# IMMUNOLOGICAL CHARACTERISTICS OF THE POLLEN OF Ambrosia artemisiaefolia

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An increase in the incidence of allergic diseases of both infectious and non-infectious origin has recently been observed. The correct diagnosis and successful treatment of the pollinoses (bronchial asthma, hay fever, etc.) are impossible without accurate knowledge of the antigenic (allergenic) properties of plant pollen. A particularly important place among these allergens is occupied by the pollen of the common ragweed (Ambrosia artemisiaefolia L.), the cause of many allergic diseases in Europe and America [3, 6, 8, 9]. This species of ragweed is widespread in the USSR also, especially in Krasnodar Province [1, 2]. However, there is no information in the literature concerning the allergic properties of the pollen of the ragweeds found in the USSR.

The object of this investigation was to study the antigenic properties of the pollen of Ambrosia artemisiaefolia and to develop a method of specific desensitization of animals sensitized to this species of allergen.

It is very difficult to obtain an allergic reaction in animals or to induce antibody formation to plant pollen in animals. Most investigators report that allergic reactions and antibody production can only be produced by immunization of animals with alum-precipitated pollen extracts or by administration of extracts mixed with Freund's complete adjuvant [7, 10]. Some authors [4] consider that extracts of the pollen of the ragweed are antigenically active even without preliminary precipitation with alum and without the addition of adjuvant. Others [5] state that a suspension of dry pollen in "stimulator" is highly effective for immunization.

## METHOD

The anaphylactogenic properties of common ragweed pollen were studied in experiments on 73 guinea pigs weighing 250-300 g. The animals were sensitized with a 5% suspension of ragweed pollen and with an extract of the pollen prepared on the basis of 5 parts of pollen to 100 parts of extracting fluid. The 5% suspension was prepared treating the pollen with ether and carefully mixing it in sterile conditions with "stimulator" (mineral oil 40 g, anhydrous lanoline 20 g, killed Mycobacterium tuberculosis cells 60 mg). The stimulator was sterilized by autoclaving. The pollen suspension was rendered aseptic by addition of merthiolate in a dose of 10 mg/100 ml suspension. The extract was prepared by extraction with Kok's fluid for 3 days from pollen defatted with ether, the resulting product then being passed through a Seitz filter and tested for sterility.

Preliminary experiments on intact animals showed that the extract did not possess toxic properties, so that it could be used for the reacting injection.

## RESULTS

The anaphylactogenic properties of ragweed pollen were tested in the experiments of series I. The animals were sensitized with two subcutaneous injections of 5% pollen suspension in a volume of 0.5 ml and a single subcutaneous (24 guinea pigs) or intraperitoneal (10 guinea pigs) injection of a 5% extract of ragweed pollen in the same dose. The preparations were injected on alternate days. On the 21st day after the beginning of sensitization,

TABLE 1. Anaphylactic Reaction in Guinea Pigs Sensitized with Ragweed

| Animals | Number of animals | Anaphylactic reaction | | | | |
|---|---|---|---|---|---|---|
| | | − | ± | + | ++ | +++ |
| Experimental | | − | − | 8 | 6 | 20 |
| Control | | 10 | − | − | − | − |

Note: − no reaction, ± very slight reaction, + anaphylactic signs in the form of scratching, sneezing, pawing movements, with no obvious signs of bronchospasm, ++ marked anaphylactic reaction with obvious signs of bronchospasm, +++ anaphylatic shock ending in death.

TABLE 2. Effect of Specific Desensitization on Development of Anaphylactic Reaction in Guinea Pigs

| Animals | Number of animals | Anaphylactic reaction | | | | |
|---|---|---|---|---|---|---|
| | | − | ± | + | ++ | +++ |
| Experimental | | 19 | − | − | − | − |
| Control | | − | − | − | 2 | 8 |

the guinea pigs were injected intravenously with the reacting dose of antigen – 5% extract of ragweed pollen in a dose of 1 ml. Control guinea pigs were sensitized in the same manner, but instead of the reacting dose of antigen they were injected with an equal volume of extracting fluid (5 guinea pigs) or extract of timothy-grass pollen (5 guinea pigs).

