

necrosis apparently is influenced by the extent of diffusion of the toxin.

This study of the effect of rabbit tissues on staphylococcal toxin shows that the toxicity decreases in the presence of some of these tissues. The apparent tissue-adsorption may be due largely to the degree of attenuation attained in the presence of different diluents. These *in vitro* observations with staphylococcal toxin are interesting in view of the interpretation of similar experiments of others with tetanus toxin.

10282 P

Studies on the Chemistry and Immunology of Ragweed Pollen Proteins.

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The opinion has been held for some time in our laboratories that many allergens responsible for atopic symptoms have present two types of protein molecules. An extended study of these allergens has led us to the conclusion that there are usually present representatives of the larger variety, loosely typified by crystallizable egg albumin and also representatives of a type of protein molecule much smaller, approaching the size of the peptone group. In the work of the staff of the Allergy Clinic on the preparation of purified proteins from the various foods and environmental materials, it was found that the more these proteins were purified by repeated precipitation and subsequent dialysis, the less skin reactive they became. Some of them lost practically all of this property. Other investigators have recorded this observation. The usual explanation that has been offered is that the protein has been denatured in the process of purification. This explanation is not valid in the light of experimental evidence in connection with crystallizable egg albumin secured by Cole and Aaronson¹ in this laboratory. They found that with each successive crystallization the skin reaction diminished in certain types of egg sensitive cases until with the sixth crystallization this reaction was quite feeble. The precipitinogenic power of the albumin remained unaltered throughout all the crystallization processes.

¹ Cole, A. G., and Aaronson, A. L., personal communication.

From this we conclude that the antigenicity of the crystallizable egg albumin was not modified and that the skin reacting substance, either in chemical combination or adsorbed on the albumin, was released during the crystallization processes.

This study was undertaken to determine whether a similar condition existed in the protein content of pollen.

Equal parts of giant and short ragweed pollen were defatted with ether and extracted with distilled water. Rabbits were injected until a serum of a high precipitin titer was obtained against this pollen extract.

Portions of the pollen extract were acidified with various buffers. The precipitates that occurred reached the maximum at a pH between 3.8 and 4.2, usually at 4.0 with a definite diminution in the total nitrogen content of the solution. An additional slight precipitate occurred on heating, reaching its maximum at a temperature between 55 and 60°C. No further precipitate occurred even if this final filtrate was boiled for an hour.

The filtrates from pollen extracts, which had been adjusted to various hydrogen ion concentrations, when brought back to a pH of 6 showed progressive diminutions of precipitin reaction with pollen antiserum as the hydrogen ion was increased, with a maximum decrease at pH 4. The filtrates from the experiment at pH 4 when heated at 60°C for one hour and brought back to a pH of 6 gave no reaction whatever with the pollen antiserum. The precipitates obtained by acidification of the original pollen extract were partly soluble in 0.5% sodium carbonate. The solutions of these precipitates gave definite precipitin reactions with the pollen antiserum.

The physico-chemical properties of the proteins precipitated at pH 3.8-4.2 at 60°C are those that are associated with the larger protein molecules—relative ease of precipitation, quantitative absorption with aluminium cream, very little diffusibility through a permeable membrane such as collodion, and marked viscosity of the solution. On the other hand, the protein fraction remaining in solution has the general characteristic of the smaller protein molecule with more difficulty of precipitation from solution (it is impossible to precipitate quantitatively the skin reacting protein with aluminium cream), moderate power of diffusion through collodion membranes, and less viscosity of the solution.

Skin tests were done on ragweed sensitive patients with the original untreated pollen extracts and the filtrate from which all precipitable material had been removed at a pH of 3.8 and heating. The

filtrate obtained was brought back to a pH of 6.0 to prevent non-specific irritation. Scarifications were made with a special instrument which produces superficial cuts of practically the same depth and length. The filtrate gave practically undiminished skin tests when compared with an original untreated pollen solution of equivalent nitrogen content. Nor was the skin reaction diminished in the filtrate which had been boiled for an hour. The skin tests were specific for ragweed pollen.

Twelve ragweed sensitive patients were treated preseasonally with the filtrate from which all precipitable material had been removed. None of these had any relief. During the past season a few patients were treated with the filtrate to which gelatin had been added. Some evidence of success was present. However, the number of cases treated was too small and the amount of treatment not sufficient to permit definite conclusions.

Summary. The protein of ragweed pollen has been separated into fractions. The one fraction has relatively large molecular size and is responsible for the precipitin reaction with pollen antiserum. The other fraction is made up of proteins of relatively small molecular size and is the skin reactive protein of pollen. This last observation is similar to that of Benjamins and his collaborators,² who in their work on grass pollen, came to the conclusion that the skin reacting substance is of small molecular size. Our results differ from those obtained by Harley.³ He concludes that in timothy pollen the skin reactive fraction consists of proteins of the albumin type. We failed to produce hyposensitization of ragweed sensitive patients by the use of the fraction containing small protein molecules. There is an indication that it may be possible to do so when it is used in connection with large protein molecules. Benjamins and his collaborators found that the skin reactivity of the small protein molecule from grass pollen could be enhanced by the addition of protein of large molecular size. The immunizing properties of the fraction consisting of the larger protein molecules are now being studied.

² Benjamins, C. E., von Dishoeck, H. A. E., and German, J. L. M., *J. Allergy*, 1935, **6**, 335.

³ Harley, David, *Brit. Exp. Path. J.*, 1937, **18**, 469.