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ALLERGENS IN POLLEN

*Allergic Reactions in Mucous Membranes
(Eye and Nose) and in the Skin Provoked by Conventional
Pollen Extracts and by Extracts Derived from
Microorganisms Cultivated from Pollen*

By

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The nature of the allergenic substances in pollen is not known. Observations made at the Cernelle Laboratory have shown that different microorganisms can be cultivated from certain kinds of pollen. Certain species of microorganisms can be cultured more often from certain particular pollens. As bacterial products can provoke asthmatic reactions in the bronchi (2, 3, 4), it was felt desirable to find out if extracts from microorganisms cultivated from pollen could in the absence of any other pollen-derivatives provoke reactions of an allergic type.

METHOD

The methods used for skin titration and provocation tests (nasal and conjunctival tests) are the same as those used earlier (1).

The conventional extracts have been prepared by shaking 10 grs of pollen and 90 ml of physiologic salt solution mostly containing 0.5 per cent of phenol.

The extracts from microorganisms have been prepared in the following way. Some days before the pollen is ready the stalks or twigs are placed in water inside a room which is as sterile as possible. The room is sealed and the pollen production is observed through a glass-window in the door. The pollen falls down on agar-plates. The different colonies of microorganisms are grown in dextrose solution (1 per cent). For the main culture different substrates have been used, one of which is soya bean extract.

The different microorganisms have been identified in different laboratories. The extracts 3119 is prepared from *Cryptococcus luteolus*, cultivated from birch

pollen (*Betula alba*). Extract 3166 is prepared from *Pseudomonas maltophilia*, cultivated from timothy pollen (*Phleum pratense*).

Other microorganisms have also been used. Extracts from dried organisms as well as filtrates of culture media have been tested.

The nitrogen content of the extracts or filtrates prepared is as follows:

Extract of soya bean 1:10	contains 0.539 mg N/0.5 ml
Extract 3166 (<i>Pseudomonas maltophilia</i>) 1:10*	contains 1.131 mg N/0.5 ml
Extract 3119 (<i>Cryptococcus luteolus</i>) 1:10*	contains 1.359 mg N/0.5 ml
Extract 2770 (A conventional birch extract) 1:10	contains 0.830 mg N/0.5 ml
Extract 2818 (A conventional timothy extr.) 1:10	contains 0.383 mg N/0.5 ml
Extract 3235 (A conventional timothy extr.) 1:10	contains 1.129 mg N/0.5 ml

* = undiluted filtrate

RESULTS

Skin Titrations and Provocation Tests

It is desirable that the medium used for the cultivation of the microorganisms does not give either a positive skin reaction or a positive eye or nasal test. 3166 and 3119 are both cultivated on soya bean extract. This extract does not produce a positive reaction either in provocation tests 1:10 or by skin titration in a concentration 1:1,000 in the patients tabulated in Table 1.

A soya bean extract may however sometimes give a positive reaction in skin tests and provocation tests on pollen sensitive patients. Patients not allergic to pollen have up to now not given positive reactions to soya bean extracts (with only one exception). This subject is now under investigation.

It has clearly been possible to show that extracts prepared from certain microorganisms cultivated from pollen have allergenic properties and are capable of producing reactions both in the skin and in the mucous membranes in patients sensitive to pollen (See Table 1). These reactions cannot be separated from the reactions provoked by ordinary pollen extracts. After the injection in the skin an immediate reaction occurs in 10–15 minutes with a wheal and an erythema. In nasal tests itching, sneezing and watery discharge are produced in 10–15 minutes. In eye tests redness, itching and tears in the conjunctiva appear after 10–15 minutes.

TABLE 1

Results of skin titrations and provocation tests with different pollen extracts and extracts from microorganisms cultivated from pollen. Details concerning the allergens used are given on page 388. Timothy - Microorganism 3166 (Timothy - M 3166) is an extract from *Pseudomonas maltophilia* cultivated from timothy pollen. Birch - Microorganism 3119 (Birch - M 3119) is an extract from *Cryptococcus luteolus* cultivated from birch pollen. The conjunctival and nasal tests are evaluated as follows: + = a weak but clear reaction, ++ = a reaction of medium severity, +++ = a strong reaction. The skin reactions are evaluated as in Table 2.

