

Immediate Type Hypersensitivity to Common Moulds

Comparison of Different Diagnostic Materials

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Extracts of five common atmospheric moulds (*Alternaria*, *Aspergillus*, *Cladosporium*, *Mucor* and *Penicillium*) obtained from different manufacturers were compared by means of skin prick testing and, partly, with the radioallergosorbent test. Considerable differences were found between the extracts with respect to positive tests found in mould-allergic individuals and controls. A purified allergen preparation of *Cladosporium herbarum* was superior to the crude allergen extracts. The results suggest that the allergen contents of the extract differed both with respect to total allergenic potency and to allergenic composition.

Key words: diagnostic tests, immediate hypersensitivity, moulds.

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The diagnosis of respiratory allergy to moulds is often difficult because the history may be equivocal, and the allergen extracts available for objective diagnosis may be far from satisfactory, as pointed out more than 30 years ago by Browning (12) and by Nilsson & Aas (17). Studies in our laboratories, and by others, have demonstrated a marked heterogeneity of mould extracts, and that the quality and composition of the extracts depend on many variables (8, 18, 24).

This led us to investigate in more detail to what degree various commercial allergen extracts of five of the most common atmospheric moulds (*Alternaria*, *Aspergillus*, *Cladosporium*, *Mucor* and *Penicillium*) differ with respect to total allergenic activity in diagnostic skin prick test (SPT) and radioallergosorbent test (RAST). Since it has been shown that the purified allergen preparation of timothy grass

pollen is representative of and superior to the crude aqueous pollen extract, both in diagnosis and therapy (13, 14), a similar type of purified preparation of *Cladosporium herbarum* was included in the study in comparison with conventional aqueous and glycerinated crude extracts. Similar high quality allergen preparations were not available for the other moulds.

PATIENTS

The study included a total of 80 patients aged 8-15 years referred with asthma $\pm$ rhinitis and a history suggestive of allergy to moulds, and with an informed consent to participate given by the patient and parents. Twelve patients with allergic asthma without any history suggesting mould allergy were selected as controls. The latter had no previous SPT or

RAST reactions to any of the common moulds when skin prick tested with commercial allergen extracts used in the present study and with commercially available Phadebas® RAST.

MATERIAL

Commercial extracts of five of the most common atmospheric moulds (10, 15, 18, 21) were purchased from three different producers, two of which delivered two different modifications of their extract. These extracts were labelled B, C, D, E, and F, respectively (Table 1). They were used in SPT in the concentrations delivered for this purpose by the manufacturer in question. B and C were 50% glycerinated and aqueous extracts respectively, delivered by one manufacturer. D and E were aqueous extracts produced by different methods by another producer, and F was an aqueous extract delivered by a third manufacturer (25).

An internal reference extract from *Cladosporium herbarum* was prepared according to methods previously described (7). It was diluted to a concentration corresponding to 200 µg/ml of dry weight corresponding to 1000

BU/ml in SPT activity from previous assessments performed in 18 mould allergic individuals (5, 16).

This preparation was denoted A. A purified preparation of *Cladosporium herbarum* of so-called Spectralgen quality was kindly provided by Nyegaard & Co., Oslo, and was denoted S. This was delivered as a white, freeze-dried and easily soluble material which had been quantitated by means of SPT and RAST inhibition by the manufacturer and delivered in a concentration considered optimal for SPT (5, 13, 16). All preparations were used before the expiration dates stated.

Allergosorbents for RAST

Phadebas RAST allergosorbents from one production batch for each of the five moulds were purchased from Pharmacia, Uppsala. Allergosorbents were also prepared with the purified *Cladosporium herbarum* preparation S and with the reference *Cladosporium herbarum* material A, according to standard methods (22). The concentration of A used to prepare the A allergosorbents were 5 mg/ml (dry weight) and of S for S allergosorbents 1 mg/ml, with 33 discs/ml.

