IMMUNOCHEMICAL CHARACTERISTICS OF AN ALLERGOID FROM PLANT POLLEN

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Investigations aimed at studying the possibility of using preparations with reduced allergenic properties, but preserving the ability to induce synthesis of specific antibodies against the original allergen, and possessing high hyposensitizing activity, for the treatment of pollinoses and other allergoses, are currently in progress [6, 8, 10-16]. Therapeutic preparations of this sort have been obtained by treatment of allergens from plants with haloformates [16], glutaraldehyde [12, 17, 18], and formaldehyde [7, 8], and they have been called allergoids [9]. Residual allergenicity and immunogenicity have been studied experimentally [2, 3, 7-10], and clinical trials of preparations of this kind have been undertaken [6, 11, 13-15]. However, the immunochemical nature of allergoids and the connective mechanisms of their formation have not yet been adequately investigated, and this has retarded to some degree the development of a scientifically based technology of their commercial manufacture.

Some immunochemical properties of allergoids from ragweed pollen were studied in the investigation described below.

EXPERIMENTAL METHOD

The basic principles of preparation of allergoid from ragweed pollen were as follows: extraction of the pollen defatted with ether, dialysis of the extract, freeze-drying, redissolving in diluting fluid, the addition of formaldehyde, and incubation for 32 days [7]. To assess the properties of the allergoid, the following tests were used: Ouchterlony's precipitation test, electrophoresis and immunoelectrophoresis in agarose [4], electrophoresis in polyacrylamide gel [19]; antisera for these tests were obtained by intravenous hyperimmunization of rabbits with ragweed allergen.

EXPERIMENTAL RESULTS

Previous observations that allergoid have lost much of their ability to interact with precipitins were confirmed by Ouchterlony's precipitation test [7]. For instance, Fig. 1 shows that the allergen formed clear precipitation lines with hyperimmune serum, whereas the allergoid, with the same protein nitrogen concentration (0.2 mg/ml), had lost these properties. Immunoelectrophoresis showed that after treatment with formaldehyde antigens with relatively slow electrophoretic mobility were blocked (protein nitrogen concentration in the preparations 1 mg/ml). The allergen formed six precipitation lines with the hyperimmune serum, but the allergoid formed only one line. Mainly those precipitation lines which were located near the cathode disappeared in the test with allergoid (Fig. 2). Electrophoresis of this preparation likewise did not reveal protein fractions with a relatively higher positive charge, whereas they were present in the allergen.

A marked decrease in the hapten-specific activity of the allergoid, which was observed in previous investigations [7, 8, 10], evidently took place through interaction between formaldehyde and the Σ-amino groups of the lysine residues of the allergen molecule, which may lead both to an increase in size of the molecule and to an increase in the rigidity of its structure. This view is in agreement with the opinion of workers who have suggested a technology for preparation of allergoid, and it also agrees with data on the mechanism of antitoxin formation [1, 5].

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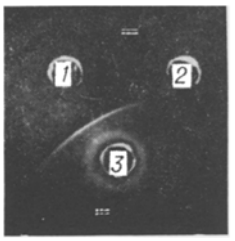

Fig. 1

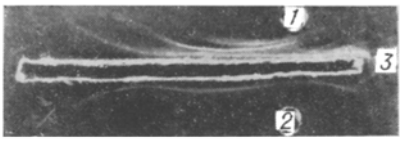

Fig. 2

Fig. 1. Precipitation test in agar. 1) Allergen; 2) allergoid; 3) hyperimmune rabbit serum against allergen.

Fig. 2. Immunoelectrophoresis in agarose. 1) Allergen; 2) allergoid; 3) hyperimmune serum against allergen.

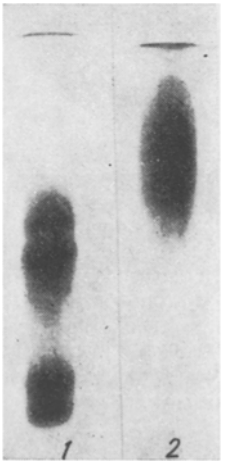

Fig. 3. Electrophoresis in polyacrylamide gel. 1) Allergen; 2) allergoid.

The present investigation of ragweed allergoid and of the original allergen by electrophoresis in polyacrylamide gel after preliminary treatment with 8 M urea gave the following results: the unmodified allergen, after treatment with urea, gave four electrophoretic lines, whereas these fractions could not be found under identical conditions in the allergoid (Fig. 3). This is evidence that the allergoid molecule is more resistant than the allergen molecule to the action of denaturing agents. The location of the protein fraction of the allergoid nearer to the cathode is an indirect indication of an increase in size of the allergoid molecule.

The results are evidence that the reduction of hapten specificity is the result of interaction between formaldehyde and the amino groups of the allergen, leading in turn to increased stability and enlargement of the protein molecules of the allergen. These factors are responsible for the reduction of allergenicity and the increase in immunogenicity of the allergoid.

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