Initials, date of birth, sex and diagnosis	Allergens	Intracutaneous tests	Conjunctival tests	Nasal tests	Intracutaneous tests	Dates for Provocation tests
A. R. 42 02 18 Male Hay fever	Timothy 3235 " - M 3166 Birch 2770 " - M 3119 Soya bean	+ 1:1000 + 1:1000 - 1:1000 - 1:1000 - 1:100	+ + 1:30 + + 1:100 - 1:10 - 1:10 - 1:10	+ + + 1:30 + + 1:100 - 1:10 - 1:10 - 1:10	Sept., 15, 1967 "	Oct., 26, 1967 " 17 " " 24 " " 20 " " 16 "
J. U. 46 04 10 Male Hay fever and asthma	Timothy 3235 " - M 3166 Birch 2770 " - M 3119 Soya bean	- 1:1000 - 1:1000 + + 1:1000 + + 1:1000 - 1:1000	- 1:10 - 1:10 + + 1:10 + + 1:30 - 1:10	- 1:10 - 1:10 + + 1:10 + + 1:30 - 1:10	" "	" 26 " " 23 " " 24 " " 20 " " 19 "
S. G. 15 04 17 Male Hay fever and asthma	Timothy 3235 " - M 3166 Birch 2770 " - M 3119 Soya bean	- 1:100 - 1:100 + 1:1000 + 1:1000 - 1:100	- 1:10 - 1:10 + (+) 1:30 + (+) 1:30 - 1:10	- 1:10 - 1:10 - 1:10 - 1:10 - 1:10	Dec., 5, "	Febr., 21, 1968 " 14 " March, 8 " " 6 " " 13 "
P. B. 34 10 24 Female Hay fever and asthma	Timothy 3235 " - M 3166 Birch 2770 " - M 3119 Soya bean	+ 1:1000 + + 1:100 - 1:100 - 1:100 - 1:100	+ (+) 1:10 (+) 1:10 - 1:10 - 1:10 - 1:10	+ + 1:10 + 1:10 - 1:10 - 1:10 - 1:10	Febr., 20, 1968 " 27 " Jan., 23 " March, 1 " " 8 "	Febr., 2 " " " " " " " " " " " " "

TABLE 2

Results of skin titrations (intracutaneous tests) on pollen sensitive patients. Details of the allergens used are given on page 388 and in Table 1. The skin reaction is evaluated by taking the sum of two diameters of the urticarial wheel measured at right angles to each other.

If the result is between 10-20 mm the reaction is indicated by (+)

If the result is between 20-30 mm the reaction is indicated by +

If the result is between 30-40 mm the reaction is indicated by ++

If the result is between 40-50 mm the reaction is indicated by +++

Initials, date of birth, sex diagnosis. (H = Hay fever A = Asthma)	Allergens used				Dates for intracutaneous tests
	Timothy 3235	Timothy - M 3166	Birch 2770	Birch - M 3119	
M. L. Male 30 04 05	-1:1000	-1:1000	-1:10.000	-1:10.000	Jan., 1, 1968
E. B. Male 50 09 21	-1:100 (+):1:1000	-1:100 -1:1000	+ + + + 1:1000 -1:10.000	+ + + + 1:1000 (+):1:10.000	Oct., 20, 1967
L. J. Male 27 09 22	+1:100 -1:1000	+1:100 -1:1000	+ + + 1:100 -1:100.000	+1:1000 -1:1 mil.]	Oct., 23, 1967
W. H.-V. Female 34 05 17	+1:100 -1:10.000	+1:100 -1:1000	+1:10.000 -1:100.000	+1:100.000 -1:100.000	Sept., 21, 1967
A. R. Male 23 02 24	+1:1000 -1:10.000	+1:100 (+):1:1000	+1:10.000 -1:100.000	+1:10.000 -1:100.000	Sept., 20, 1967
K. J.-E. Male 33 07 13	-1:100 -1:1000	-1:1000 (+):1:100	-1:10.000 + + + + 1:1000	+1:10.000 (+):1:10.000	Febr., 6, 1968
W. A. Male 20 04 02	(+):1:1000 +1:100	-1:1000 +1:100	(+):1:10.000 + + + + 1:1000	(+):1:10.000 + + + + 1:1000	Febr., 5, 1968