Table 1
Mould extracts used for skin prick testing

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|----|---|
| A. | <i>Cladosporium herbarum</i> extract. Freeze-dried dialysed preparation produced in author's laboratory (7). Solved in 0.9% NaCl with 0.5% phenol to a dry weight concentration of 200 µg/ml. |
| B. | Commercial aqueous extract with 50% glycerol, 1:20 (w/v)*. (<i>Alternaria iridis</i> , <i>Aspergillus fumigatus</i> , <i>Cladosporium herbarum</i> , <i>Mucor racemosus</i> , <i>Penicillium expansum</i>). |
| C. | Commercial aqueous extract containing 0.5% phenol, 1:100 (w/v)*, dialysed. Moulds as in B except: <i>Penicillium roqueforte</i> . Delivered by same manufacturer as for B. |
| D. | Commercial aqueous extract containing 0.5% phenol, 1:50 (w/v)*, dialysed. (<i>Alternaria tenuis</i> , <i>Aspergillus spinosus</i> , <i>Cladosporium herbarum</i> , <i>Mucor mucedo</i> , <i>Penicillium notatum</i>). |
| E. | Commercial aqueous extract with 50% glycerol, 20,000 PNU/ml*, dialysed. (<i>Alternaria tenuis</i> , <i>Aspergillus spinosus</i> , <i>Cladosporium herbarum</i> , <i>Mucor mucedo</i> , <i>Penicillium notatum</i>). Delivered by same manufacturer as for D. |
| F. | Commercial aqueous extract containing 0.5% phenol, 1:10 (w/v)*, non-dialysed. (<i>Alternaria tenuis</i> , <i>Aspergillus mix: fumigatus</i> , <i>terreus</i> , <i>niger</i> , <i>nidulans</i> ; <i>Cladosporium herbarum</i> , <i>Mucor racemosus</i> , <i>Penicillium mix: digitatum</i> , <i>expansum</i> , <i>glaucum</i> , <i>roseum</i> , <i>notatum</i>). |
| S. | <i>Cladosporium herbarum</i> preparation (Spectralgen quality). Freeze-dried purified (refined) preparation solved in 0.9% NaCl with 0.5% phenol to dry weight concentration of 250 µg/ml corresponding to 1,000 BU/ml (4, 5, 13). |

* Highest concentration available.

Patient serum

Serum was separated from venous blood drawn at the time of consultation when skin testing was performed. Serum was stored at -20°C until analysed.

Skin test procedure

A drop of the allergen preparation was applied in duplicate on the volar aspects of the forearms of the patient. The distance between each test site was more than 2 cm. A sterile short-bevelled needle (Scimitar No. 26 disposable) was introduced through the droplet at an angle of approximately 20° in such a way that the tip of the needle slightly touched the skin, penetrating the surface of it without causing bleeding. The needle was raised so that the angle between the needle and the skin surface increased to approximately $60-90^{\circ}$ and was then removed. During this movement, the skin was very slightly lifted by the point of the needle. The droplet was wiped off immediately after the puncture had been performed. This technique has been shown to give reproducible results (4).

The size of the wealing reaction was determined and recorded 15–20 min after the puncture test. The outlines of the resulting weal were marked on the skin with a soft ballpoint pen. The erythema was not measured. The largest diameter (D) and the diameter perpendicular to this (d) were recorded. The marks of the area of the weal were transferred by means of a translucent scotch tape to a record sheet.

Histamine HCl 1 mg/ml (5.43 mmol/l) was used as standard reference for SPT (4, 6). The wealing reaction to the histamine test was read and recorded after 12–15 min.

The bronchial provocation test (BPT) was performed only with the C extract, using techniques previously outlined (2). The C extract was chosen because it has previously been used in routine BPT in our department (17).

Radioallergosorbent test (RAST) was performed in duplicate following the procedures described by the manufacturer. Phadebas RAST was recorded in RAST classes 0–4 as advocated by the manufacturer. RAST class ≥ 1 was recorded as positive.

EXPERIMENTS AND RESULTS

A total of 110 duplicate (220 single) SPT were performed with the histamine reference solution. The mean diameter $(D+d)/2$ ranged from 3.5 mm to 12.5 mm. In no instances was there a deviation of as much as 20% between duplicates (mean 5% deviation).

For practical reasons all extracts could not be used in SPT at the same time on all the children included in the study. Three or four of the different preparations, however, were used in parallel in series of 20–40 patients. Bronchial challenges were performed in 26 patients and in eight of the control individuals, all of the latter with negative results. All the bronchial provocation tests were performed with the aqueous C-extract adjusted to suitable concentrations not giving false positive irritant reactions in the bronchi (2, 17). The BPT results with the *Cladosporium herbarum* extract are shown in Fig. 1 as compared with the results of SPT with the same extract and with the results of Phadebas RAST indicated as well.

