EXPERIMENTAL STUDY OF THE ALLERGENIC ACTIVITY OF *Cyclachena xanthifolia* POLLEN

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Successful specific hyposensitization in pollinoses largely depends on the completeness of discovery of the allergens concerned [1]. Besides the well-known weeds associated with the risk of allergy, the aerial spectrum of Saratov from August through October has been found to contain a high concentration (352 pollen grains/cm^2) of pollen of *Cyclachena xanthifolia* (Nutt) Fresen, belonging to the Astraceae family.

Previously, on the basis of results of skin testing of patients with pollinoses by an extract of cyclachena pollen it was suggested that this could be concerned in the formation of pollen allergy [9]; however, a morphological study of cyclachena and ragweed pollen revealed no clear differences between them, and no attempt has been made to study the allergenic properties or immunologic characteristics of this species.

It was accordingly decided to study the structural features of *C. xanthifolia* pollen and its sensitizing properties.

EXPERIMENTAL METHOD

Pollen was collected in the usual way [4, 23]. The morphological features of the pollen of *C. xanthifolia* and of *Ambrosia artemisifolia* (ragweed) were compared by study with the HI-12A (Hitachi) scanning electron microscope. Fresh pollen from the two species of plants, after appropriate preparation [5], were taken for examination. Ultrathin sections were cut on the LKB-480-2A Ultrotome by means of glass knife after preliminary treatment, and examined in the LEM-7A electron microscope. The allergen was obtained from the pollen by saline extraction as described by Coca and Milford [2]. To study the allergenic properties of the cyclachena pollen guinea pigs were sensitized in accordance with the usual scheme [6]. The response of the lymphoid system to antigenic stimulation was evaluated according to changes in the cytological composition of the lymph nodes by examination of squash preparations 8-25 days after injection of the antigen [7]. The presence of reagin-like antibodies in the sensitized guinea pigs was judged from changes in rabbit basophils under the influence of an allergen (extract of cyclachena pollen)—antibody (serum of a sensitized guinea pig) complex in the indirect basophilic test [11]. To confirm the allergenic properties of the cyclachena pollen extract the method of passive cutaneous anaphylaxis [10] was used. Changes in the skin to the allergen—antibody reaction were observed after intradermal injection of serum of sensitized guinea pigs (diluted 1:10 and whole). After fixation of the antibodies in the skin for 4 h a mixture of 1 ml of 1% trypan blue solution and 1 ml of cyclachena allergen, containing 15,000 PNU was injected, and after 10-20 min the diameter of the foci of erythema was measured. The degree of allergenicity of the pollen was assessed from ability to reproduce anaphylactic shock and its severity, using the equation in [6] for the calculation. In animals dying from anaphylactic shock a histological study was made of tissues of the intestine, lungs, and skin.

EXPERIMENTAL RESULTS

The morphological study of pollen of *C. xanthifolia* and of *A. artemisifolia* in the light and electron microscopes showed that with respect to the principal morphological fea-

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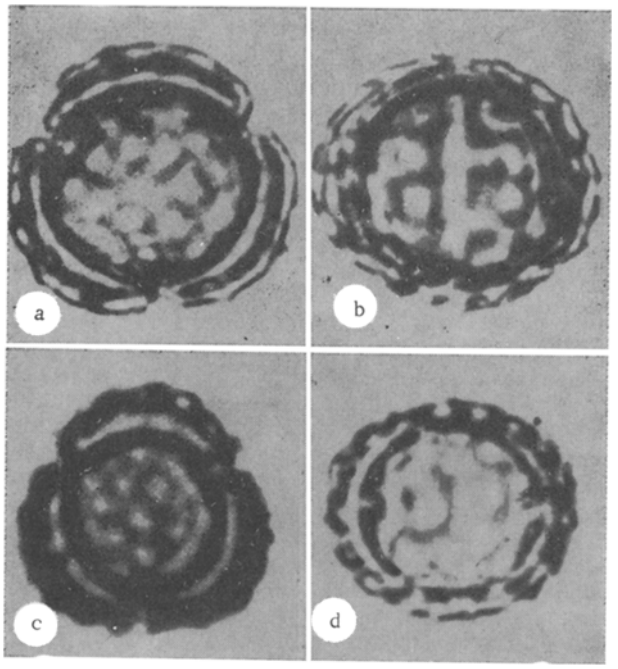

Fig. 1. Pollen grains of *Cyclachena xanthifolia* (a, b) and of *Ambrosia artemisifolia* (c, d) under the light microscope. 500×. a, c) viewed from the pole; b, d) from the equator.

