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THE ALLERGEN(S) OF HOUSE DUST:

COMPARISON WITH PRODUCTS DERIVED FROM MOULDS.

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IN 1924 Storm van Leeuwen (1924) called attention to the part played by mould allergens in the cause of "climatic asthma," prevalent in Holland. He tested such asthmatics intracutaneously with extracts of six different moulds of the genera *Aspergillus*, *Mucor* and *Penicillium*, and obtained reactions in 50 per cent of the cases. In the same year Cadham (1924) reported instances of asthma due to sensitization to grain rusts. These observations, following upon the demonstration by Cooke (1922) of allergic reactions to household dusts, led several allergists to believe that moulds were the cause of dust sensitivity.

This hypothesis was encouraged by Brown (1936), who based it, however, on the observation of a single case, an asthmatic subject reacting to dust extract and to *Alternaria* cultured from his house dust. Cohen (1929), too, in his early work, believed that infestation with moulds caused dust to develop allergenic properties. He observed that asthmatics reacted to dust collected from the kapok of their cotton mattresses, but not to dust of the fresh material. After new mattresses had been substituted for the old ones, dust collected from them after several months again gave positive tests in these patients.

Neither Cohen nor Brown could substantiate their hypothesis in later work. Brown obtained mostly negative results when he tested dust-sensitive patients with moulds and bacteria cultured from the dusts to which they were sensitive. Furthermore, cotton seeds inoculated with all the micro-organisms isolated from

the reacting dust and kept for one month yielded extracts which failed to react in dust-sensitive patients. Cohen, Nelson and Reinarz (1935) claimed that cotton linters left in autoclaved, airtight jars for several years acquired the capacity to react with dust-sensitive individuals, but this was apparently due to ageing without the interference of moulds.

Much work has been done since on the various types of moulds which give positive skin tests in allergic individuals, but the question whether the reactions to moulds and to dust are caused by the same factor has not yet been decided. Practically nothing is known concerning the chemical nature of the substances which cause sensitization produced by moulds, although Hassid, Baker and McCready (1943) have described the preparation of an immunologically active polysaccharide produced by *Coccidioides immitis* (Rixford and Gilchrist). This preparation had a nitrogen content of 2-3 per cent, and on hydrolysis yielded 20-40 per cent of reducing sugars. The compound gave a negative biuret test for protein, like our dust antigen, on the strength of which the authors concluded that a non-protein nitrogenous compound was associated with the polysaccharide. No attempt was made to detect or identify amino-acids following hydrolysis of the complex.

It seemed of interest, particularly in view of the above-mentioned chemical observations, to include in our work on the dust antigen some investigations upon common moulds.

EXPERIMENTAL.

Investigations with extracts of moulds grown in pure culture and with their culture fluids.

It was decided that suitable patients should be tested with standard crude antigen, and with pooled extracts from cultures representing eight common genera. Pure strains of these moulds (Table I) were kindly supplied by Prof. Raistrick, of the London School of Hygiene and Tropical Medicine.

TABLE I.—*Moulds Used for Preparing Extract for Biological Testing. All Strains Grown Separately, Extracted and the Extracts Pooled.*

<i>Aspergillus</i> spp.	13 strains.
<i>Penicillium</i> spp.	6 „
<i>Mucor spinosus</i>	1 strain.
<i>Rhizopus nigricans</i>	1 „
<i>Cladosporium herbarum</i>	1 „
<i>Alternaria</i> spp.	2 strains.
<i>Trichoderma viridans</i>	1 strain.
<i>Caldariomyces fumago</i>	1 „

Each mould was grown on Czapek-Dox medium for one month at 25° C., the growth removed and ground in a mortar with sand and 10 ml. normal saline, and the suspension then filtered through a sintered glass funnel. The extract so obtained was sterilized by steaming at 100° for 0.5 hour on three successive days, after the addition of phenol (0.5 g./100 ml.).

The medium on which the mould had grown was filtered through a No. 1 Whatman filter paper, and the filtrate sterilized as above after the addition of phenol to the same concentration.

The absence of irritation in the normal skin by any of the extracts used was proved by injecting pooled mould extracts (approx. 3×10^{-4}), mould culture fluids and media controls into 15 non-allergic persons; in no case was a reaction observed. Solutions of dust antigen of concentration 1×10^{-4} were included in the present survey, although it is realized that at present we are not able to state whether a threshold reaction to dust antigen at this dilution represents a low grade sensitivity to dust (i.e. a weak reactor) or a non-specific reaction. For discussion of such reactions see Maunsell, Whetnall and Rimington (1947).

