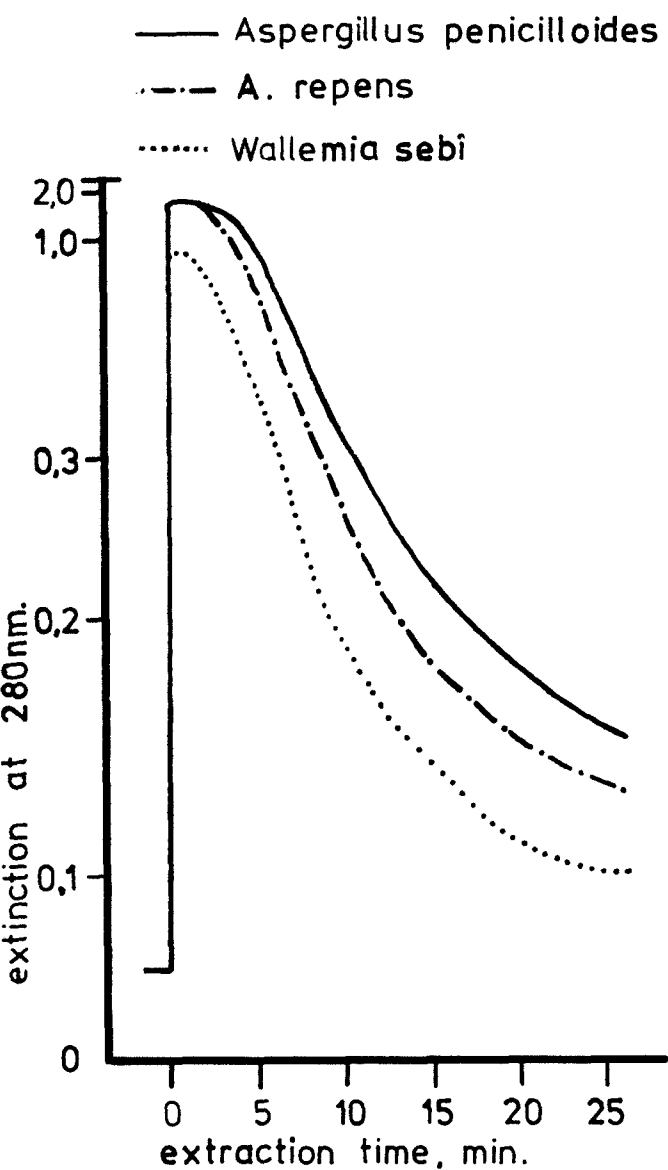


Fig. 1. Extinction at 280 nm during the first 26 min of extraction of 500 mg dry material of *Aspergillus penicilloides* and *A. repens* and of 1000 mg dry material of *Wallemia sebi*. (Extraction fluid: PBS; temperature: 4°C; pumping speed of fluid: 90 ml/h).

The radioallergosorbent test (RAST) has been carried out in cooperation with the *Centraal Laboratorium van de Bloedtransfusiedienst* in Amsterdam. The combined fractions at 0–6, 6–12, 12–18 and 18–40 min were dialysed against aqua bidest. during 48 h at 4°C, lyophilised, redissolved in buffer (composition: 8 g NaHCO₃ + 29 g NaCl/liter aqua dest., pH=8.0) and coupled to CNBr-activated sepharose. The protein content of the fractions varied from 300 to 2,000 µg as measured according to Lowry (8). The coupled fractions were sent to the *Bloedtransfusiedienst*. There 0.5 mg sepharose (with antigen) was challenged with 50 µl serum from two patients

hypersensitive to *Aspergillus fumigatus*. Earlier experiments have shown that a cross reactivity exists between extracts of *Aspergillus penicilloides*, *A. repens*, *Wallemia sebi* and *A. fumigatus* (unpublished data). The binding of added radioactivity (%) was judged as follows:

- 0 – 2%: negative
- 2 – 5%: weakly positive
- 5 – 10%: positive
- 10 – 20%: strongly positive
- >20%: very strongly positive

RESULTS

The main part of the protein-containing material is set free within the first 20 min (Fig 1).

The corrected coefficient E_{305}/E_{280} (Table 1) shows that both combined fractions contain lysine-sugar conjugates. The later fractions (10–20 min extraction) contain more of these compounds than the first fractions (0–10 min extraction).

In all cases the highest percentages of binding in the RAST (Table 2) were seen in the first fractions, irrespective of the protein content. The last fractions (18–40 min extraction) had a low percentage of binding, although they contained a large amount of protein.

Table 1
Corrected coefficient E_{305}/E_{280} , calculated for extracts of *Aspergillus penicilloides*, *A. repens* and *Wallemia sebi*

Fungal species extracted	Extraction time (min)	Corrected E_{305}/E_{280}
<i>A. penicilloides</i>	0–10	1.44
	10–20	1.72
<i>A. repens</i>	0–10	1.45
	10–20	1.72
<i>Wallemia sebi</i>	0–10	1.42
	10–20	1.77

Table 2
Binding of added radioactivity (%) in the RAST of sera of two patients, hypersensitive to *Aspergillus fumigatus*, challenged with extracts of *A. penicilloides*, *A. repens* and *Wallemia sebi*

Fungal species extracted	Extraction time (min)	μg Protein	Binding of added radioactivity (%)	
			1	2
<i>A. penicilloides</i>	0-6	1000	4.7	15.8
	0-6	2000	4.9	17.5
	6-12	1000	3.1	9.6
	12-18	700	2.5	4.7
	18-40	1400	1.8	3.3
<i>A. repens</i>	0-6	500	3.6	17.4
	0-6	1000	3.5	17.4
	6-12	550	3.5	14.8
	12-18	300	3.4	11.8
	18-40	1000	3.1	9.5
<i>Wallemia sebi</i>	0-6	600	3.4	14.7
	6-12	600	2.2	4.7
	12-18	400	1.4	1.8
	18-40	600	1.1	1.5

DISCUSSION

Most of the protein-containing material and most of the RAST-active compounds are released within the first 20 min of the extraction. This is in accordance with the research done on pollen (4, 5, 6, 7). The results of the RAST could be negatively influenced by the fact that the test is performed with sera of patients hypersensitive to *Aspergillus fumigatus*.

The corrected coefficient E_{305}/E_{280} shows the presence of a considerable amount of lysine-sugar conjugates in the two combined fractions. The coefficient increases with the extraction time, but is always higher than the one found for house dust (extracted at room temperature during 48 h): 1.42-1.77 against 1.22 (2). The Folin reagent measures all proteins, whether immunologically (IgE) active or not. The total amount of protein increases with the extraction time just as the coefficient does for the lysine-sugar conjugates.

Apparently the nature of the allergenic factors recovered depends upon the extraction time. Commercial fungal allergen preparations are usually obtained after 24 to 48 h extraction. The patient, however, only "extracts" the fungal material for 1 h. *In vivo*

therefore the IgE-active material could be more prominent than the lysine-sugar conjugates.

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