

SEROLOGICAL STUDIES ON POLLEN EXTRACTS

II. *Demonstration by a serological method of the specificity of the antigen-mosaic of the extracts of six grass pollens.*

By

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INTRODUCTION

Serological experiments have been performed with the object of demonstrating the specificity or otherwise of the antigenic components of the extracts of six grass pollens.

Rabbits were inoculated with the various pollen extracts and the antisera so produced examined serologically using Boyden's (1951) modification of the "coated particle technique" as previously described (Hoet, Mynors, Coombs, Bell and Milner, 1952). The sera were examined, in the first place, for their direct agglutinating activity against tanned red cells "coated" with the individual pollen extracts. By this method, no serological specificity could be demonstrated as strong cross reactions were observed in all cases. However, when the different extracts were examined for their ability to inhibit the homologous reactions, the identity of each extract was clearly shown.

These experiments demonstrated that although the six

grass pollen extracts studied possess common antigens, each has a specific mosaic, due probably to the fact that each possesses, besides the common antigens, a specific antigen (or antigens) of its own.

It must be stressed that these experiments have serological implications only. The factors studied were those components of grass pollen extracts antigenic in the rabbit and these may, of course, be quite unrelated to the allergenic fractions of pollen causing hay-fever in man. On the other hand the allergenic fractions may subsequently be found to coincide with the components found to be antigenic in the rabbit.

MATERIALS AND METHODS

Pollen Extracts.

These were the standard concentrated glycerine extracts as prepared by C. L. Bencard Ltd.

Pollen extracts of the following grasses were used in this study:—

1. Rye grass (*Lolium perenne*).
2. Cocksfoot (*Dactylis glomerata*).
3. Yorkshire fog (*Holcus lanatus*).
4. Timothy (*Phleum pratense*).
5. False oat (*Arrhenatherum avenaceum*).
6. Foxtail (*Alopecurus pratensis*).

Comparable extraction processes were carried out on each pollen. The total nitrogen contents of the concentrated extracts were determined by the micro-Kjeldahl method. Aliquot portions of the extract were treated with tungstate to precipitate protein nitrogen, the residual nitrogen in the treated extract was determined by micro-Kjeldahl and the protein nitrogen figures obtained by difference.

Each c.c. of extract contained the extractive from 50mg. of the appropriate dry pollen. On clinical skin test the extracts were all equally potent, as judged by the average wheal diameter obtained by the prick test technique on patients known to be sensitive to the particular grass pollen used.

Grass Pollen Extract (Concentrated)	Nitrogen Content mg./c.c.		
	Total	Non-protein	Protein
1. Rye grass	0.81	0.78	0.03
2. Cocksfoot	1.02	0.80	0.22
3. Yorkshire fog	1.01	0.64	0.37
4. Timothy	1.06	0.78	0.28
5. False oat	0.94	0.94	trace
6. Foxtail	1.73	0.75	0.98

Production of rabbit anti-pollen extract sera.

Pairs of fully grown rabbits were injected with individual pollen extracts. Three courses of intraperitoneal injections were given. Each course consisted of 5–8 injections of a dose ranging from 1–2 c.c. of the extract diluted 1–10 with saline. The animals were bled for serum on the 9th or 10th day after the last injection of each course.

For the main experiments reported in this paper only the 2nd course antisera were used for the reason explained in our previous paper (1952 loc.cit.).

Before being used in any test, the sera were heat inactivated at 56°C for half an hour and absorbed free of agglutinins for human red cells. The absorbed sera were stored at –20°C without the addition of a chemical preservative, until required for testing.

Direct agglutination tests and agglutination inhibition tests using tanned cells 'coated' with pollen extract.

The methods used were those described previously (Hoet, Mynors, Coombs, Bell and Milner, 1952).

EXPERIMENTAL STUDY

1. *Examination of the rabbit anti-grass pollen extract sera for their ability to agglutinate tanned red cells 'coated' with the different grass pollen extracts.*

The results with the second course antisera are shown in Table 1, and were very much as expected. The strong cross

TABLE 1

Examination of Second Course Rabbit Anti-Grass Pollen Extract Sera for their ability to agglutinate Tanned Red Cells "Coated" with the Different Grass Pollen Extracts.

Anti-Sera to Pollen Extracts		Antigen Tanned Red Cells "Coated" with Pollen Extracts of:—					
Rabbit Anti: (Second Course)		Rye	Cocks- foot	Yorkshire- fog	Timothy	False oat	Foxtail
Rye	2397	2560	2560	1280	2560	2560	640
Rye	2398	640	640	320	320	640	640
Cocksfoot	2399	320	640	320	640	640	640
Cocksfoot	2394	640	640	320	640	640	640
Yorkshire fog	3322	640	640	2560	1280	1280	640
Yorkshire fog	2323	320	640	2560	640	1280	1280
Timothy	3764	1280	1280	2560	5120	2560	1280
False oat	3765	640	1280	1280	2560	5120	1280
False oat	2334	1280	1280	1280	1280	2560	1280
Foxtail	2279	2560	2560	2560	2560	5120	5120
Foxtail	3771	320	1280	1280	640	640	2560

Numbers indicate the titre of the serum producing agglutination.

antigenic relationship completely overshadowed any individual antigenic difference which each pollen might possess.