Cases of vasomotor rhinorrhoea (62 in all) were now tested for dust and mould sensitivity, 45 being found dust sensitive, and of these 15 also gave reactions to moulds (extracts and media). None of the 62 patients displayed sensitivity to moulds accompanied by insensitivity to dust. These results are collected in Table II.

TABLE II.—*Skin Tests with Dust Allergen, Pooled Mould Extract and Culture Fluids upon Patients Suffering from Vasomotor Rhinorrhoea: Number of Individuals in Brackets.*

Subjects (62)	$\left\{ \begin{array}{l} + \text{ to dust (45)} \\ - \text{ to dust (17)} \end{array} \right.$	$\left\{ \begin{array}{l} + \text{ to moulds (extract and culture) (15)} \\ - \text{ to moulds (extract and culture) (30)} \\ + \text{ to moulds (0)} \\ - \text{ to moulds (17)} \end{array} \right.$

It seemed possible that the differences in sensitivity to dust and moulds might be only of a quantitative nature. Skin titrations were therefore carried out with dust antigen, and a test injection was also made with a standard dilution of pooled mould extracts in a series of 36 dust-sensitive patients, who suffered from bronchial spasm and rhinorrhoea, to see whether the dual sensitivity was detectable in those reacting strongly to dust as opposed to those more feebly dust-sensitive.

In this series 12 of the 36 patients reacted to both dust and moulds, and titrations showed that no such quantitative relationship existed as that postulated above. Some of the strongest dust-reactors gave no reaction to moulds, whereas some weakly dust-positive patients did so (see Table III). In addition to the strains of fungi used in the above studies, Prof. Raistrick also kindly put at our disposal five preparations of material, predominantly polysaccharide in character, obtained from the following organisms:

Penicillium roqueforti.

„ *capreolinum*.

„ *tardum*.

„ *funiculosum*.

Caldariomyces fumago.

It appeared of interest to test them on suitable patients, particularly in view of our own work on the chemical nature of the dust antigen and the report of Hassid, Baker and McCready (1943), already mentioned, concerning the nitrogen-containing polysaccharide material which they obtained from *Coccidioides immitis*. The preparations were dissolved in carbol-saline (1 mg./ml.), sterilized in the same way as the dust antigen, and serial dilutions prepared. Intradermal tests using a concentration of 1×10^{-3} were negative in 5 non-allergic controls, and in two

TABLE III.—*Sensitivity to Moulds of Subjects Exhibiting Varying Degrees of Sensitivity to Dust Antigen.*

Case.	Threshold of dust sensitivity.	Reaction to pooled mould extract.
1 .	1×10^{-4}	—
2 .	"	—
3 .	"	—
4 .	"	—
5 .	"	—
6 .	"	—
7 .	"	++
8 .	1.25×10^{-5}	++
9 .	6.25×10^{-6}	—
10 .	"	—
11 .	"	—
12 .	"	—
13 .	"	++
14 .	"	++
15 .	"	++
16 .	3.13×10^{-6}	—
17 .	"	—
18 .	"	—
19 .	"	—
20 .	"	—
21 .	"	—
22 .	"	—
23 .	"	++
24 .	1.56×10^{-6}	++
25 .	"	++
26 .	7.8×10^{-7}	—
27 .	"	—
28 .	"	—
29 .	"	—
30 .	"	++
31 .	"	++
32 .	"	++
33 .	1×10^{-7}	—
34 .	"	—
35 .	"	—
36 .	"	++

cases who had been found sensitive to dust antigen but insensitive to pooled mould extract. Positive tests were obtained, however, with 5 persons who reacted to both dust antigen and moulds. Some differences were seen in the strength of the reactions to the polysaccharides from the different species, but the number of cases tested was too small to allow of significant conclusions on this point. The results are collected in Table IV.

