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Fractionation of Pollen Proteins and Their Skin Reactions on Sensitive Individuals.

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Dunbar¹ showed that hayfever was caused by sensitiveness to the proteins of pollen. We are attacking the problem on the following assumption: (1) That hayfever is due to sensitivity of an individual to one or more proteins of pollen. (2) That this sensitivity is due only to substances of protein nature (we are aware that evidence has been presented that sensitivity may be due to non-protein substances. (3) That the isolation of the offending protein or proteins in pure form should be the ideal therapeutic agent. Our present paper is concerned only with the separation of the proteins of giant (*Ambrosia trifida*) and common (*Ambrosia elatior*) ragweed pollen, and the results of skin reactions of the proteins obtained on sensitive individuals.

Twenty-eight gm. of pollen was exhaustively extracted in a ballmill with the following solvents in the order indicated: Distilled water, 5% NaCl, 1% NaF, 0.25% Na₂CO₃, and 0.1% HCl. It was determined clinically that water extracted practically all of the active substances. Hence, our interest is mainly in the water soluble proteins.

To a solution of the water soluble proteins an equal volume of a saturated solution of ammonium sulphate was added. The precipitated proteins were filtered off, dissolved in water, and reprecipitated from the water solution 3 times by adding an equal volume of ammonium sulphate as indicated, finally the proteins were dissolved in water and dialyzed free from salt. The proteins which precipitated between these concentration limits with ammonium sulphate are the globulins. The filtrate from the above fraction was raised to 64% by adding saturated ammonium sulphate. The proteins which were precipitated between these limits were reprecipitated 3 times, finally dissolved in water and dialyzed the same as the previous fraction. The proteins of this fraction are no doubt a mixture of globulins and albumins. The filtrate from the above fraction was completely saturated with ammonium sulphate and the precipitated proteins of

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¹ Dunbar, Berlin klin. Wchnschr., 1903, 24 and 26.

this fraction, precipitating above 64% saturation with ammonium sulphate, are predominantly of the albumin type.

The subjects used for the experiment were consecutive cases giving a history of autumnal hayfever and showing a skin reaction to solutions of ragweed pollens. After the first few cases the tests were limited to 6 solutions of the water soluble proteins, the albumin, globulins and the protein precipitated at 50-64% of ammonium sulphate from the giant and common ragweeds. Each of these solutions was used in a concentration of 0.0025% nitrogen. A drop of the material placed on the volar surface of the forearm was stabbed into the superficial layers of the skin with a sharp, specially designed instrument making a 1 mm. cut not deep enough to produce bleeding. Care was taken to prevent contamination of each drop with the material preceding it. The reactions to the different protein solutions were classified in pluses in each individual by measuring the size of the wheal, the presence or absence of pseudopods, the elevation, and the extent of the erythema produced. The strongest reaction was marked a 4+, and the others graded in proportion.

The 40 cases divided themselves into 2 distinct groups, 20 being present in each group. The individuals of one group were sensitive to the protein of the common ragweed only, those of the other to the proteins of both weeds.

In the group sensitive only to the common ragweed the reaction was distinctly limited to the globulins only, a slight reaction being present in the 50-64% fraction, and no reaction in the albumin fraction.

In the group sensitive to both weeds the reaction was less limited. The skin sensitivity to the common ragweed in this group of cases again was most marked to the globulins, where invariably the reaction was classified as a 4+, and a 2+ to the albumin as well as to the 50-64% fraction of the common ragweed. The reaction to the protein of the giant ragweed given by this group was almost completely limited to the albumin fraction, very little reaction being present to any of the other proteins of the giant ragweed.

The limited amount of pollen material available for this preliminary work compelled the use of solutions of a 0.0025% N concentration limited to the technique described. This permits the conclusion that there are 2 groups of cases sensitive to the ragweed pollens. The difference may be only relative, one group being more sensitive than the other. More concentrated protein solutions and possibly the use of intradermal technique on some cases will be used to clarify this question.