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MOULD ALLERGY

III. *Extract preparation of moulds*

By

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INTRODUCTION

In clinical studies on allergy, it is important to use extracts produced by a mild and efficient extraction procedure. Preliminary investigations on extract preparation of moulds have shown submerged cultivated mycelia to be valuable in skin tests (1). There are many advantages to use mycelia instead of spores for the extract preparation: mycelia can easily be obtained in pure form and in great quantities (1) though spores sufficient for the extract preparation only can be assembled with great labour. Since spores, however, seem to be the most frequent airborne forms of fungi (2), they may be thought to be the causative agent of allergic diseases. For this reason it is of importance to know whether there are any great differences between mould spores and mycelia from an allergological point of view.

The following is a report on the extractability of mycelia and spores and includes information on skin reactions in humans and on some diffusion-in-gel studies.

MATERIAL AND METHODS

Antigen.

Mycelia were derived from *Botrytis cinerea* Pers., *Penicillium frequentans* Westl., *Aspergillus niger* v. Tieghem and *Aspergillus repens* (Cda.) de Bary, by submerged cultivation as described in a previous publication (1). Following cultivation, the medium was filtered off from the mycelia through a Büchner funnel until the mycelia formed a hard felt-like mat. It was quickly frozen to

-20°C . The dry weight of the mat varied between 20 % and 25 % of the wet weight (P_2O_5 , 90°C , 24 h). One portion of the mycelia was disintegrated in the X-press described by Edebo (3). The X-press was operated at -25° to -30°C and the material was forced through a 2.5 mm hole, which required a pressure of 2–4 tons/cm². This was accomplished with the aid of a hydraulic jack. The result of the disintegration was controlled microscopically. It was found that three cycles were sufficient for a complete disintegration (Figs. 1 and 2). The disintegrated and non-disintegrated mycelia were extracted separately with 0.1-m sodium phosphate containing 0.015-m citrate. The citrate in the buffer was used in order to chelate polyvalent metal ions which might possibly activate degrading enzymes. The buffer was adjusted to pH 7.0. The material was homogenized with the buffer (one part wet weight, two parts buffer solution) by means of an Ultra Turrax TP 18/2 blender in a cold bath (30 seconds at 0°C). The suspension was then centrifuged at $50,000 \times g$ for 120' at 0°C (Spinco L). One extraction proved to be sufficient since it removed 80–90 % of the extractable nitrogen. The extract was poured off and sterilized through a Seitz filter (EK 6).

Spores were derived from the moulds mentioned in the preceding paragraph. They were obtained from cultures on Petri dishes using the basal medium (1) employed in the production of mycelia, with an addition of 2.5 % agar (Difco). *Botrytis cinerea* Pers. was however cultivated as surface cultures in liquid medium in Erlenmeyer flasks since this method gave heavier sporulation. After the spore formation had reached its apparent maximum, the spores were sucked off with the aid of a Thiessen filter (4). The spores which followed the air current adhered to a membrane filter and were afterwards transferred to a glass bottle. Microscopic control showed that the spores were free from mycelia. One portion of the spores was disintegrated in the same way as the mycelia (Figs. 3 and 4). The spores were forced through the X-press 5 times instead of 3 times, however. About 80 per cent was disintegrated, according to microscopic evaluation. Further compressions were avoided since too many cycles might cause considerable denaturation of proteins. The disintegrated and non-disintegrated spores were separately homogenized, extracted and sterile filtered in the same way as the mycelia. The amount of the buffer solution was chosen so that the spore-buffer relation was the same as for mycelia-buffer, as calculated on a dry weight basis.

Sterility was controlled aerobically and anaerobically. The sterile extracts were taken to clinical and diffusion-in-gel examinations.

Antisera were prepared by injecting rabbits intravenously with a suspension of the disintegrated spores and mycelia, respectively, containing 1 mg dry weight per ml in 0.15-m NaCl. There were eight series of rabbits (I–VIII) and each series contained three rabbits.

Each rabbit was administered 1 ml of the suspension every third day during five weeks and, after an interval of three weeks, the rabbits received a booster of 1 ml and were then bled. No rabbits succumbed during the procedure. The

immunsera from the rabbits which in each series, gave the strongest precipitation lines against homologous antigen, were used for the immunological analyses.

	Disintegrated mycelia	Disintegrated spores
<i>Botrytis cinerea</i> Pers.	I	V
<i>Penicillium frequentans</i> Westl.	II	VI
<i>Aspergillus niger</i> v. Tieghem	III	VII
<i>Aspergillus repens</i> (Cda.) de Bary	IV	VIII

The extracts and sera were stored at -20°C , dispensed in 3 ml portions and thawed only once.

Gel filtration of the extracts was performed on Sephadex G 25 coarse (5, 6, 7). The temperature in the columns was $+1^{\circ}\text{C}$. The column size was $1 \times 45\text{ cm}$ and the elution rate was 40 ml/h. The column was charged with 3 ml extract containing, in total, about 12 mg N.

Determination of the nitrogen concentration was made by the micro Kjeldahl technique.

Immunological analyses were carried out with the double diffusion method described by Ouchterlony (8, 9). The gel contained 1 % agar, 0.15 M NaCl and 1:1000 NaN_3 . The hydrogen ion concentration was adjusted to 10^{-7} . The basins in the gel were stamped out with templates according to *Feinberg* (10). The extracts and the sera in the basins were carefully sucked out and refilled once a day for three consecutive days. The diffusion took place at room temperature and the precipitation lines were recorded as drawings and photographs.

