MODEL OF EXPERIMENTAL POLLINOSIS

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A model of experimental pollinosis was created in guinea pigs. The animals were kept in a chamber in which the allergen, a dialyzed aqueous extract of ragweed pollen, was dispersed by means of a coaxial nebulizer. As a result of aerosol sensitization the guinea pigs formed homocytotropic antibodies, an indication of their sensitization to the specific allergen. Sensitization of the animals to ragweed allergen was accompanied by increased sensitivity of the bronchopulmonary system and was characterized by reflex bronchospasm to a reacting inhalation or to intravenous injection of the specific allergen.
KEY WORDS: pollinosis; ragweed; inhalation; homocytotropic antibodies.

Allergic responses of immediate type, characterized by the production of special antibodies known as reagins, are the leading mechanism of pollinosis. The discovery of the mechanisms of antibody formation in pollinosis can be largely facilitated by the creation of an experimental model. With the aid of such a model of allergic diseases, those allergic reactions which constitute the pathogenetic basis of disease can be reproduced [2].

The use of the inhalation method to produce sensitization of various animals has been reported in the literature. The authors in question used powerful allergens such as horse dander, egg albumin, and blood serum proteins [4, 8, 11, 13, 14]. No reference could be found to the use of plant pollen allergens as sensitizing agents. Yet pollen allergens, used to produce experimental pollinosis, are the most adequate material.

The object of this investigation was to create an experimental model of pollinosis and to study immunologic reactivity in this model of the disease. Guinea pigs were used as the experimental animals, for bronchospasm and disturbance of cardiovascular activity occupy a leading place in the pathogenesis of allergic reactions in these animals.

EXPERIMENTAL METHOD

Preparation of a Water-Soluble Fraction of Ragweed Pollen. Experiments were carried out on 142 albino and noninbred guinea pigs weighing 200-250 g. A dialyzed water-soluble extract of pollen of Ambrosia artemisifolia, prepared by a modified Goldfarb's method [7], was used as the allergen. The dry, nondefatted ragweed pollen was mixed with distilled water in the ratio of 1 :8 and extracted for 24 h on a magnetic mixer at 4°C. The resulting extract was separated by filtration, dialyzed against distilled water for 4 days, its pH adjusted to 7.0, and lyophilized. The dried fraction was used to sensitize the animals by inhalation.

Method of Inhalational Sensitization. The experimental animals were kept in a closed airtight chamber with a volume of 0.7 m^3. The allergen in a dose of 500 mg, dissolved in 5 ml distilled water, was dispersed in the chamber by means of a coaxial nebulizer under the pressure of 2.5-2.9 atm. The conditions of dispersion were such as to produce an aerosol consisting of particles 2-2.5 μ (83%) and over 2.5 μ (17%) in diameter [3]. The animals were sensitized by three courses, each of which consisted of four daily inhalations. One inhalation lasted 2 h. The intervals between courses were 12-14 days. The aspiration dose of the allergen obtained by the guinea pigs during inhalation for 2 h was determined by multiplying the minute volume of inspiration by the concentration of the allergen in the chamber and the exposure. Since the respiratory minute volume was 50 ml and the concentration of the allergen was 0.00071 mg/ml chamber air, during an exposure of 2 h the

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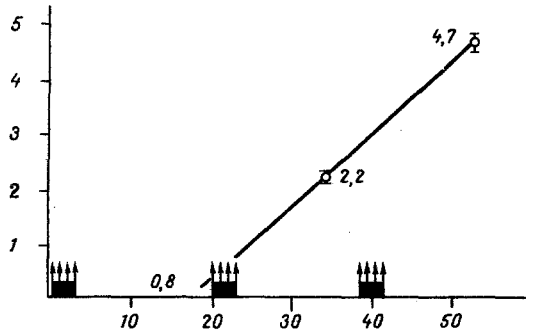

Fig. 1. Dynamics of AHA titers in guinea pigs during sensitization by inhalation. Abscissa, stage of experiment (in days); ordinate, antibody titer (in $\log_2$). Inhala- of ragweed allergen indicated by arrow.

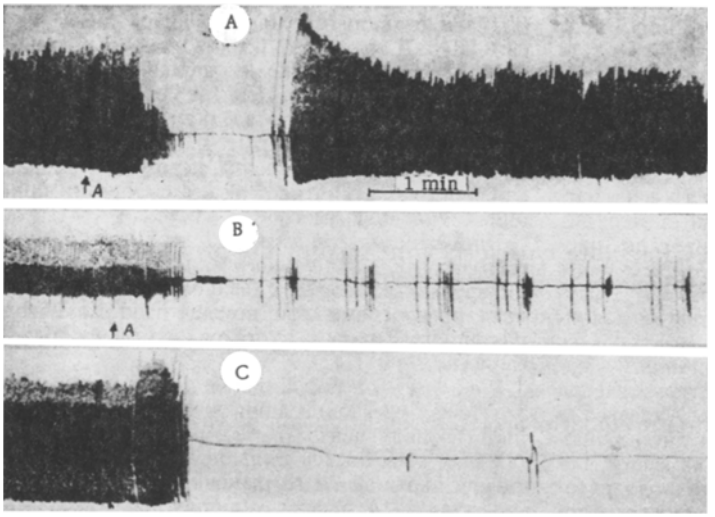

Fig. 2. Respiration of guinea pigs sensitized by the aerosol inhalation method recorded in vivo. A) One course of sensitization; B) two courses; C) three courses. Time of injection ragweed allergen indicated by arrow.

guinea pigs inhaled approximately 4.26 mg of the allergen.