All patients and controls with negative reactions both to SPT and RAST were negative in BPT.

A marked difference was found between the different extracts used as regards SPT reactions to them. In this paper, only the results with the most active of two different extracts delivered by the same manufacturer are given (Table 2) since they were quite similar. In the *Cladosporium herbarum* system clearly positive reactions $\geq 2+$ were obtained in 49% of the patients using the internal reference extract A, and in 60% of patients tested with the purified S preparation, but only in 12.5% of patients tested with extract C. The difference in SPT between the best *Cladosporium herbarum* extracts

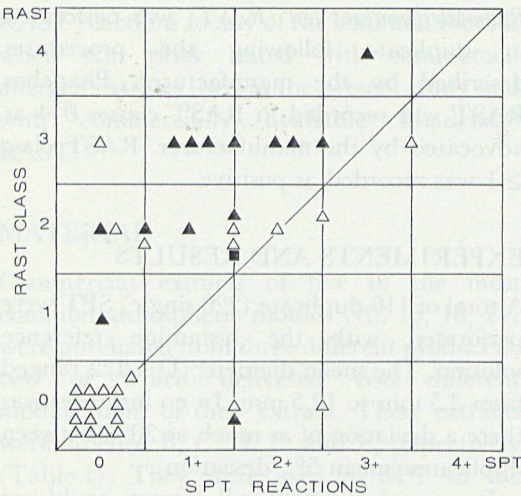


Fig. 1. Correlation between results of skin prick tests (SPT) with C extract from *Cladosporium herbarum*, and Phadebas RAST with *Cladosporium herbarum* in patients with positive (▲) or negative (△) results to bronchial provocation tests with the C extract of *Cladosporium herbarum*.

purchased from the three producers as compared with the reference preparation A and the purified preparation S, respectively, are shown in Figs 2–5. In the same Figures the results of Phadebas RAST are also indicated for comparison. The results from testing with the five most common moulds suggested that *M. racemosus* or *M. mucedo* seemed to be of negligible (1%) clinical importance when extracts B or C from one producer were

considered. The results with material from other producers, however, suggested that allergic reactions to this mould may be quite important, with SPT positive in 28% for extract F and in 40% for Phadebas RAST (Table 2).

When the results of SPT with all kinds of extracts and RAST were considered together, 18 of the 80 patients reacted only to *Cladosporium herbarum* and not to any of the other four moulds. A separate reaction to any of the others was found in only one case (*Alternaria tenuis*). In SPT, the purified preparation available from *Cladosporium herbarum* (S) was found to be diagnostically superior to the other *Cladosporium herbarum* extracts. The other particularly active type of extract (F) elicited seemingly non-specific reactions (+ to ++) in three of the control patients.

The correlation between SPT and Phadebas RAST results varied with the extract used in SPT. The correlation between SPT and RAST when the same material was used in both tests was found to be good (Fig. 4). Phadebas RAST showed a less favourable correlation with the least SPT-active one (C).

The results with extracts B and C delivered by one manufacturer indicated that the two had similar composition, extract C being

Table 2
Results of skin prick tests (SPT) with various mould extracts from different manufacturers (see text for symbols), and of radioallergosorbent test (RAST) with Phadebas® material (P) and purified materials (S), respectively. In SPT reactions ≥ class 1 is recorded as positive. For the S RAST also the results when only ≥ class 2 is considered positive is given, (S2). For Phadebas RAST (P) class 1 or more is recorded as positive

Percent positive in SPT for preparation*	A	S	C	D	F	RAST
<i>Alternaria</i>	—	—	10 (8/80)	14 (11/80)	35 (14/40)	59
<i>Aspergillus</i>	—	—	10 (8/80)	—	42.5 (17/40)	62.5
<i>Cladosporium h.</i>	49 (39/80)	60 (24/40)	12.5 (10/80)	30 (24/80)	65 (26/40)	67.5 (P) 86 (S class 1) 79 (S class 2)
<i>Mucor</i>	—	—	1 (1/80)	9 (7/80)	27.5 (11/40)	40
<i>Penicillium</i>	—	—	2.5 (2/80)	9 (7/80)	32.5 (13/40)	56

