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Proteins of timothy and orchard grass pollen and their reaction to vernal hay fever.

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The most widely accepted view regarding the causation of hay fever is that it is due to the pollen of certain grasses and weeds blooming in the summer and fall. Dunbar and his pupils showed convincingly that the proteins of the pollens are responsible for hay fever. There have been several attempts in the past to obtain the active constituent, but a complete, systematic chemical investigation has never been carried out. The most comprehensive study was made by Heyl[1] on ragweed. Unfortunately, however, the clinical part of his investigation was not carried out to completion.

The material used for our study consisted of the pollen of timothy and orchard grass. On account of the meager supply available, we can report only on a qualitative study.

For the exhaustive extraction, we used 5 gm. of each of the pollens, and extracted them successively with 10 per cent sodium chloride, 0.5 per cent sodium hydroxide, and 70 per cent alcohol. It is remarkable how large a percentage of the extracted nitrogen is non-protein nitrogen. From half to one-third of the total nitrogen of the pollen is not extractable with these solvents.

TABLE I.
Quantitative Analysis of Pollen.*

	Orchard grass	Timothy
	Per cent	Per cent
Nitrogen	4.51	4.00
Ash	3.98	4.31
Ether extract	3.0	3.21

* Analysis based on moisture-free material.

[1] F. W. Heyl, *J. Am. Chem. Soc.*, 1919, xli, 670.

In the preparation study, we used about 75 gm. of the pollen, which had been previously extracted with ether to remove fatty material. It was first extracted with water, and then with 10 per cent NaCl until there was practically no nitrogen found in the extract. The residue was extracted with alcohol, and finally with 0.2 per cent sodium hydroxide. Coagulation tests made on both the water and salt extracts of the timothy pollen indicated the presence of two proteins, one coagulating at 68.8° C., and the other at 85° C. Similar extracts of the orchard grass pollen also showed the presence of two proteins, one coagulating at 65° and another at 85.2° C. We separated the lower coagulating protein by heat, and the other by ammonium sulphate at 0.65 of saturation. The latter was redissolved and dialyzed for 10 days, but the amount of precipitate formed was too small to allow a study of its properties and composition.

After all the precipitable proteins were removed from both the water and salt extracts by coagulation by heat and filtration, the combined clear filtrate was transferred to a parchment bag, and dialyzed for 10 days to eliminate the inorganic salts. The dialysate obtained was concentrated by evaporation *in vacuo*. Alcohol was added to the sirupy residue, making the final concentration 70 to 80 per cent, and the precipitated material dehydrated with alcohol followed by ether. This material obtained from the proteose fraction was easily soluble in water, and was found by the clinical tests on hay fever patients to be the most active substance of the pollen.

The residue remaining after the exhaustive extraction with water, salt and alcohol, was extracted with 0.2 per cent sodium hydroxide. By acidification of the alkaline extract with acetic acid, a protein was obtained which was found by clinical tests to have negligible importance as an exciting factor.

The clinical findings suggest that the proteose fraction is the intoxicating and primary element in all hay fever subjects sensitive to timothy pollen, and that the albumin fraction is an added toxic factor in about 50 per cent of the subjects. The importance of the glutelin fraction is negligible.

TABLE II.
Distribution of Nitrogen.

	Per cent of Total N	
	Orchard grass	Timothy
Salt soluble (10% NaCl)	17.5	9.85
Alkali soluble (0.2% NaOH)	20.8	15.4
Non-protein N	25.7	24.9
Residual N	36.0	49.8

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The anaphylactogenic character of horse dander and its crossed relationship to horse serum.*

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The anaphylactogenic properties of horse dander have been open to question. Friedberger[1] contends that horse dander is not anaphylactogenic. Busson and Ogata[2] and, later, Longcope, O'Brien and Perlzweig,[3] have shown that horse dander is definitely anaphylactogenic. These latter studies have appeared in the literature during the progress of our work on this same subject. We should like to state that our findings definitely show horse dander to be anaphylactogenic.

During the course of our studies, we have had occasion to work on the crossed relationship between horse dander and horse serum. Longcope[2] and his co-workers definitely state that this crossed relationship does not exist and that horse dander is anaphylactogenically specific. Contrary to these findings, we have found that there is a definite crossed anaphylactic relationship between these two substances.

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1 Friedberger, E., and Kamio, *Ztschr. f. Immunitat.*, 1923, xxxvii, 379.

2 Busson and Ogata, *Wien. Klin. Wchnschr.*, 1924, xxxvii, 820.

3 Longcope, O'Brien, and Perlzweig, *J. Immunol.*, 1925, x, 599.