

# A STUDY OF THE ANTIGENIC PROPERTIES OF THE POLLEN OF CERTAIN PLANTS BY MEANS OF THE GEL-PRECIPIATION REACTION

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The investigation of the antigenic structure of the pollen of different plants is of great importance in the present state of development of the study of the pollenoses—allergic diseases caused by plant pollen. Outside the USSR investigations have been made of the antigenic properties of the pollen of herbaceous plants: timothy-grass, twitch-grass, fox-tail grass, rye-grass, false oat, dandel, dogs'-tail grass, sweet vernal grass, brome grass [1], and ambrosia [3]. The antigenic properties of tree pollen have not yet been investigated. There is no information in the literature on the antigenic structure of the pollen of plants growing in the USSR.

In order to study the antigenic structure of plant pollen, the gel-precipitation reaction of Ouchterlony is most frequently used [6]. Since plant pollen possesses relatively weak antigenic properties, in order to obtain immune sera special schemes of immunization of animals with pollen antigens are used, employing stimulators [1, 2, 4]. It has been found that immunization with a suspension of pollen is more effective than immunization with a saline extract of pollen.

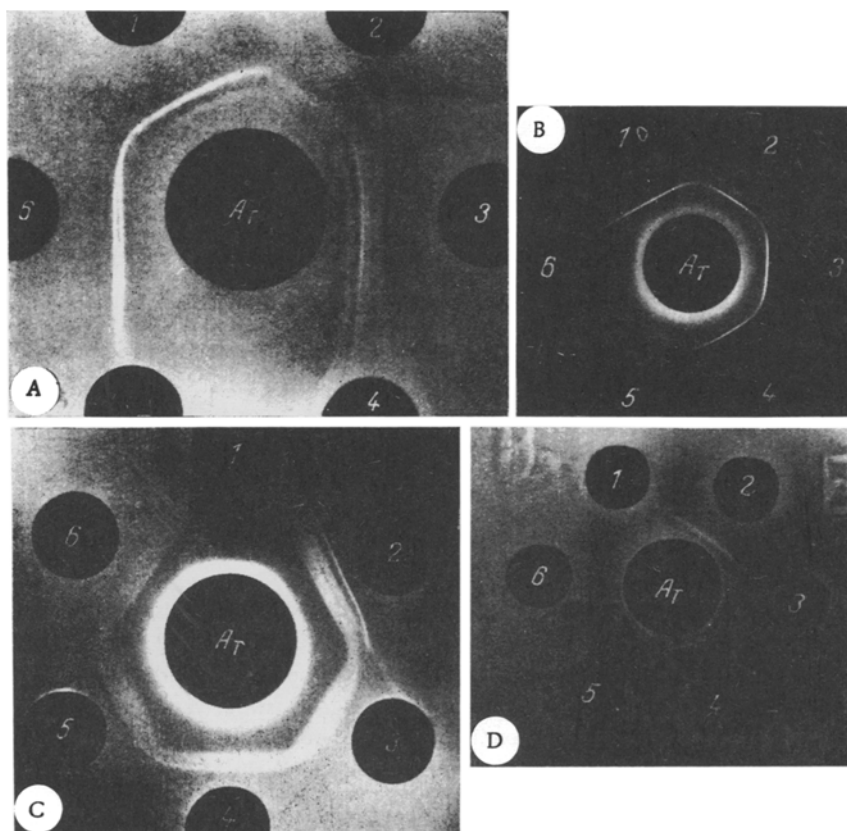
The objects of the present investigation were: 1) to obtain immune sera to antigens of pollen from the following grasses: timothy grass (*Phelum pratense*), cock's-foot grass (*Dactylis glomerata*) and false oat (*Arrhenatherum elatius*), and also to antigens of pollen of the following trees: poplar (*Populus suaveolens*), maple (*Acer pequendo I*), silver birch (*Betula verrucosa*), pine (*Pinus silvestris*), aspen (*Populus tremula*), and ash (*Fraxinus excelsior*); 2) to study, by means of the Ouchterlony gel-precipitation reaction, the antigenic composition of the pollen of these plants; 3) to discover any possible cross reactions between the antigens of the pollen of the following grasses: timothy-grass, cock's-foot grass, false oat, meadow fescue (*Festuca pratense*), rye (*Secale cereale*), couch grass (*Agropyrum repens*), upright brome grass (*Bromus erectus*), and smooth-stalked meadow grass (*Poa pratensis*); and 4) to discover any possible cross reactions between the antigens of the pollen of the following trees: poplar, maple, birch, pine, aspen, ash, hazel (*Corylus avellana*), gray alder (*Alnus incana*), and spruce (*Picea excelsa*).

## METHOD

To obtain antisera rabbits weighing 2.5-3 kg were used. Altogether 32 rabbits were immunized: 18 with grass pollen and 14 with tree pollen. The results of preliminary investigations confirmed Feinberg's discovery that immunization of animals with pollen suspension together with Freund's stimulator is more effective than immunization with a mixture of a saline extract and the same stimulator.

The pollen was defatted with ether and mixed with stimulator in the ratio of 5 g pollen to 100 ml stimulator. The composition of the latter was: mineral oil—2 parts, anhydrous lanoline—1 part, and killed tubercle bacilli—1 mg, per ml of mixture. Sodium merthiolate was used as antiseptic in a proportion of 1:10,000.