Concentration of the antisera and the extracts was performed by the cellugel method described by *Palmstierna* (11).

Absorption tests were performed by mixing disintegrated or non-disintegrated mycelia or spores with the sera in the form in which they were obtained from the culture (after filtration and air suction). It was found that the necessary amount was 100 mg dry weight of mycelia or spores per ml serum. The absorbed serum was stored at $+4^{\circ}\text{C}$ overnight before use.

Dryweights were estimated by weighing the mycelia and the spores after they had dried in a vacuum exsiccator over P_2O_5 for 24 hours at $+90^{\circ}\text{C}$.

Intracutaneous test was carried out on human beings sensitive to moulds. The extracts were also tested on non-allergic persons for control.

RESULTS

Extraction.

The result of the extractions is described in Table 1. Nitrogen values were taken as a measure of the efficiency of the extraction. It could be shown that it was more difficult to ex-

TABLE 1
Extraction results of non-disintegrated and disintegrated mycelia and spore extracts.

	Dry-weight of the mould extracted g	Total nitrogen content mg N/g		mg N extracted			
				Mycelia		Spores	
		Mycelia	Spores	Non-disintegrated	Disintegrated	Non-disintegrated	Disintegrated
<i>Aspergillus repens</i> (Cda.) de Bary	1	50.0	60.0	10.7 (21 %)	31.4 (63 %)	6.9 (13 %)	29.1 (49 %)
<i>Botrytis cinerea</i> Pers.	1	54.4	73.7	23.3 (43 %)	34.7 (64 %)	4.6 (6 %)	31.2 (43 %)
<i>Penicillium frequentans</i> Westl.	1	59.3	47.0	20.3 (34 %)	41.2 (74 %)	3.9 (9 %)	18.9 (41 %)
<i>Aspergillus niger</i> v. Tieghem	1	60.0	57.5	19.4 (33 %)	37.1 (62 %)	19.3 (34 %)	24.6 (43 %)

TABLE 2
Estimation of the amount of low molecular N-containing compounds in non-disintegrated mycelia- and spore extracts by means of Sephadex G 25 coarse.

	% N in the mould extracts belonging to low molecular N-containing compounds	
	Non-disintegrated mycelia extract	Non-disintegrated spore extract
<i>Aspergillus repens</i> (Cda.) de Bary	18 %	22 %
<i>Botrytis cinerea</i> Pers.	19 %	24 %
<i>Penicillium frequentans</i> Westl.	20 %	25 %
<i>Aspergillus niger</i> v. Tieghem	15 %	18 %

tract nitrogenous compounds from the spores than from the mycelia. This may be explained by the fact that the disintegration of the spores was not complete. The extracted nitrogen from the non-disintegrated spores and mycelia was generally much lower than that from the disintegrated ones. This was most marked when using spores. This may in part be explained by the fact that it was difficult to incorporate the spores in the buffer solution during the extraction procedure. Non-disintegrated *Aspergillus niger* spores were, however, rather easily mixed with the solution. A treatment of the spores with ethyl ether and 0.1 % Tween 80 gave, however, only slightly better extraction results and we therefore excluded ethyl ether and Tween 80 from our experiments. Chemically these substances may also be either denaturing and/or disturbing during succeeding fractionation procedures.

Gel filtration.

In the use of nitrogen as a measure of the extraction efficiency, it is of interest to know whether the medium remaining around the mycelia after the filtration (Büchner) has any influence on the nitrogen content of the extract. The medium (1) is composed of low molecular weight compounds which are easily removed by passing the extract through a Sephadex G 25 coarse column. Using this method it is possible to remove molecules up to m.w. of 4,000–10,000 (as compared to Dextran). Table 2 shows that the values of low molecular nitrogen products are about the same in the mycelia- and spore extracts, which is in accordance with *Taha* and *Knights* (41, 42). With regard to these values, it is unlikely that large amounts of nitrogen in the medium have influenced the nitrogen values of the primary extracts.

Serology.

Gel diffusion was performed in order to analyse the precipitinogen contents of disintegrated and non-disintegrated mycelia and spores.

TABLE

Results of intracutaneous tests by means of extracts of non- and disintegrated spores and N/ml). Reaction expressed as sum of horizontal and vertical "diameters" of blisters: A = non-disintegrated;

	Penicillium frequentans Westl.				Aspergillus niger v. Tieghem			
	Mycelia		Spores		Mycelia		Spores	
	A	B	A	B	A	B	A	B
D. L.	—	+	—	+	—	—	—	—
G. B.	(+)	+	(+)	+	—	—	—	—
A. R.	—	+	+	+	—	—	—	—
C. B.	+	++	(+)	++	—	+	(+)	(+)
S. B.	—	+	(+)	+	—	—	—	—
S. F.	—	+	—	(+)	—	—	—	—
Eb. E.	—	+	+	+	—	—	—	(+)
S. J.	—	—	—	—	—	(+)	—	(+)
A. K.	+	++	(+)	+	—	—	—	—
Ei. E.	—	+	—	+	—	—	—	—
E. H.	(+)	++	+	++	++	++	++	++
S. L.	—	+	—	++	—	(+)	—	+