— Not done
* See Table 1 for details

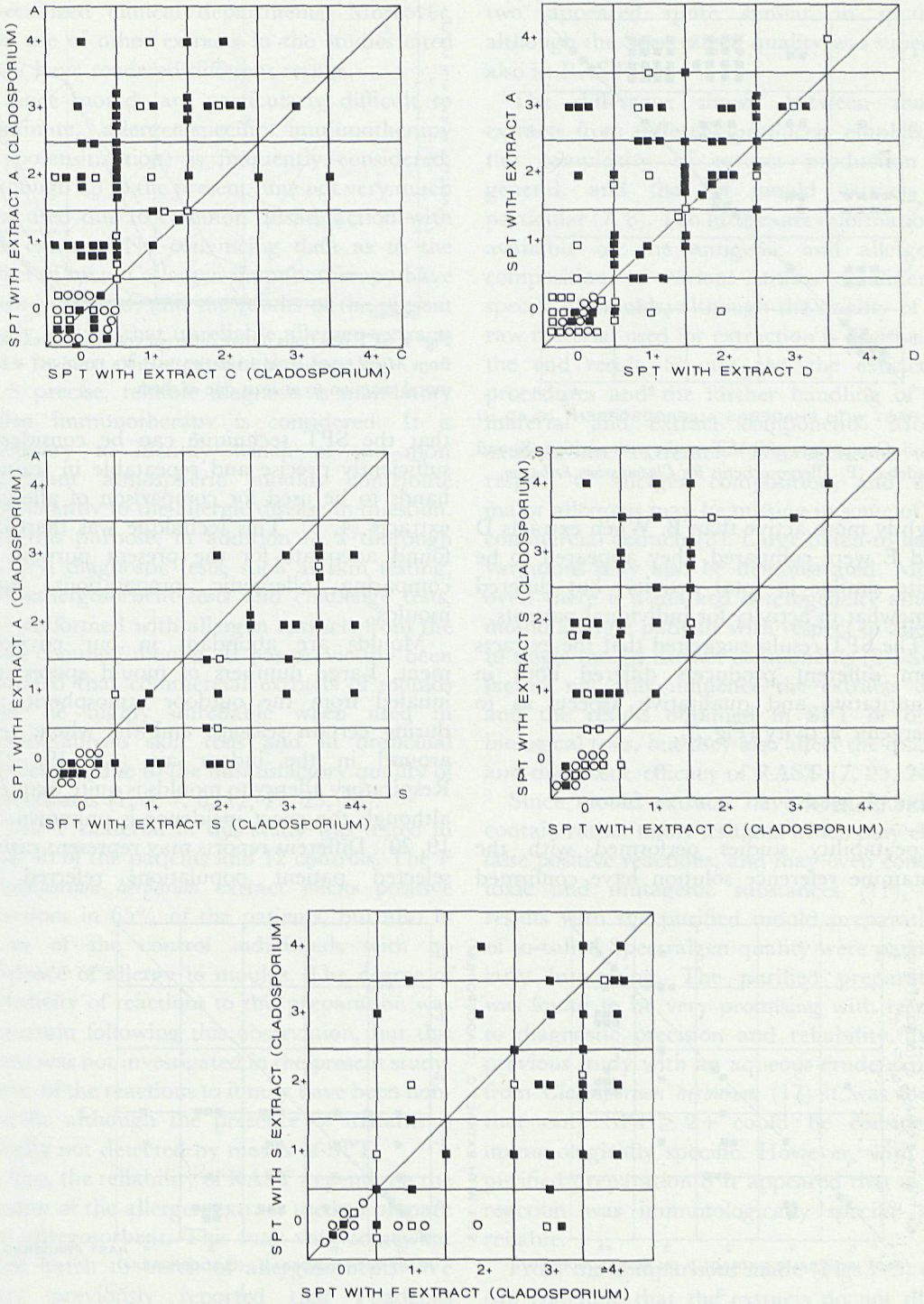


Fig. 2. Comparison of skin prick test (SPT) results with extracts A, C, D, F, and S, respectively. All tests with *Cladosporium herbarum*

□, Phadebas RAST negative patient; ■, Phadebas positive patient; ○, Control individual.