Passive Cutaneous Anaphylaxis. Antiragweed homocytotropic antibodies (AHA) were detected in the serum of all the sensitized animals by the passive cutaneous anaphylaxis test [10]. For this purpose 0.1 ml of the test serum was injected intradermally into the depilated dorsal surface of a normal guinea pig. After an interval of 24 h 0.5 ml of 6% ragweed pollen extract with 0.25 ml of 5% Evans' blue dye was injected intravenously. The reaction was read 25-30 min later by measuring the diameter of the stain. A stain 4 mm in diameter was taken as the titer of the serum.

Pneumograms of the Guinea Pigs. To assess the state of the bronchopulmonary system of the sensitized guinea pigs pneumograms were recorded by means of a two-channel plethysmograph with ink recorder of the P42-SKhÉ type.

EXPERIMENTAL RESULTS

The AHA, appearing in the serum of the experimental animals and detected by the passive cutaneous anaphylaxis test were used as the index of sensitization of the animals. Maximal fixation of AHA in the recipients' skin was observed 24 h after injection of the serum of the experimental animals. Considerable variability in AHA production by individual groups of experimental animals was noted, but nevertheless there was a clear increase in the AHA titers in the course of repeated inhalations of the allergen. As a rule, after the first course of inhalations AHA were detected extremely rarely (in 5 of 142 guinea pigs, 3.5%) and their mean titer was 0.8 ± 0.06*. After the second course of inhalations antibodies were found in 56.2% of animals (in 68 of 121 guinea pigs) and their mean titer was 2.2 ±0.23. After three courses of inhalations antibodies were discovered with a mean titer of 4.7 ± 0.3 in 82.3% of animals (in 70 of 85 guinea pigs) (Fig. 1).

*Here and later antibody titers are given as $\log_2$ of the number expressing the titer.

To study the properties of AHA their response to heating and to treatment with mercaptoethanol was investigated. During heating of the sera of the sensitized guinea pigs for 4 h at 56°C their skin-sensitizing activity diminished or disappeared altogether. Before heating, the mean titer of the sera was 4.6 ± 0.42, whereas after heating it was 0.16 ± 0.12 (7 sera). After treatment of the sera with 0.2 M mercaptoethanol [9], a decrease in activity of AHA was observed. The mean titer of AHA before treatment with mercaptoethanol was 4.2 ± 0.53, but after treatment it was 1.3 ± 0.46 (7 sera). Thermolabile guinea pig AHA, not resistant to mercaptoethanol treatment, if injected into the skin of a normal guinea pig remained there and produced sensitization of an area of skin which lasted 20 days [12]. With respect to their thermolability, sensitivity to treatment by reducing agents, and ability to remain fixed for a long time in the skin of normal guinea pigs, guinea pig AHA resemble human IgE. The connection between AHA and human IgE is also supported by results obtained by other workers [5, 6, 12].

One of the severest clinical manifestations of pollinosis is pollen asthma, manifested principally by bronchospasm and edema of the mucous membrane with hypersecretion of the small and smallest bronchi. The state of the bronchopulmonary system of the sensitized animals was judged by recording the pneumograms. After intravenous injection of ragweed pollen extract into normal animals, in no case was any appreciable change observed in respiration as recorded by plethysmogram, whereas after intravenous injection of 100-300 μg histamine, a profound disturbance of respiration developed in every case and was observed during the recording of histamine shock. The reactions of the bronchopulmonary system of the experimental animals resembled in character those which develop during typical anaphylactic shock of asphyctic type [1]. For instance, mild forms of respiratory disturbances were expressed as a gradual decrease in the amplitude of the respiratory movements and slowing of their rate or transient apnea, lasting 20-30 sec, followed by complete restoration of the respiratory movements (Fig. 2A). Severe forms of respiratory disturbance in the guinea pigs were characterized by longer respiratory arrest (2-3 min) with the subsequent addition of periodic or terminal respiration, frequently ending in paralysis of the cardiovascular and respiratory centers (Fig. 2B, C).

Reactions of this last type were observed in 33.3% of guinea pigs sensitized by one course of inhalations, in 47.6% of animals after two courses, and in 100% after three courses of inhalations. The strongest allergic reactions, accompanied by bronchospasm, emphysema of the lungs, and a fall of blood pressure, were detected after three courses of sensitization in response to a reacting inhalation or to intravenous injection of the allergen.

It can be concluded from the results that inhalational sensitization of guinea pigs with dialyzed aqueous extract of ragweed pollen is characterized by the formation of AHA, which serve as an indicator of sensitization of the animals to the specific allergen. Sensitization of animals with ragweed allergen is accompanied by increased sensitivity of the bronchopulmonary system and is characterized by a reflex bronchospasm to the reacting injection of allergen. The model of experimental pollinosis thus created corresponds to the natural pathway of sensitization during development of the disease and can be recommended for the study of the pathophysiological mechanisms of pollinosis.

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