TABLE

Results of intracutaneous tests by means of extracts of non- and disintegrated spores and of the mycelia and the spores. 1 g extracted with 9 ml buffersolution = 1:10. The extracts expressed as sum of horizontal and vertical "diameters" of blisters: —: no A = non-disintegrated;

	Penicillium frequentans Westl.				Aspergillus niger v. Tieghem			
	Mycelia		Spores		Mycelia		Spores	
	A	B	A	B	A	B	A	B
D. L.	+	++	—	(+)	—	—	—	—
G. B.	+	+++	—	+	—	—	—	—
A. R.	—	++	—	+	—	—	—	—
C. B.	+	++	—	+	+	++	+	+
S. B.	—	++	—	(+)	—	—	—	—
Eb. E.	—	+	—	—	+	++	(+)	+

3
mycelia on mould sensitive patients. Intracutaneous injection of 0.05 ml solution (0.05 mg
—: no reaction; (+): < 20 mm; +: 20–30 mm; ++: 30–40 mm; +++: 40–50 mm.
B = disintegrated.

Aspergillus repens (Cda.) de Bary				Botrytis cinerea Pers.				Hist-amine 1:10000	Buffer 1:1	Sub- strate 1:1
Mycelia		Spores		Mycelia		Spores				
A	B	A	B	A	B	A	B			
+	++	—	+	—	—	—	—	++	—	—
+	++	(+)	+	(+)	+	—	+	+	—	—
(+)	+	(+)	+	—	—	—	—	+	—	—
—	+	(+)	+	++	++	+	++	+	—	—
—	—	—	—	—	—	—	—	+	—	—
+	++	—	+	—	(+)	(+)	(+)	+	—	—
+	++	+	+	—	—	—	—	+	—	—
+	+	—	(+)	—	—	—	—	+	—	—
+	++	+	+	+	++	+	++	+	—	—
—	—	—	—	—	(+)	—	(+)	+	—	—
+	+++	++	+++	+	++	—	++	+	—	—
—	—	—	—	—	—	—	—	+	—	—

4
mycelia on mould sensitive patients. The extracts are diluted with regard to the dryweight
used for testing are diluted 1:1000. Intracutaneous injection of 0.05 ml solution. Reaction
reaction; (+): < 20 mm; +: 20–30 mm; ++: 30–40 mm; +++: 40–50 mm.
B = disintegrated.

Aspergillus repens (Cda.) de Bary				Botrytis cinerea Pers.				Hist-amine 1:10000	Buffer 1:1	Sub- strate 1:1
Mycelia		Spores		Mycelia		Spores				
A	B	A	B	A	B	A	B			
+	++	—	+	—	—	—	—	++	—	—
++	+++	—	+	+	++	—	+	+	—	—
—	++	—	—	—	—	—	—	+	—	—
+	++	—	+	++	+++	(+)	++	+	—	—
—	—	—	—	—	—	—	—	+	—	—
+	++	—	(+)	—	—	—	—	+	—	—

Extracts from disintegrated mycelia were found to give very strong precipitation lines in comparison to the other extracts, when nitrogen values were used as a measure of the concentration. Extracts from non-disintegrated mycelia had to be concentrated 3-4 times in order to give precipitation lines of similar intensity.

The anti-sporesera had to be concentrated 4-5 times in order to give precipitation lines against corresponding antigens. However, in spite of concentration experiments the non-disintegrated spore extract still gave weak lines and therefore poorly reproducible results.

Disintegrated and non-disintegrated mycelial extracts were analysed against their homologous immunsera, but, in the comparison between mycelia and spores, they were analysed against a mixture of equal amounts of anti-mycelial serum and a fourfold concentrate of anti-sporeserum.

The results are shown in Figs. 5-12. No reactions of immunological non-identity could be detected. The results do not, however, exclude possible partial identity reactions. Support for the immunological relation between spores and mycelia was provided by the fact that all precipitation lines disappeared after absorption of the sera with both disintegrated and non-disintegrated spores or mycelia.

Skin tests.

The mycelial and spore extracts were employed in intracutaneous tests on mould sensitive patients. Table 3 shows that there is no greater difference in skin reactivity between disintegrated mycelia and spores, as estimated on nitrogen basis. Disintegrated mycelia- and spore extracts, however, gave stronger skin reactions than the non-disintegrated ones (Fig. 13).

If the extracts were diluted on the dry weight basis, it could be shown that the disintegrated mycelia gave a stronger skin reaction than the other kinds of extracts (Table 4). The extracts and the culture medium gave no positive skin reaction on non-allergic persons.

