contains combined fatty acid or an ester. This ester has suffered more hydrolysis when acid instead of diastase was used in decomposing the starch. In the light of these data, very likely the unsaturated constituent present in the fat, labeled "X," is combined with the palmitic acid and accounts for the presence of the ester.

The fact that the palmitic acid, which is a mono-basic acid, is linked as an ester to the unsaturated compound ("X") precludes the possibility of direct carbohydrate fatty acid union, and since it has been shown that the entire fatty material (fatty acid and unsaturated substance "X") is combined with the carbohydrate and only liberated by hydrolysis, the substance "X" must serve as a connecting link between the acid and the carbohydrate.

Starches from other sources than corn also contained combined fat.

Samples of various representative starches were purified as described under Part I and the "fat by hydrolysis" determined. In the following table are enumerated the results of these analyses. Fifty g. samples were used, except in the case of potato starch.

Starch.	"Fat by hydrolysis."	Acid No.	Iodine No.
Corn	0.61	182.5	92.5
Rice	0.83	283.4	84.7
Sago	0.11	151.0	..
Cassava	0.12	168.0	..
Potato (200 g. sample)	0.04	109.4	..
Horse chestnut	0.56	21.9	..

From the above results it is evident that corn starch is not unique in having combined fat, but that starches of widely differing genera also contain combined fatty material.

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THE RAGWEED POLLEN PROTEINS.

BY FREDERICK W. HEYL AND HARRIS H. HOPKINS.[1]

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In considering any drug from a pharmaceutical point of view, two distinct schools of thought are always in evidence. First, the progressive school which is largely dominated by chemistry and to whom pharmacy owes much in the matter of the isolation or preparation of pure or crystalline principles. Secondly, there is the conservative group, dominated

[1] Holder of The Upjohn Coöperative Fellowship at Kalamazoo College (1919–1920). This paper is based upon the thesis presented by Mr. Hopkins to the Faculty of Kalamazoo College, in partial fulfillment of the requirements for the degree of Master of Science.

by the simpler pharmaceutical methods and favoring the preparation of galenical extractives. It is well for us to understand that however desirable the progressive results due to chemical investigation in all cases may be, that there are a number of drugs (*e. g.*, ergot and digitalis) the actions of which we do not comprehend, and for which it were well if the pharmacist adopted and the clinician used, a strictly galenical product until the chemist has first worked out the problem involved. Where the pharmacist attempts to precede the chemist, as has been done with both the drugs mentioned, there arises a chaotic condition which actually retards the advance of knowledge.

Ragweed pollen is such a drug. The uncertain state of our knowledge does not justify the preparation or use of pure principles from this drug, even though at present we may view the albumin fraction as the constituent of chief interest. In the texts we find pollen toxalbumin classified with the albuminous substances ricin, abrin, and crotin on the basis of their similar activity in producing antitoxic substances in the animal body. This classification is due to the work of Dunbar,[1] who perhaps succeeded in showing that he could produce a pollen antitoxin using rye pollen, but it need not necessarily hold for ragweed pollen.

It has been shown that rye pollen toxin which is extracted with water will withstand autolysis in the presence of the proteases of pollen. Kamman[2] claims that this increases its activity 100 fold, whereas, on the contrary, artificial digestion with trypsin leads to the loss of activity exactly as in the case of ricin. Kamman noted a loss of activity for rye pollen toxin when it was allowed to stand in the presence of alcohol and attributes this to the coagulation of the albumin. On the other hand, he states that his product was thermostabile, a finding which is difficult to understand if the activity resides in the coagulable albumin fraction.

The conclusive work of Osborne, Mendel and Harris[3] on ricin leads to the thought that in an analogous manner the pure pollen albumin is probably the so-called toxin in this case, but when we find, not 40% as in the rye pollen, but only 1–2%, we feel more than ever the logic of adhering to the galenical extract until the required information shall have been gained. In the case of Kamman's so-called "pollentoxin," the usual protein reactions (except the biuret) were lacking and it is evident that Kamman's thought is influenced by the work of Kyes[4] and of Faust,[5] who have established the non-nitrogenous, thermostabile nature of the neurotoxic constituent of cobra venom. This substance "ophiotoxin,"

[1] *Zur Ursache und specifischen Heilung des Heufiebers," München,* 1903.
[2] *Biochem Z.*, 46, 151 (1912).
[3] *Am. J. Physiol.*, 14, 259 (1905).
[4] *Biochem. Z.*, 4, 99 (1907); 8, 42 (1908).
[5] *Arch. expt. Path. Pharm.*, 56, 236 (1907).