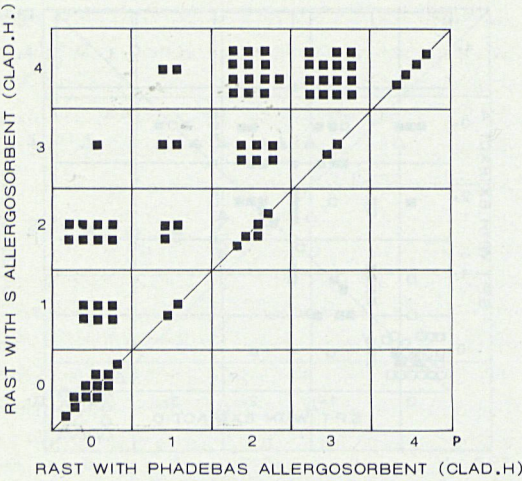


Fig. 3. Comparison of RAST results with purified (S) and Phadebas (P) allergosorbents for *Cladosporium herbarum*.

slightly more active than B. When extracts D and E were compared, they appeared to be quite similar in total activity, but differed somewhat in activity for individual patients.

The SPT results suggested that the extracts from different producers differed both in quantitative and qualitative aspects as to allergenic activity (Fig. 5).

DISCUSSION

Repeatability studies performed with the histamine reference solution have confirmed

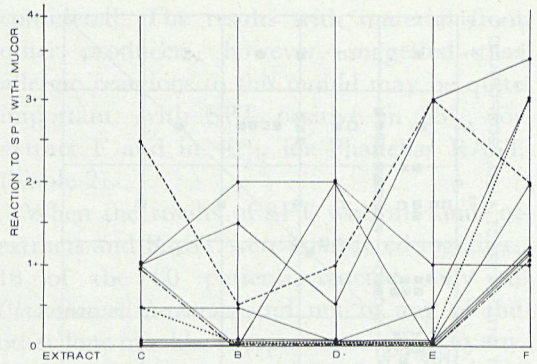


Fig. 5. SPT reactions to five different commercial extracts from *Mucor racemosus* in 10 patients with a positive (+1 or more) reaction to at least one of them.

that the SPT technique can be considered sufficiently precise and repeatable in trained hands to be used for comparison of allergen extracts (4, 6). This technique was therefore found adequate for the present purpose of comparing allergenic preparations from moulds.

Moulds are abundant in our environment. Large numbers of mould spores are inhaled from the outdoor atmospheric air during certain seasons, and the whole year around in the indoor air (15, 19, 21). Respiratory allergy to moulds is quite frequent although the exact incidence is unknown (2, 19, 20). Different reports may represent rather selected patient populations referred to

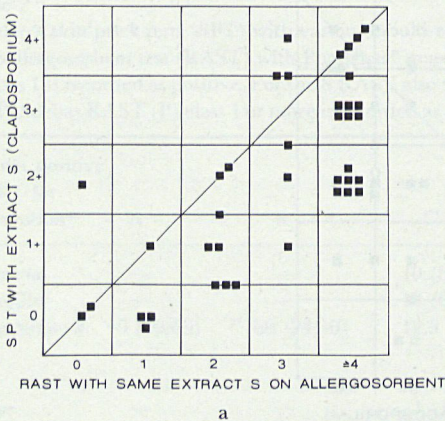
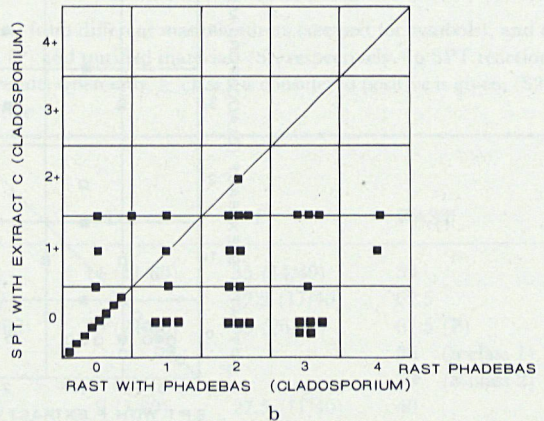


Fig. 4. Correlation between SPT and RAST to *Cladosporium herbarum*,
a) when same purified material (S) is used in both tests,



b) when C extract is used in SPT and correlated to Phadebas RAST (with another allergen preparation).

specialized clinical departments. Moreover, the use of other extracts in the studies cited may have rendered different results.

Since moulds are particularly difficult to eliminate, allergen-specific immunotherapy (hyposensitization) is frequently considered, although up to the present time not very much practised due to common dissatisfaction with the extracts. No convincing data as to the effect of mould allergen immunotherapy have been presented, and the results of the present study suggest that unreliable allergen extracts may be part of the explanation for this.