The scheme of immunization of the rabbits was as follows. A 5% suspension of pollen, 0.6 ml by volume, was injected subcutaneously into one hind limb and intramuscularly into the other. The injections were repeated weekly for 3 weeks. The course of immunization was repeated every 2 months. During immunization, blood was taken from the animals and the gel-precipitation reaction carried out by Ouchterlony's method with corresponding antigens. Animals with a high antibody titer were sacrificed. Their serum was frozen at  $-20^{\circ}$  and used when required.



Gel-precipitation reaction with extracts of grass and tree pollens. A:  $A_T$ ) antiserum to timothy-grass dust; 1) extract of false oat pollen; 2) cock's-foot grass; 3) timothy grass; 4) ash; 6) fescue; 5) extracting fluid; B:  $A_T$ ) antiserum against timothy-grass pollen; 1) extract of fescue pollen; 2) timothy-grass pollen; 3) cock's-foot grass; 4) false oat; 5) rye; 6) birch; C:  $A_T$ ) antiserum against false oat pollen; 1) extracting fluid; 2) extract of false oat pollen; 3) timothy-grass; 4) cock's-foot grass; 5) rye; 6) fescue; D:  $A_T$ ) antiserum against birch dust; 1) extract of elm pollen; 2) birch; 3) hazel; 4) alder; 5) poplar; 6) ash.

This method was used to immunize 22 rabbits: 5 rabbits with timothy-grass pollen, 4) with cock's-foot grass pollen; 3) with false oat pollen; 3) with birch pollen; 1) with maple pollen; 2) with aspen pollen; 1) with pine pollen, and 3 rabbits—with ash pollen.

Another group of rabbits was immunized with a suspension of pollen with stimulator, receiving two courses of injections, and 2 months later each rabbit was given an injection of 2 ml of a saline extract of the corresponding pollen daily for 3 days: on the first day, intraperitoneally, and on the next 2 days intravenously. The series of injections was repeated every week for 4 weeks. This alternation of the courses of immunization with pollen suspension and extract was repeated to obtain an immunological reaction of high intensity. Altogether 10 rabbits were immunized by this combined method: 4 rabbits with timothy-grass pollen, 1) with cock's-foot grass pollen; 1) with false oat pollen; 1) with birch pollen; 2) with maple pollen, and 1 rabbit—with poplar pollen.

Saline extracts of various species of pollen were used for the Ouchterlony gel-precipitation reaction. The total nitrogen concentration in the extracts was determined by Kjeldahl's method. The extracts prepared from the different species of pollen contained from 0.21 to 0.63 mg nitrogen/ml.

## RESULTS

Comparison of the two variants of immunization which were used showed that the more effective was the combination of courses of immunization with pollen suspension and stimulator and with a series of injections of saline

extract. For example, the antisera of rabbits receiving 6 courses of immunization with timothy-grass pollen and stimulator gave 1 and 2 precipitation lines respectively with the homologous antigen, but with antiserum of rabbits when the combined method was used, 3-5 specific precipitation lines were obtained (see figure, A).

As a rule the number of precipitation lines increased in the process of immunization. For example, in rabbit No. 856 the first precipitation line appeared after the 2nd course of immunization, the next four lines—after the 5th course, and the remaining lines—after the 8th course of immunization.

In order to detect cross reactions with the immune sera obtained (sera against timothy-grass, false oat, and cock's-foot grass antigens) extracts of pollen from rye, fescue, couch grass, smooth-stalked meadow grass, and upright brome grass were used. The precipitation lines were clearer and most numerous in the reactions between extract of timothy-grass pollen and the corresponding antiserum. With an increase in the number of specific precipitation lines an increase in the number of cross reactions was observed.

Antisera against timothy-grass pollen most frequently gave precipitation lines with extract of fescue pollen, and less frequently with extract of false oat and cock's-foot grass pollens. No cross reactions were observed between antisera and extracts of tree pollens (see figure, B). Cross-precipitation lines appeared frequently between antiserum against cock's-foot grass and antigens from timothy-grass, fescue, and false oat pollens, and between antiserum against false oat and antigens from timothy-grass, fescue, and cock's-foot grass pollens (see figure, C). Cross reactions were also obtained with antigens from tree pollens. For example, antiserum of rabbit No. 846, immunized with birch pollen (see figure, D), gave cross precipitation lines with antigens from alder and hazel pollens. In control tests in which the antiserum was replaced by normal rabbit serum, or extracting fluid was used instead of antigens, no precipitation lines appeared.

Tree pollens caused a less intensive production of antibodies by rabbits than grass pollens. Antisera against tree pollens gave a positive reaction after 4-5 courses of immunization, whereas clear precipitation lines with antisera against grass pollens appeared as a rule after the 2nd course of immunization. The grass pollens also possessed more marked allergenic properties than the tree pollens. Comparison of the frequency with which positive cutaneous allergic reactions were obtained showed that of 72 patients with hay fever 68 gave reactions to antigen from grass pollens, but only 4 reacted to antigen from tree pollens; the highest percentage of positive tests was obtained with extract of timothy-grass pollen.

The cross reactions obtained by Ouchterlony's method evidently indicate that the pollens of related plants possess common antigenic properties. The presence of common antigenic properties in different species of pollen may, in our opinion, explain the polyvalency of cutaneous reactions in patients with allergic diseases.

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