($C_{17}H_{26}O_{10}$) is probably combined as an ester or salt with the albumin-like substances which are present, and with which it can be precipitated by saturation with ammonium sulfate. As is frequently the case, it is known that the native substance is more stable and has greater activity than the isolated fraction owing to the protective action of the protein moiety.

As has been previously[1] shown, the aqueous extract from pollen when precipitated with alcohol or when saturated with ammonium sulfate yields a small quantity of a coagulable albumin and a larger quantity of substance which was formerly classified as a proteose. The analytical results reported in this paper show that the albumin is a normal protein except that it is incomplete and lacks histidine and perhaps tryptophane. But the "proteose" or non-coagulable part of this fraction differs from the corresponding ricin proteoses or any other substances previously described in this fraction.

We have analyzed by the Van Slyke process 2 "proteose" products. The first was the most soluble part precipitated at 5/10–10/10 saturation with ammonium sulfate and was found to contain only 5.43% nitrogen. A second sample represented the entire fraction and was further purified by dialysis and it contained 6.0% nitrogen. These samples are possibly mixtures or uncoagulable combinations of the albumin and a non-nitrogenous moiety, there being a higher percentage of protein in the entire fraction than in that precipitated at higher saturations. On the basis of the nitrogen content these products correspond to a mixture or compound containing 38.3% and 35.1% of the albumin, respectively. Recalculating our base and tyrosine analysis we can make the following comparisons.

	Calc. for albumin × 0.383.	Found entire proteose.	Calc. for albumin × 0.351.	Found soluble proteose.
Arginine	2.36	2.08	2.15	1.48
Histidine	0.00	0.00	0.00	0.00
Lysine	3.36	4.48	3.07	3.70
Tyrosine	1.08	1.10	0.98	0.78

The stability of these substances is remarkable and it is difficult to decide definitely whether we have here an ester, a salt-like substance or a mixture of albumin with a nitrogen-free substance.

We have analyzed the pollen glutelin, which is the predominant protein, and found it to differ from the water-soluble albumin in that histidine is present among the bases and that it is therefore more complete. We have in pollen, therefore, a series that may represent 3 stages of protein development.

Native compound proteose (N = 5.4%) ⟶

Albumin(-histidine) ⟶ glutelin.

[1] THIS JOURNAL, 41, 670 (1919).

Experimental.

1. **Proteose.**—The aqueous extracts of ragweed pollen, after the removal of coagulable albumin by heating and filtering, contain an appreciable quantity of proteose-like material which can be precipitated by saturation with ammonium sulfate. As we observed considerable loss of material by dialysis, we thought we might procure a representative analysis on the material which had not been subjected to this treatment. The material[1] precipitated at 5/10–10/10 saturation was therefore redissolved in water and reprecipitated with alcohol. In this condition a considerable quantity of ammonium sulfate was present in the final product. It was, therefore, redissolved and a slight excess of barium hydroxide solution was added and the ammonia was removed at 40°. The precipitation was then made quantitative and after removal of the barium sulfate, the proteose solution was evaporated to dryness in a weighed flask.

The subsequent analysis gave such low results that we proceeded to study the dialysed sample[2] of the entire proteose product obtained in the first pollen extraction, which had been purified by a lengthy dialysis. The results were of the same nature and prove the conjugated nature of this substance. Both products gave a strong Molisch test. The fact that the analysis of the second product gave higher results than the first indicates the admixture *in the total proteose* of more protein. This is, of course, precipitated at lower saturations and is less extensive in the first sample.

(*a*) A sample of the undialyzed proteose (which had been prepared by precipitation between 5/10–10/10 saturation with ammonium sulfate) weighing 4.4258 g., and equivalent to 3.3595 g. ash and moisture-free, was hydrolyzed for 48 hours with 100 cc. of 20% hydrochloric acid. (Amino N = 4.48 and 4.39%.) The hydrolysis solution was subjected

Analysis of Proteose, 5/10-10/10.