No agglutination was obtained with any of the pre-inoculation sera.

2. *The ability of each grass pollen extract to inhibit specifically the homologous agglutination reaction.*

All six grass pollen extracts were tested for their ability to inhibit the homologous agglutination reactions. In these tests, as in the previous work (Hoet et al. loc.cit.) 4 and 16 agglutinating doses were chosen in order to study the inhibition produced by the extracts. The results of these are shown in Tables 2 and 3.

An examination of these tables shows the serological identity of each of the extracts. To account for these results it would seem that each extract has a characteristic antigen-

TABLE 2

Inhibition of the Homologous Agglutination Reactions with Grass Pollen Extracts using 16 Agglutinating Doses of Second Course Antisera.

Anti-Serum Rabbit Anti:		Antigen. Tanned Red Cells "Coated" With Pollen Extracts of:-	Dilutions at which the following extracts inhibited the reaction						
			Control Fluid	Rye	Cocksfoot	Yorkshire fog	Timothy	False oat	Foxtail
Rye	2397	Rye	o	1:20	o	o	o	o	o
Rye	2398	Rye	o	1:40	1:5	1:10	1:5	o	1:5
Cocksfoot	2399	Cocksfoot	o	o	1:40	o	o	o	o
Cocksfoot	2395	Cocksfoot	o	o	1:80	o	o	o	o
Yorkshire fog	2323	Yorkshire fog	o	1:5	o	1:40	o	o	o
Yorkshire fog	2322	Yorkshire fog	o	1:5	o	1:40	o	o	o
Timothy	3764	Timothy	o	o	o	o	1:80	o	o
False oat	3765	False oat	o	o	o	o	o	1:60	o
False oat	2334	False oat	o	o	o	o	o	1:40	o
Foxtail	2297	Foxtail	o	o	o	o	o	o	1:80
Foxtail	3771	Foxtail	o	o	o	o	o	o	1:40

o = No inhibition at 1:5.

mosaic of its own. It is difficult to be certain that this characteristic mosaic is an expression of a qualitative rather than of a quantitative nature. However, if it were simply quantitative then it must be postulated that there are at least six different antigens in the extracts studied. In all probability the individual pollen extracts vary in their content of the common antigens which they possess and at the same time possess certain qualitatively distinct antigens characteristic of their own individualities.

DISCUSSION

Clinical experience with skin tests and with specific hypo-sensitization of hay-fever patients with grass pollen extracts has divided allergists on a point of opinion. One group holds the view that the pollens of the different grasses, although

TABLE 3
Inhibition of the Homologous Agglutination Reactions with Grass Pollen Extracts using 4 Agglutinating Doses of Second Course Antisera.

Anti-Serum Rabbit Anti :		Antigen. Tanned red Cells „Coated“ With Pollen Extracts of:—	Dilutions at which the following extracts inhibited the reaction						
			Control Fluid	Rye	Cocksfoot	Yorkshire fog	Timothy	False oat	Foxtail
Rye	2397	Rye	o	1:80	1:5	1:5	1:5	o	o
Rye	2398	Rye	o	1:160	1:40	1:40	1:20	o	1:10
Cocksfoot	2399	Cocksfoot	o	1:5	1:160	1:5	o	1:5	1:5
Cocksfoot	2395	Cocksfoot	o	o	1:320	1:5	o	o	o
Yorkshire fog	2323	Yorkshire fog	o	1:20	1:5	1:320	1:20	1:5	1:5
Yorkshire fog	2322	Yorkshire fog	o	1:40	o	1:160	1:20	1:5	1:5
Timothy	3764	Timothy	o	o	o	o	1:160	o	o
False oat	3765	False oat	o	o	o	o	o	1:640	o
False oat	3334	False oat	o	1:10	1:5	1:10	o	1:320	o
Foxtail	2297	Foxtail	o	o	o	o	o	o	1:160
Foxtail	3771	Foxtail	o	o	o	o	o	o	1:160

o = No inhibition at 1:5.

possessing common allergens, also possess specific allergens peculiar to the species or genus and that these specific allergens also play a role in hay-fever. The other group considers that the allergens responsible for grass pollen hay-fever are the same in the different grass pollens.

The experiments reported here merely give what may be considered as corroborative evidence to the former body of opinion. They demonstrate by serological *in-vitro* tests that the pollen of each of the six grasses studied possesses a characteristic antigen-mosaic composed of antigens common to other grass pollens and an antigen or antigens peculiar to the grass pollen in question.

SUMMARY

1. Rabbits were inoculated with the pollen extracts of six different grasses. The antisera so produced were used

to study serologically the antigenic nature of the pollen extracts.

2. Direct agglutination reactions using the "antigen coated particle" technique of Boyden (1951) demonstrated the presence of common antigens in each of the six different grass pollen extracts. However, using an inhibition method, an antigen-mosaic characteristic for each of the six grass pollen extracts could be shown.

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