A precise, reliable diagnosis is mandatory when immunotherapy is considered. It is necessary to identify which of the most abundant atmospheric moulds contribute significantly to the allergic disease in question. For this purpose, in addition to a thorough history, diagnostic tests, such as skin testing, radioallergosorbent tests and challenge tests, are performed with allergen extracts from the most common moulds. It has previously been reported that commercial extracts of moulds may be highly unreliable when used in intracutaneous skin tests and in bronchial challenges, due to the unsatisfactory quality of the extracts (1, 3, 7, 8, 12, 17, 23, 24).

The F material in this study was tested in only 40 of the patients and 12 controls. The F *Cladosporium herbarum* extract elicits positive reactions in 65% of the patients, but also in three of the control individuals with no evidence of allergy to moulds. The degree of specificity of reactions to this preparation was uncertain following this observation, but this point was not investigated in the present study. Some of the reactions to it may have been non-specific although the presence of irritants is usually not detected by means of SPT.

Also, the reliability of RAST depends on the quality of the allergen extract used to prepare the allergosorbent. This may vary somewhat from batch to batch of allergosorbents. We have previously reported that Phadebas allergosorbents from *Cladosporium herbarum* were less active than our own reference (A) allergosorbent (9), but in the present study the

two appeared quite similar in quality, although the Spectralgen quality was superior also in RAST.

The difference shown between mould extracts from different producers emphasizes the complexity of extract production in general, and that of mould extracts in particular (7, 8). Too little exact information is available on the antigenic and allergenic composition of various strains of different species of moulds, although the quality of the raw material used for extraction is decisive for the end result. So are also the extraction procedures and the further handling of the material and extract components. Mould extracts are extremely heterogeneous with respect to allergen composition, and even major allergens may be missing in some of the commercial extracts (8). Large batch-to-batch variations may also be demonstrated. Moreover, there is a marked heterogeneity among mould allergic patients with respect to allergy to single mould extract components (8). Such factors not only influence the extracts used and the results obtained in SPT or other biological tests, but they also affect the quality and diagnostic efficacy of RAST (7, 23, 24).

Since mould extracts have been found to contain rather many irritants which may elicit false positive reactions, and may even contain toxic and mutagenic substances (11), the results with the purified mould preparations of so-called Spectralgen quality were particularly interesting. The purified preparation was found to be very promising with respect to diagnostic precision and reliability. In a previous study with an aqueous crude extract from *Cladosporium herbarum* (17) it was found that only SPT $\geq 2+$ could be considered immunologically specific. However, with the purified preparation S it appeared that a 1+ reaction was immunologically specific and reliable.

From the comparisons made (Figs 1-5) one can conclude that the extracts do not differ only with respect to total allergenic activity. There seem also to be differences in the content and concentration of single allergenic com-

ponents being of different significance for individual patients (as specifically illustrated in the SPT with *Mucor* shown in Fig. 5). This confirms results reported previously (7, 8). Such marked differences in extract qualities are likely to affect not only clinical diagnostic work but also scientific investigations concerned with different aspects of mould allergy. The correlations obtained between RAST and SPT with different extracts clearly illustrate the need to use the same extract in both tests for studies concerned with the reliability of the methods in question. For our study the preparation used for Phadebas RAST was not available for SPT.

Investigators using only one or the other of the extracts in SPT or RAST might report differences in incidence for *Cladosporium herbarum* allergy of approximately 12.5 and 86% respectively, in identical populations. This sheds doubt on the value of several studies of this kind reported in the literature. It also explains the difficulties in obtaining convincing results from hyposensitization with mould extracts.

As calculated from the SPT results, the allergenic activity of the biologically quantitated and purified S preparation, and that of the reference preparation A, seems a little lower than that of the histamine reference as tested in the given paediatric population. This may be due to slightly too low actual activity of the preparations, or it may be that the skin responsiveness (skin IgE antibody concentrations) was somewhat lower in the population included in the present study than that used for the preliminary standardization of the preparations in question. Still, these preparations were superior to the other ones.

Different manufacturers use different representatives of *Alternaria*, *Aspergillus*, *Mucor* and *Penicillium* in their extracts. The implication of such differences for the results of diagnostic tests and clinical practice needs further elucidation.

The present study adds to previous reports showing the need for more basic information on the allergen composition of extracts

produced for clinical use and employed in studies of diagnostic efficacy as well as for use in hyposensitization for allergy to moulds.

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