Female G. M.	H+A 45 01 11	(+): 1: 100 —1: 10.000	(+): 1: 100 —1: 10.000	+ + 1: 100 —1: 10.000	April, 1, 1968
Female	H	—1: 100	(+): 1: 100	+ 1: 1000	
P. N.	16 05 28	—1: 1000	—1: 10.000	—1: 10.000	May, 10, 1968
Male	H+(A)	(+): 1: 100	+ 1: 100	+ 1: 1000	
P. G.	45 04 02	—1: 1000	—1: 10.000	—1: 10.000	March, 21, 1968
Female	H+A	—1: 100	+ + 1: 1: 100	+ 1: 1000	
S. B.	54 08 05	(+): 1: 10.000	—1: 10.000	—1: 1000	Dec., 12, 1967
Male	H	+ 1: 1000	+ 1: 100	(+): 1: 100	
F. C.	43 01 04	(+): 1: 10.000	—1: 10.000	—1: 1000	Febr., 6, 1968
Male	H	+ + 1: 1000	+ 1: 100	+ 1: 100	
G. B.	49 02 12	—1: 10.000	—1: 10.000	—1: 1000	Febr., 29, 1968
Male	H	+ 1: 1000	+ + 1: 1: 100	(+): 1: 100	
C. G.	14 05 30	—1: 100.000	—1: 10.000	—1: 1000	May, 10, 1968
Male	H	+ 1: 10.000	+ 1: 100	(+): 1: 100	
S. G.	38 10 07	(+): 1: 10.000	—1: 10.000	(+): 1: 10.000	May, 6, 1968
Female	H+(A)	+ + 1: 1000	+ 1: 10.000	+ + 1: 1000	
N. A.	50 07 19	—1: 10.000	(+): 1: 1000	(+): 1: 10.000	Dec., 28, 1967
Female	H	+ 1: 1000	+ 1: 100	+ 1: 1000	
L. A.	52 06 09	(+): 1: 10.000	—1: 10.000	—1: 10.000	Jan., 22, 1968
Female	H	+ 1: 1000	+ 1: 1000	+ 1: 1000	
J. S.-O.	35 06 16	—1: 100.000	—1: 10.000	—1: 10.000	March, 13, 1968
Male	H+A	+ 1: 10.000	+ 1: 1000	+ 1: 1000	
E. K.	50 12 23	—1: 100.000	—1: 10.000	(+): 1: 100.000	April, 23, 1968
Male	H+A	+ 1: 10.000	+ 1: 10.000	+ + 1: 10.000	
L. A.	30 07 29	—1: 1000	—1: 1000	(+): 1: 10.000	Dec., 19, 1967
Female	H+A	+ 1: 100	(+): 1: 100	+ 1: 1000	
K. L.	52 01 09	(+): 1: 1000	—1: 10.000	(+): 1: 1000	Dec., 11, 1967
Male	H	+ 1: 100	+ 1: 1000	+ 1: 100	

The extracts prepared from the microorganisms have a specific action. A patient who is sensitive to timothy but not to birch pollen reacted positively only to extracts from microorganisms cultivated from timothy but not to extracts from microorganisms cultivated from birch (Pat. AR and PB, Table 1). The reverse was the case for a patient sensitive to birch but not to timothy (Pat. JU and SG, Table 1). The same culture medium (soya bean extract) has been used for the extracts 3119 and 3166.

A great number of pollen sensitive patients have been skin titrated with both 3235, 3166, 2770 and 3119. Skin sensitivity (by skin titration) shows a good correlation between the extracts 3235 and 3166 and between the extracts 2770 and 3119 (Table 2). 4 patients not sensitive to pollen reacted negatively to both 3166 and 3119. One of these 4 patients was very sensitive to horse dander. The other 3 patients had no allergy.

DISCUSSION

The reactions provoked in pollen sensitive patients by skin titration, conjunctival and nasal tests are quite similar if conventional pollen extracts or extracts prepared from certain microorganisms (bacteria and fungi), cultured from pollen are used. The antigenic substances responsible for the clinical effect may therefore be bacterial or fungal in origin. If future studies show that the only antigenic fraction of importance in provoking the clinical symptoms in pollen sensitive patients is in fact bacterial or fungal in origin, then antigen extracts for diagnostic or therapeutic procedures in patients with pollen allergy may be obtainable direct from certain microorganisms. Further studies will throw light on this point.