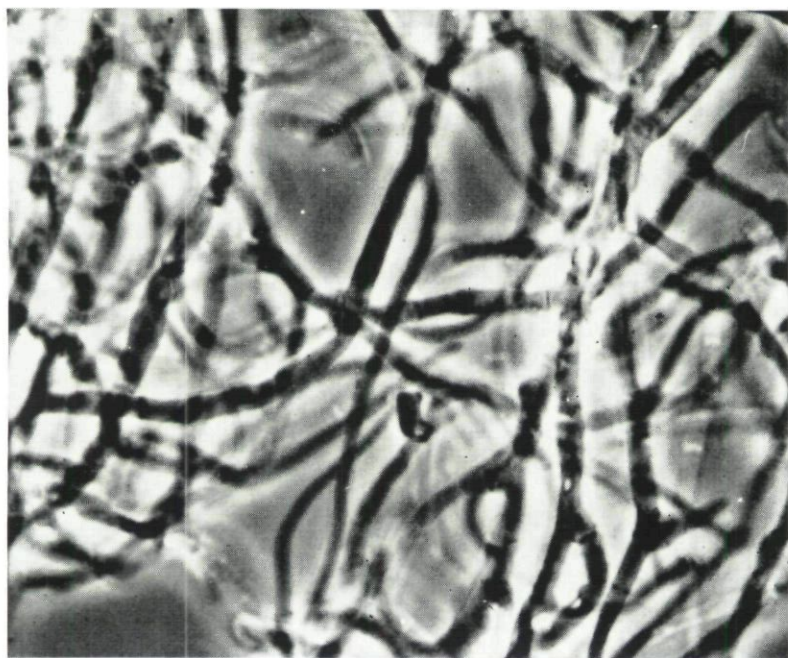


Fig. 1.

Non-disintegrated submerged cultivated mycelia of *Botrytis cinerea* Pers.
(Phase contrast photomicrograph) $\times 500$.

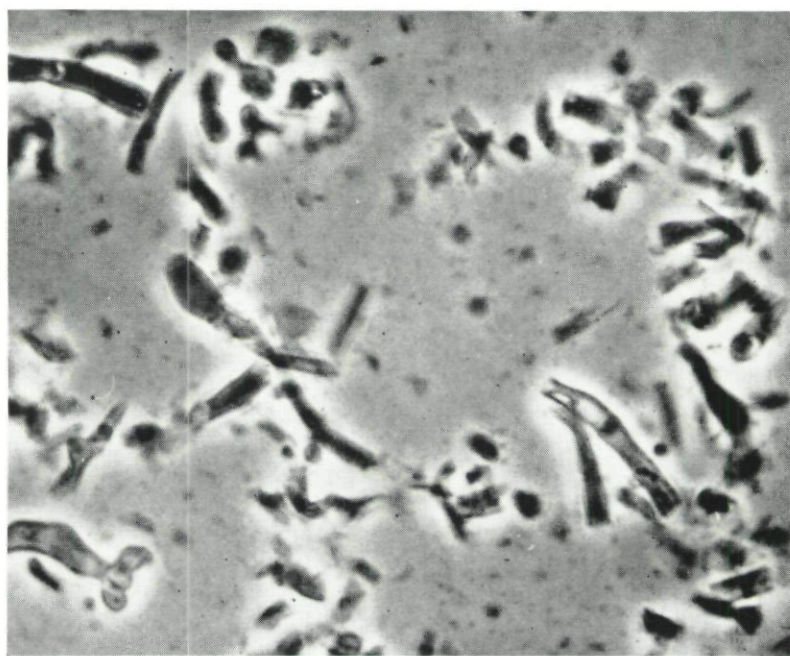


Fig. 2.

Disintegrated submerged cultivated mycelia of *Botrytis cinerea* Pers.
(Phase contrast photomicrograph) $\times 500$.

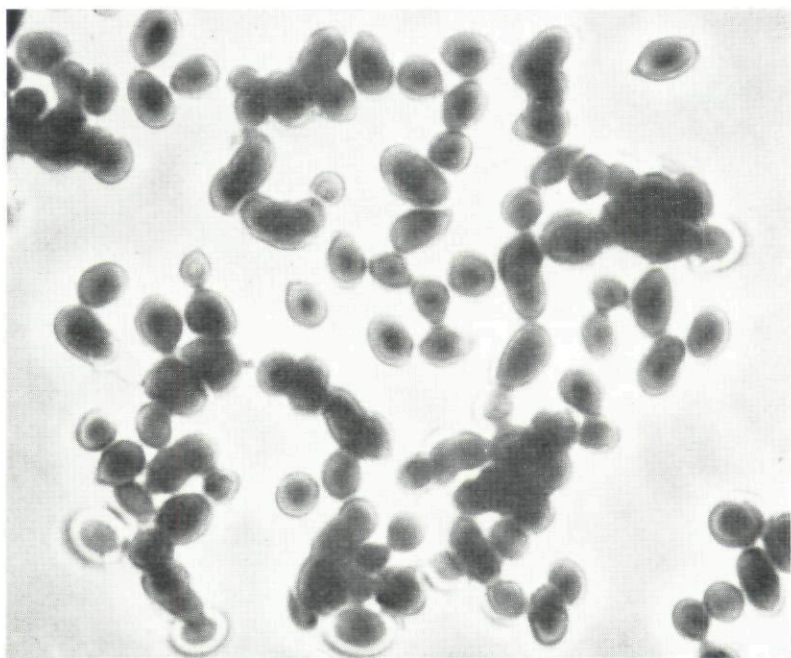


Fig. 3.

Non-disintegrated spores of *Botrytis cinerea* Pers.
(Phase contrast photomicrograph) $\times 1000$.