TABLE IV.—*Titration in Subjects Sensitive to Dust Antigen and to Moulds of Threshold Concentrations with Polysaccharides Isolated from Moulds.*

Case.	Threshold concentration of polysaccharides derived from—				
	<i>Penicillium roqueforti</i> .	<i>Penicillium tardum</i> .	<i>Penicillium capreolium</i> .	<i>Penicillium funiculosum</i> .	<i>Caldariomyces fumago</i> .
1	1×10^{-4}	1×10^{-4}	1×10^{-4}	1×10^{-4}	1×10^{-3}
2	1×10^{-5}	1×10^{-5}	1×10^{-5}	1×10^{-5}	1×10^{-4}
3	1×10^{-4}	1×10^{-4}	1×10^{-4}	1×10^{-4}	Not tested
4	1×10^{-5}	1×10^{-5}	Not tested	Not tested	„
5	1×10^{-4}	1×10^{-4}	„	„	„
6	1×10^{-3}	1×10^{-3}	1×10^{-3}	1×10^{-3}	1×10^{-3}

Five non-allergic subjects and 3 subjects sensitive to dust but not reacting to moulds gave negative tests with each polysaccharide at 1×10^{-3} .

Chromatographic Investigations.

The "polysaccharide" materials derived from *Caldariomyces fumago*, *Penicillium roqueforti* and *Penicillium tardum* were investigated by partition chromatography as described by Rimington, Stillwell and Maunsell (1947), and the results obtained are presented in Table V.

TABLE V.—*Examination of Amino-acids by Partition Chromatography after Acid Hydrolysis of Materials Predominantly Polysaccharide in Character Isolated from Moulds (Raistrick).*

	<i>Caldariomyces fumago</i> .	<i>Penicillium roqueforti</i> .	<i>Penicillium tardum</i> .
Nitrogen content	1.08%	2.80%	..
Amino-acids present	Cysteic acid,*	Cysteic acid,*	..
after 24 hours' hydrolysis with approx.	Glutamic acid	Glutamic acid	Glutamic acid
6 N hydrochloric acid	Aspartic acid	Aspartic acid	Aspartic acid
	Serine	Serine	Serine
	Glycine	Glycine	Glycine
	Threonine	Threonine	Threonine
	Alanine	Alanine	Alanine
	Valine	Valine	Valine
	Leucine	Leucine	Leucine
Histidine and tyrosine†	Absent	Absent	Absent

* Probably derived from cystine.

† Destruction of tyrosine during hydrolysis under these conditions cannot be excluded.

DISCUSSION.

It is apparent that a general chemical similarity exists between the three polysaccharide products derived from moulds of which we had sufficient material to carry out a chromatographic examination after hydrolysis and the preparations of dust antigen which we have studied (Rimington, Stillwell and Maunsell, 1947). All consist of a polypeptide-like grouping of simple amino-acids associated with a polysaccharide complex. The usual colour reactions for proteins are not given by these materials.

It is of interest that when a comparison was made of the sensitivity of patients

to house dust antigen and to moulds or the isolated mould products, some individuals were found to be sensitive to both dust and moulds, but no quantitative relationship existed between these manifestations of allergic sensitivity. Other patients were sensitive to dust only. As mentioned above, no patient was found sensitive to moulds but insensitive to dust.

From these results it is clear that much more work will be required upon the chemical extraction and purification of the antigens present in various allergenic inhalants, and then an extensive examination of the most highly purified products obtainable for cross-reactions when tested upon sensitive individuals, before any conclusions may be reached concerning the origin of the allergen present in household dusts. We feel that the pollens which have already been so extensively investigated should also be submitted to more rigorous chemical examination along the lines which we have shown to be useful in the present instances.

SUMMARY.

1. A comparison has been made of the reactions given by allergic individuals to purified house dust antigen, the pooled extracts of 26 strains of mould grown in pure culture, the culture fluids obtained from the latter, and also to materials predominantly polysaccharide in character isolated from 4 species of *Penicillium* and 1 species of *Caldariomyces*.

2. Some patients gave positive reactions to both house dust and mould products, others were sensitive only to house dust. No patient was sensitive to moulds and insensitive to house dust.

3. Titration of threshold values in the skin showed a lack of quantitative relationship between dust and mould sensitivity.

4. Examination of 3 of the nitrogen-containing polysaccharide materials from moulds by chromatography after acid hydrolysis revealed the presence in them of several of the simple amino-acids. In this respect they resembled, chemically, the purified antigen from house dust.

We wish to thank Prof. Raistrick and Mr. G. Smith of the London School of Hygiene and Tropical Medicine for kindly providing us with several strains of micro-organisms, and also for the metabolic products which we have examined chemically and biologically. Dr. Dent performed the chromatographic analysis. Our thanks are due to Dr. Loutit and Mr. Daggett for their helpful interest. One of us (D. E. S.) wishes to thank the Medical Research Council for a personal grant.

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