The above mentioned hypothesis that the antigenic substances responsible for the clinical effect may be bacterial or fungal in origin is supported by immunological studies performed with gel diffusion, immunoelectrophoresis and indirect fluorescent antibody tests. These studies have shown that rabbit antisera to conventional birch or timothy pollen extracts

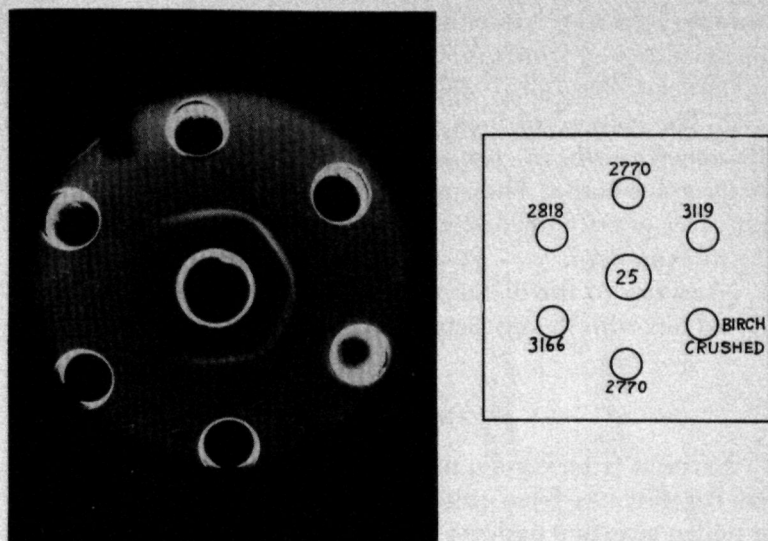


Fig. 1.

Gel diffusion according to Ouchterlony. Rabbit antiserum (25) against a conventional birch extract (2770) was in the centre well. Different antigens according to the figure in the peripheral wells. Some details of these antigens are found on page 388 and below. For the crushed birch antigen the same pollen was used but crushed before the extract was prepared. The antigens used were as follows:

- Birch pollen 2770
- Birch-Microorganism 3119
- Birch pollen, crushed
- Timothy pollen 2818
- Timothy-Microorganism 3166

The allergens were used in a concentration 1:10 and the rabbit-antiserum was undiluted.

contain antibodies against certain microorganisms cultivated from the same kind of pollen but not against microorganisms from the other. The results will be published in detail later. An example of a gel diffusion experiment is given here (Fig. 1). In this figure it is seen that a conventional birch extract has antigenic factors in common with a culture filtrate from microorganisms cultivated from birch, but not with a con-

ventional timothy extract or a culture filtrate from microorganisms cultivated from timothy. In Fig. 1 rabbit antiserum (25) against a conventional silver birch extract was in the centre well. The culture medium used in the tests seen in Fig. 1 was the same (soya bean) for microorganisms cultivated from both birch and timothy. The soya bean extract itself therefore has not after injection produced any antibodies in the rabbit with the method used.

(Antisera to the different extracts were obtained by injecting rabbits with the extracts together with Freund's adjuvant.)

CONCLUSIONS

1. Extracts from certain microorganisms cultivated from pollen (or filtrates from cultures of these microorganisms) can in pollen sensitive patients by both skin titration and provocation tests provoke immediate reactions. These reactions cannot be distinguished from the reactions provoked by ordinary pollen extracts. In this study patients sensitive to birch and/or timothy pollen have been investigated.

2. Patients sensitive to timothy but not to birch react to microorganisms cultivated from timothy pollen, but not to microorganisms from birch pollen. Similarly patients sensitive to birch pollen react to microorganisms cultivated from birch pollen but not to microorganisms from timothy pollen.

3. Up to now the strongest reactions have been obtained by filtrates from cultures from *Cryptococcus luteolus* (cultivated from birch pollen) and *Pseudomonas maltophilia* (cultivated from timothy pollen).

4. The antigenic substances responsible for the clinical effect of pollen may be bacterial or fungal in origin. This is supported by immunological tests, not yet published in detail.

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