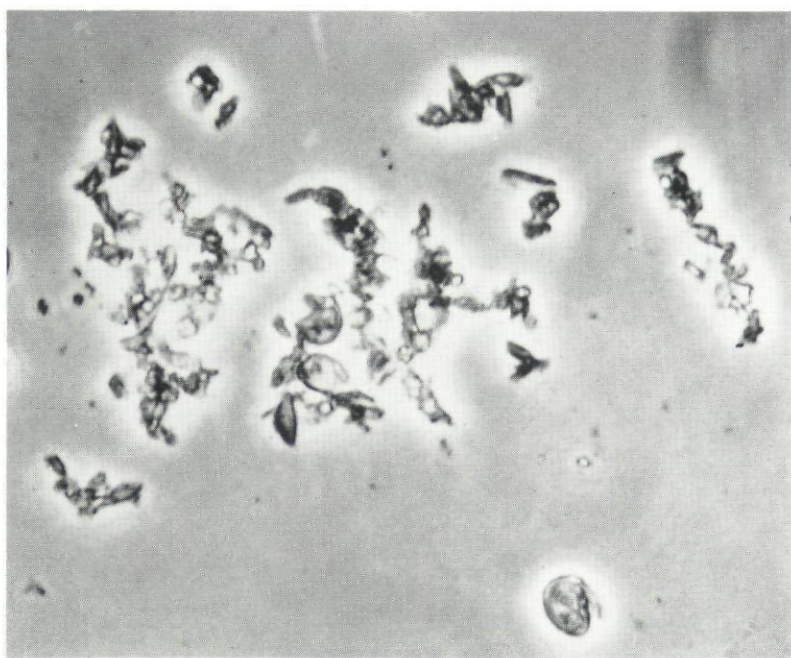


Fig. 4.

Disintegrated spores of *Botrytis cinerea* Pers.
(Phase contrast photomicrograph) $\times 1000$.

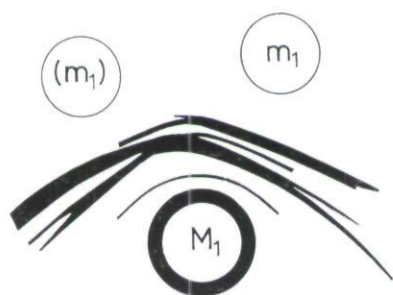
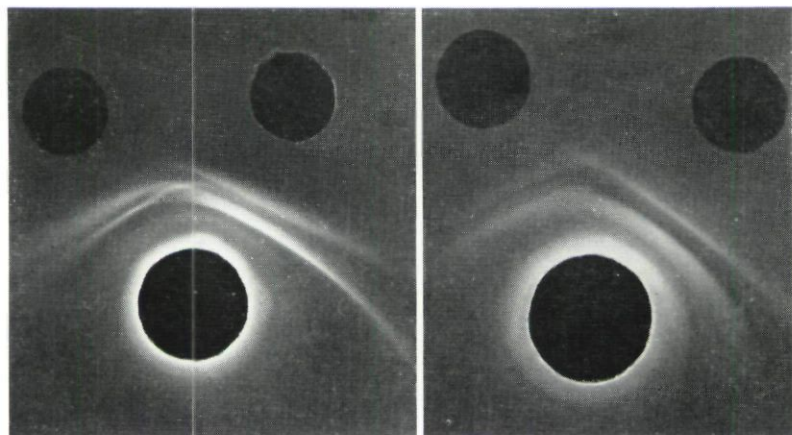


Fig. 6.

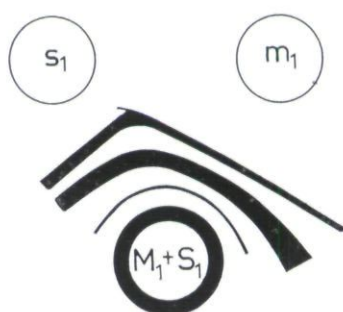


Fig. 5.

Aspergillus repens (Cda.) de Bary.

Figs. 5-12.

Photographs and schematic representations of precipitation patterns obtained by comparative analyses of a) non-disintegrated- disintegrated mycelia, b) disintegrated mycelia-disintegrated spores with their respective immusera.

Immune systems: (m) = non-disintegrated mycelia extract.

m = disintegrated mycelia extract.

s = disintegrated spore extract.

M+S = equal amount of anti-mycelia rabbitserum and four time concentrated anti-spore rabbitserum.

M = anti-mycelia rabbitserum.

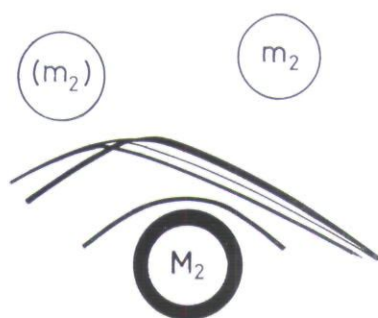
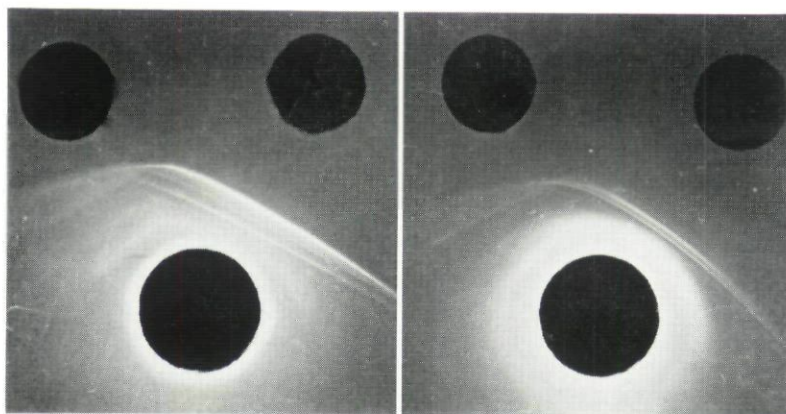


Fig. 7.