The results given in Table 1 show that after intravenous injection of the reacting dose of antigen into the sensitized animals, in every case an anaphylactic reaction developed, and 20 animals developed fatal shock. No anaphylactic reaction was observed in the control animals.

In the experiments of series II, in order to ascertain the possibility of specific desensitization, 19 guinea pigs sensitized as described above were injected intravenously with a 5% extract of ragweed pollen in a dose of 0.1 ml, 2 h before the reacting injection of antigen. The control for this series of experiments consisted of guinea pigs which received an intravenous injection of extracting fluid in a volume of 0.1 ml or 5% extract of timothy-grass pollen in the same volume 2 h before injection of the reacting dose of antigen.

The results given in Table 2 show that the preliminary intravenous injection of 5% extract of ragweed pollen before the reacting injection prevented the development of the anaphylactic reaction in all the experimental animals. Injection of the reacting dose into the control guinea pigs, on the other hand, in all cases caused the development of severe anaphylactic shock, which in 8 cases out of 10 led to death.

The antigenic properties of ragweed were also studied in 8 rabbits. The animals were immunized with a 5% suspension of pollen in "stimulator." The suspension was injected subcutaneously and intramuscularly into the rabbit's hind paw in doses of 0.6 ml. The injections were repeated three times at intervals of 7 days. Three such courses of immunization were given, with intervals of 2 months between them. Two weeks after the last course, blood was taken from the animals for testing for the presence of precipitating and hemagglutinating antibodies.

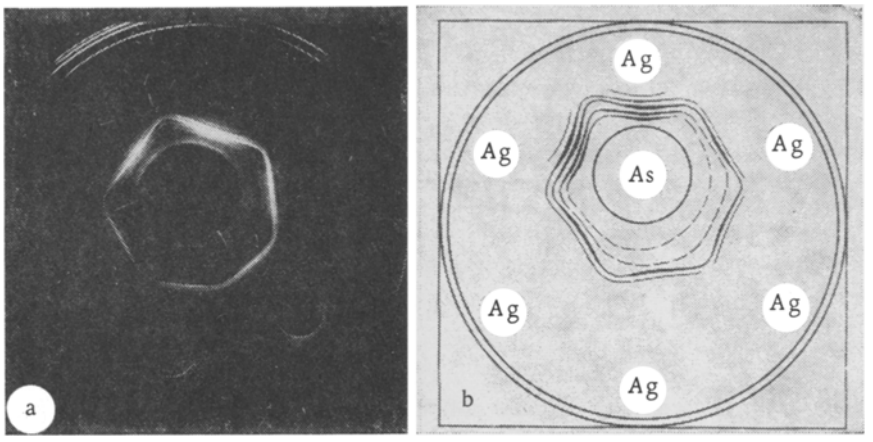


Fig. 1. Gel-diffusion reaction with eccentric central well. a) Photograph; b) scheme of reaction (As-antiserum, Ag-extract of ragweed pollen).