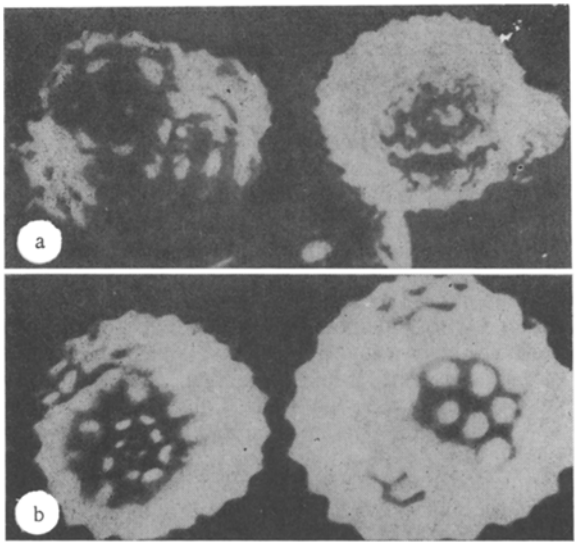

Fig. 2. Pollen grains of *Cyclachena xanthifolia* (a) and *Ambrosia artemisifolia* (b) in the scanning electron microscope. 2000×.

tures (length of the polar axis and equatorial diameter, the dimensions of the apocolpia and mesocolpia, the length and width of the grooves, the diameters of the pores, the height and diameter of the base of the spine, the thickness of the ect- and endexine, the air cavity) the pollen grains of these two species differed statistically significantly from each other; the principal and clearest difference related to the length of the grooves. Pollen grains of cyclachena have long grooves; the grooves are almost equal in length ($x = 19.87$, $\sigma = 4.35$, $S_x = 0.97$) to the transverse axis, they narrow toward the poles, where they almost join together. In ragweed the grooves are very short ($x = 1.59$, $\sigma = 5.92$, $S_x = 0.12$) and in them can be found a pore, the diameter of which is only a little less than the