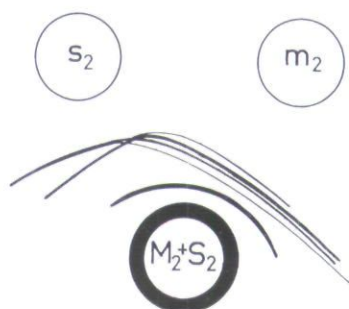
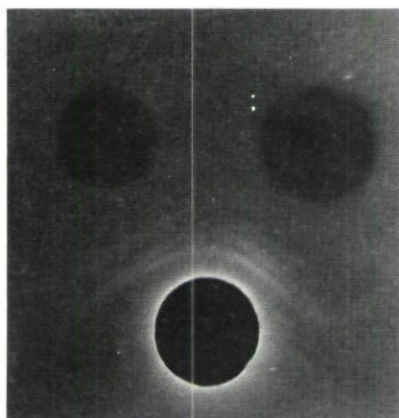


Fig. 8.

Botrytis cinerea Pers.



* Because of the dark *Aspergillus niger* spore pigments which colour the gel no photograph could be obtained from the spore-mycelia precipitation pattern.

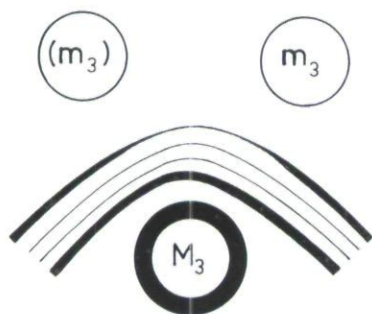


Fig. 9.

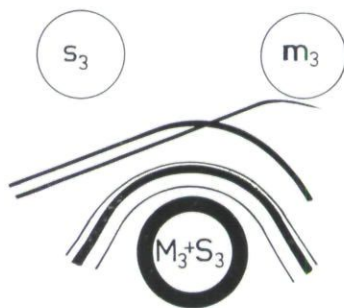


Fig. 10.

Aspergillus niger v. Tieghem.

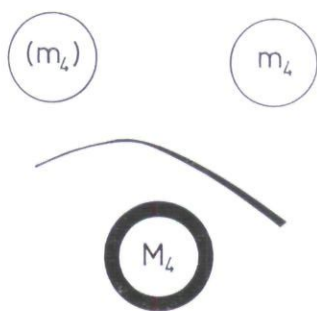
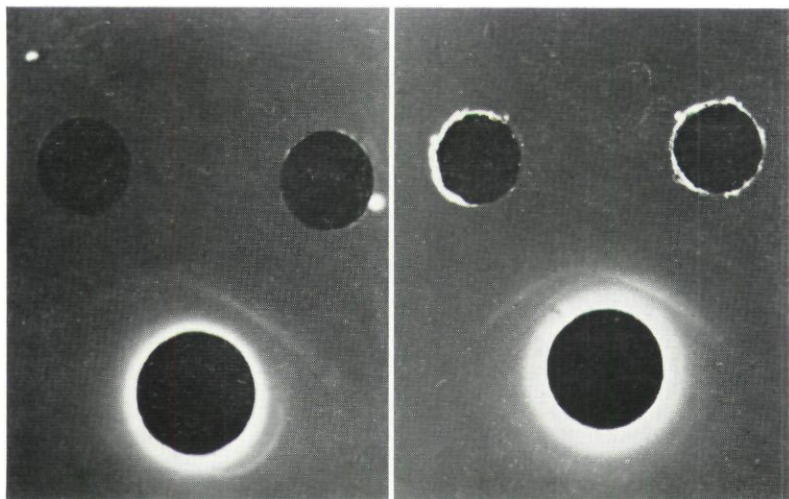


Fig. 11.

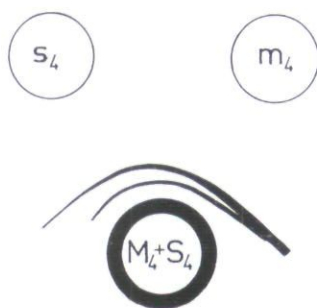
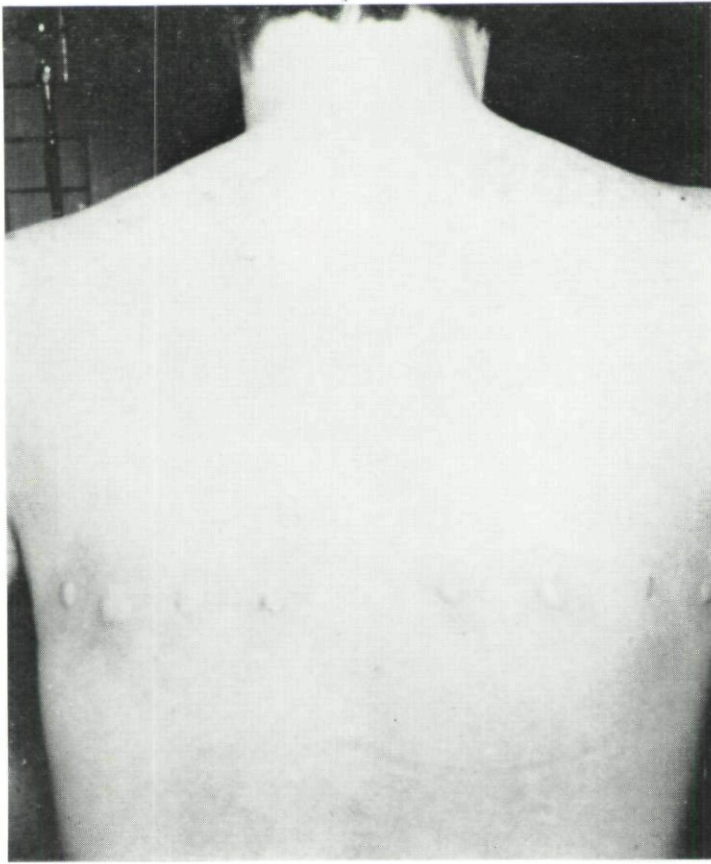