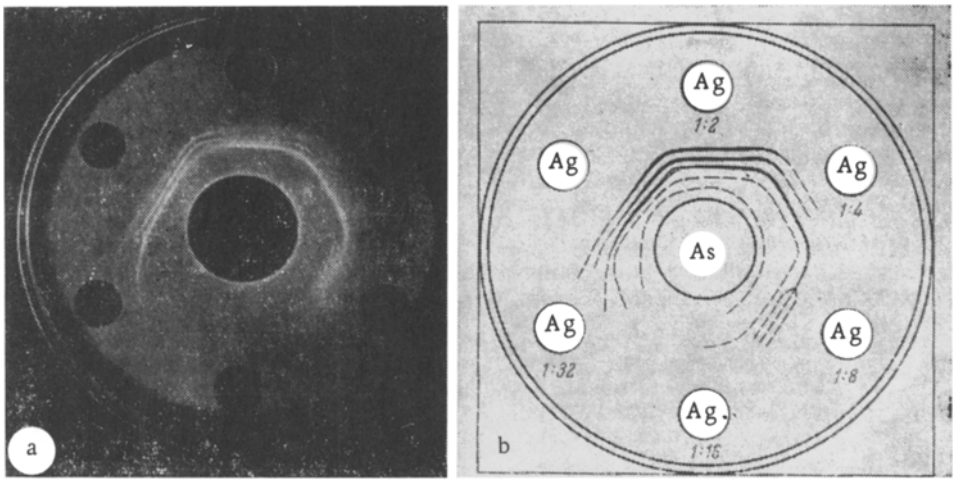


Fig. 2. Gel-diffusion reaction between antiserum and ragweed pollen extract. Numbers on scheme—dilutions of extract of ragweed pollen. Rest of legend as in Fig. 1.

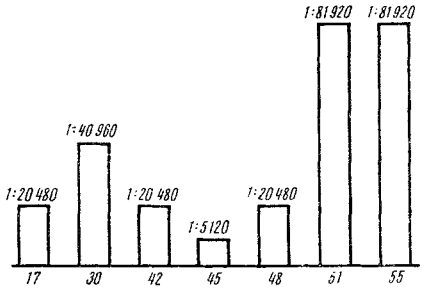


Fig. 3. Titer of hemagglutinating antibodies in serum of 7 rabbits immunized with ragweed pollen.

Precipitating antibodies were determined in the serum by the double diffusion method in gel as described by Ouchterlony. The test was carried out in the usual way in Petri dishes, using 1.5% agar. The central well, 20 mm in diameter, was filled with antiserum, and the peripheral wells, 10 mm in diameter and situated 20 mm from the central well, were filled with extract of ragweed pollen. In some experiments different dilutions of the extract were used.

To obtain a more definite idea of the number of precipitation lines, the reaction was also carried out with an eccentric central well. As control, instead of antiserum a normal rabbit serum was used.

Ouchterlony's reaction gave positive results with antisera of all the immunized rabbits. Precipitation lines appeared on the day after the reaction was set up. The intensity of the precipitation lines increased over a period of 2 weeks. At least 5 precipitation lines were found, two of which were weak (Figs. 1 and 2). After dilution of the ragweed pollen extract a gradual reduction in the intensity of most of the precipitation lines was observed, and when the antigen was diluted 1:16, four of the lines disappeared completely.

When the antiserum was replaced by normal rabbit serum no precipitation lines appeared.

The presence of agglutinating antibodies in the serum of the rabbits was detected and their titer determined by the method of passive hemagglutination of tanninized erythrocytes, introduced by Boyden. As antigen a 5% extract of ragweed pollen in a dilution of 1:50 was used. The titer of antibodies was taken to be the dilution of serum at which a hemagglutination reaction assessed as + was observed. It is clear from the diagram in Fig. 3 that hemagglutinating antibodies were detected in high titer in the serum of the rabbits.

In control experiments with normal serum or erythrocytes untreated with antigen, and also in reactions in which extract of timothy-grass pollen or twitch pollen was used as antigen, negative results were obtained.

The investigations showed that the pollen of Ambrosia artemisiaefolia, growing in Krasnodar Province, possesses marked antigenic and anaphylactogenic properties. Extracts of ragweed pollen contain not less than five antigenic components. It is considered that the detection of precipitating (by Ouchterlony's method) and hemagglutinating (by Boyden's method) antibodies in the serum of a patient sensitized to ragweed pollen may be of considerable value in the diagnosis of increased sensitivity to this species of allergen. It is evident that good results may be expected in sensitization of ragweed pollen by the use of specific therapy.

## LITERATURE CITED


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