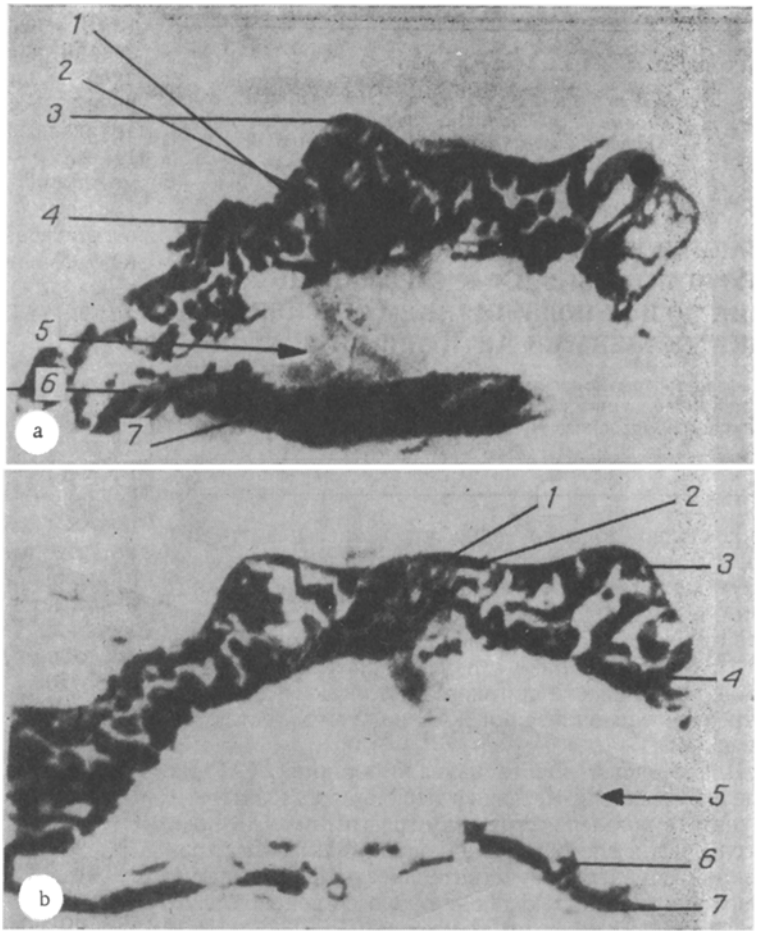

Fig. 3. Ultrathin sections through exine of *Cyclachena xanthifolia* (a) and *Ambrosia artemisifolia* (b) pollen. 20,000×. 1) columnar layer; 2) tectum; 3) spines; 4) foot layer; 5) air cavity; 6) lower layer of ectexine; 7) endexine.

width of the groove (Fig. 1). Under the electron microscope the pollen grains of the two species are spherical, trilobate, and covered with conical spines of equal size. Cyclachena pollen grains have three long deep grooves which narrow toward the poles. The grooves of ragweed pollen grains have the appearance of a hollow with slit-like pore inside (Fig. 2). Examination of ultrathin sections through the exine showed that the thickness of the exine of *C. xanthifolia* pollen is greater than that of ragweed, but the size of the inner cavity is smaller (Fig. 3). If the fact is recalled that the exine and its inner cavity may be the containers of allergenic proteins [9], it can be tentatively suggested that *Cyclachena* pollen grains should possess somewhat weaker allergenic activity than ragweed. An experimental study of the allergenic properties of cyclachena showed that in the early stages of antigenic stimulation (6th-18th days after injection of pollen) marked plasmatization developed in the regional and distant lymph nodes. The number of immunocompetent cells capable of actively synthesizing antibodies increased to 2.44-4.04% compared with 0.10-0.15% in intact animals (on statistical analysis of the data the difference between the experimental and control groups was significant by the chi-square test; $P < 0.05$).

Changes in the lymphoid tissue were accompanied by liberation of circulating reagin-like antibodies into the blood stream. After incubation of basophils of a healthy rabbit for 10 min in medium containing guinea pig serum and cyclachena pollen allergen in optimal proportions, changes were observed in the arrangement, staining, and mobility of the granules, and also in the shape of the basophils themselves: irregularly pear-shaped cells with pseudopodia appeared. In control investigations the number of altered basophils varied between 10 and 15%, but did not exceed 20%. In the experimental group the number of altered basophils increased to 20-50%. Statistical analysis by the chi-square test showed a

significant difference between control and experimental group ($P < 0.05$). An important biological property of reagin-like antibodies is their ability to induce passive cutaneous sensitization — this was observed in Ovary's phenomenon [1]. Foci of intensive erythema, the diameter of which was greatest (up to 10 mm) in cases when undiluted serum of guinea pigs sensitized with cyclachena pollen was injected, appeared 3-4 min after intravenous injection of trypan blue and cyclachena allergen in animals sensitized intradermally by serum containing reagin-like antibodies. The intensity of the reaction was lower when sensitization was carried out with serum diluted 1:10. The diameter of the foci of erythema in areas where healthy guinea pig serum and physiological saline were injected did not exceed 3-4 mm.

During reproduction of anaphylactic shock, four of the twelve sensitized guinea pigs developed lethal anaphylactic shock in response to injection of the reacting dose of allergen, and six animals developed severe shock. The anaphylactic index, calculated by the equation used, was 3.5 [6]. In response to injection of allergen into healthy guinea pigs (control) none of the animals showed any features of anaphylaxis.

Cyclachena pollen thus possesses marked allergenic properties. When injected into experimental animals immunologic changes take place in lymphoid tissue, skin-sensitizing antibodies capable of injuring cells are produced, and if the allergen is injected into sensitized animals they develop anaphylactic shock.

These experiments, and subsequent clinical observations, showed that *Cyclachena xanthifolia* pollen, together with other pollen allergens, plays an important role in the development of pollinoses.

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