Fig. 12.

Penicillium frequentans Westl.



1 2 3 4 5 6 7 8

Fig. 13.

Intracutaneous test by means of non- and disintegrated mycelia and spores.
0.05 mg N/ml.

From left to right:

- | | | |
|------------------------------|---|--|
| 1. Non-disintegrated mycelia | } | <i>Aspergillus repens</i> (Cda.) de Bary |
| 2. Disintegrated mycelia | | |
| 3. Non-disintegrated spores | | |
| 4. Disintegrated spores | | |
| 5. Non-disintegrated mycelia | } | <i>Botrytis cin.</i> Pers. |
| 6. Disintegrated mycelia | | |
| 7. Non-disintegrated spores | | |
| 8. Disintegrated spores | | |

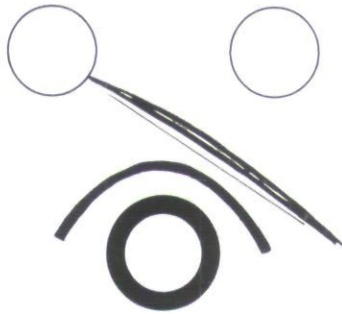
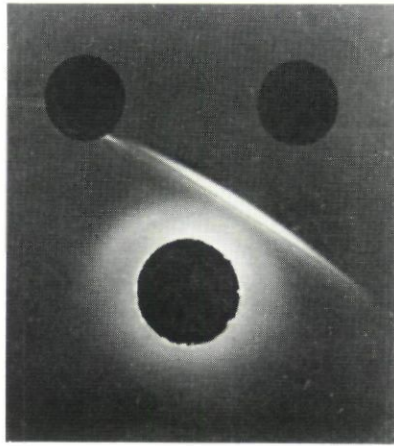


Fig. 14.

Photograph and schematic representation of precipitation pattern obtained by comparative analyse of a purified fraction (left) and the crude extract (right) of *Botrytis cinerea* Pers with rabbit anti-serum.

DISCUSSION

Moulds have been extracted by different means (12, 13, 14, 15, 16, 17, 39, 41, 42).

It is not difficult to extract most of the cell content (such as nitrogen products) from the mould, but it is difficult to do it without causing a degradation of the cell content. Many of the high molecular weight protein compounds are labile and are easily degraded at room temperature (18). Actively growing cells contain degrading enzymes, the activity of which it is important to repress. For that reason we have worked with the extracts at as low temperatures as feasible.

We have used nitrogen data as a measure of the efficiency of the extractions. These data were also used as a basis for the test dilutions. The reason for this choice was that the allergens might possibly be nitrogen-containing substances or combined with nitrogen-containing compounds (19, 20, 21, 22, 23). We have also found during our attempts to isolate some of the principal allergens in *Penicillium frequentans* Westl., *Aspergillus niger* van Tieghem and *Botrytis cinerea* Pers. by counter-flow electrophoresis, chromatography and gel filtration through Sephadex G 75 coarse (7) that the activities of those allergens purified, tested on mould sensitive patients, are associated with substances with molecular weights higher than 30.000-40.000, and preliminary analyses of these substances have shown them to contain aminoacids as well as carbohydrates. The investigations do not exclude, however, that the allergenic activity may be caused by a low molecular weight compound, coupled to a high m.w. carrier. The purification attempts of pollen extracts described by different investigators (7, 24, 25, 26, 27, 28, 29, 30, 31) including ammonium sulfate or alcohol precipitation, dialysis, electrophoresis, chromatography and gel-filtration have also shown that the allergens seem to be proteins or polypeptides combined with carbohydrates.

The skin test did not show any significant difference between the reactivity of disintegrated spores and mycelia. Dif-

ferent investigators (12, 32, 33) have found, however, that the spore extracts give a stronger skin reaction. It is impossible to compare these results with ours, since they have used different extraction- and standardization methods. In our case there was a stronger skin reaction, when the patient was tested with the disintegrated spores and mycelia than with the non-disintegrated spores and mycelia, as estimated from nitrogen values. (Table 3, fig. 13). This may possibly be dependent upon insufficient extraction of the non-disintegrated mould with low concentration of allergens.

Many authors have used the diffusion-in-gel technique in order to compare the precipitinogen content in different bacteria, pollen, moulds and other substances which may cause allergic diseases (23, 25, 34, 35, 36, 37, 38), but no comparison has in our knowledge been carried out regarding relationships between the precipitinogens derived from mould spores and mould mycelia. Our study on the precipitinogens in disintegrated and non-disintegrated mycelia and disintegrated spores and mycelia has not shown any reactions of non-identity between the samples thus compared. The antispora-sera had to be concentrated 4-5 times in order to give precipitations-lines against corresponding antigens. This is in accordance with the results of *Fukui et al.* (43) who showed that spores gave poor sera under their conditions also.

The number of lines was in most cases higher in the disintegrated material. All the precipitation lines did, however, disappear after absorption. Some of the precipitation lines had a different diffusion position from respective antigen basins. This may indicate a quantitative difference between the precipitinogens in disintegrated and non-disintegrated spore and mycelia extract.

Several investigators have studied the chemical composition of mould spores and mycelia. *Maruyama* and *Alexander* (39) have investigated the distribution of protein and nucleic acids in hyphae and microconidia from *Fusarium oxysporum*. They found a difference in the distribution of RNA and DNA in the spores and mycelia, but that "the general patterns of distri-

bution of protein, nucleic acid and polysaccharides in the conidia, however, were similar to those previously observed in the hyphae". *Meyers* and *Knight* (40) have found, during their studies on the "pool" of the moulds, that the "pool" was similar in both vegetative cells and spores. *Taha* and *Knights* (41, 42) found that, in their albumin and globulin contents, mycelial proteins did not differ from the spore proteins. *Fukui* and *Yasuda* (43) studied the antigen equipment of conidial and mycelial suspensions of *Aspergillus fumigatus* by means of precipitation and complement fixation tests and found that the conidial suspension reacted with antimycelial serum from *Aspergillus fumigatus*. They found that the reactivity of conidial suspension was far lower than that of the mycelial extract. All these investigations and the reported results of diffusion-in-gel studies and the intracutaneous tests support a close relationship between spores and mycelia.

The antigenic structure of the bacterial spore and the vegetative cells have been studied through agglutination and precipitation experiments by several investigators. By agglutination, *Howie et al.* (44) and *Bekker* (45) did not find that the bacterial spore antigens possess antigens related to the vegetative cells. Biochemical differences between spores and vegetative cells have been described (46, 47) and have thus supported these findings. In contrast to this, however, are the investigations of *Krauskopf et al.* (48) and *Doak et al.* (49) who found a serological relationship between spores and vegetative cells using agglutination- and precipitation tests. In a review (50), J. R. Norris has summed up all the investigations in this field and has established that most of the investigations indicate that the endospores possess antigens which are not represented in the vegetative growth phase. *Doak et al.* (49) have, however, made an attempt to reconcile the two opinions by stating that the bacterial spore has an antigenically complex structure, a complexity extending to the cell surface. Common spore and vegetative cell agglutinogens can be shown to exist inside the cells.

If consideration is given to the difference in morphology

and the aforesaid findings of the bacterial spore and the vegetative cell, it should be reasonable to expect that a difference also exists in surface antigens of mould spores and mycelia. In our experiments, however, the non-disintegrated spore extract gave weak lines and therefore poorly reproducible results. Disintegrated spores and mycelia did not, however, show any reactions of non-identity.

It is, of course, not certain that all the precipitinogens are allergenic for man; it is in fact, not certain that the precipitinogens are allergenic for man at all. Preliminary experiments with one of our purified fractions from *Botrytis cinerea* Pers. (which gave a skin reaction about 100–200 times stronger than that of the crude extract when it was diluted according to nitrogen values (7)) gave in spite of concentration experiments only one precipitation line with its homologous rabbit antiserum instead of four with the crude extract (Fig. 14). During their investigations with pollen and horse dandruff allergens and diffusion-in-gel studies *Goldfarb et al.* (51, 52), *Augustin et al.* (28) and *Stanworth* (53) also found that many of the precipitinogens are not allergenic for man.

Extreme caution must be exercised when comparing the gel diffusion tests with the skin reactions. The precipitating antibodies are not the same as the skin-sensitizing antibodies (re-agins) (54, 55, 56, 57) and the reactivity in rabbits is not the same as in human beings.

SUMMARY

The results of an extraction procedure of disintegrated and non-disintegrated mould spores and mycelia, and the skin reactivity on mould-sensitive patients to the spore and mycelia extracts, are described. A serologic comparison is performed employing the diffusion-in-gel technique of the precipitinogen contents of disintegrated and non-disintegrated spores and mycelia. It is shown that

- a) the efficiency of the extraction is highest in the disintegrated mycelia as estimated on the nitrogen basis,

- b) no reactions of immunological non-identity between disintegrated and non-disintegrated mycelia and disintegrated spores and mycelia could be detected,
- c) the concentration of precipitating antibodies seems to be lower in rabbits immunized with spores than with mycelia,
- d) there is no significant difference between disintegrated spores and mycelia as shown by intracutaneous tests on mould-sensitive patients, as estimated on the nitrogen values, but
- e) there is a weaker skin reaction when using non-disintegrated instead of disintegrated spores and mycelia, as estimated on the nitrogen basis.

Disintegrated submerged cultivated mycelia was found to be valuable in the clinical investigation of mould allergy, due to the fact that they can be obtained in reasonably large amounts, they do not consist of mixtures of different growth forms, they are easily extracted and they give reproducible results.

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