	Found.		Average.	Parts per 100.
Total Nitrogen	5.45	5.42	5.43	100.00
Amide N	0.45	....	0.45	8.29
Humin N	0.56	....	0.56	10.31
Arginine N	0.48	....	0.48	8.84
Cystine N	0.00	....	0.00	0.00
Histidine N	0.00	....	0.000	0.00
Lysine N	0.71	....	0.71	13.08
Mono Amino N	2.47	2.55	2.51	46.22
Total Filtrate N	3.08	....	3.23[a]	...
Non Amino N	....	....	0.72	13.26

[a] Calculated.

[1] *Loc. cit.*, p. 679.

[2] *Ibid.*, p. 676.

to the Van Slyke process, with the results tabulated below. These results are corrected for the solubilities of arginine and lysine phosphotungstates.

(*b*) A sample of dialyzed proteose (o — 10/10 saturation with ammonium sulfate) weighing 3.1765 g. and equivalent to 2.7099 g. ash- and moisture-free, was hydrolyzed by boiling with 100 cc. of 20% hydrochloric acid for 24 hours. (Amino N = 5.6 and 5.35%.) The results tabulated below were obtained by the Van Slyke process and were corrected for the solubilities of arginine and lysine phosphotungstates.

Analysis Proteose, o/10 to 10/10.

	Found.		Average.	Parts per 100.
Total Nitrogen	6.00	6.01	6.00	100.00
Amide N	0.35	....	0.35	5.83
Humin N	0.57	....	0.57	9.50
Arginine N	0.67	....	0.67	11.17
Cystine N	0.00	....	0.00	0.00
Histidine N	0.00	....	0.00	0.00
Lysine N	0.86	....	0.86	14.33
Mono Amino	2.92	2.92	2.92	48.67
Total filtrate N	3.37	3.64	3.55[a]	....
Non amino N	....	....	0.63	10.05

[a] Calculated.

2. **Glutelin.**—This protein was prepared by alkaline extraction from the first sample of pollen studied.[1] Of this material, 3.75 g. (Moisture = 18.59%; ash = 0.98%) equivalent to 3.0161 g. was hydrolyzed by boiling with 100 cc. of 20% hydrochloric acid for about 48 hours. (Amino N = 9.09 and 9.12%.) For the check analysis 2.0856 g., equivalent to 1.6774 g. moisture- and ash-free protein, was used. In calculating the results tabulated below the values have been corrected for the solubilities of all the phosphotungstates. We have persistently encountered low results in the determination of the total nitrogen of the filtrate from the phosphotungstates and this is a calculated value. (Found = 6.97%.)

Analysis Glutelin.

	Found.		Average.	Parts per 100.
Total Nitrogen	12.88	13.10	13.00	100.00
Amide N	1.30	1.14	1.22	9.40
Humin N	0.84	0.90	0.87	6.69
Arginine N	1.51	1.52	1.52	11.69
Cystine N	0.12	....	0.12	0.92
Histidine N	0.44	0.60	0.51	3.92
Lysine N	1.47	1.27	1.37	10.54
Mono amino N	6.92	7.33	7.12	54.77
Total filtrate N	7.20	7.55	7.37[a]	....
Non amino N	0.28	0.22	0.25	1.92

[a] Calculated.

[1] *Loc. cit.*, p. 676.

3 **Albumin.**—The sample analyzed was prepared from the second and third pollen extractions by coagulation. Of this 3.1976 g. (moisture = 10.99; ash = 0.0), equivalent to 2.8462 g., was hydrolyzed by boiling with 100 cc. of 20% hydrochloric acid for about 48 hours. (Amino N = 12.16 and 12.38%.) In the table given below the total nitrogen of the filtrate from the phosphotungstates is calculated by difference, and a solubility correction is made for only arginine and lysine phosphotungstates.

Analysis of Albumin.

			Parts per 100.
Total Nitrogen	15.68	15.63	100.00
Amide N	1.42	...	9.05
Humin N	0.47	...	3.00
Arginine N	1.98	1.99	12.63
Cystine N	0.00	...	0.00
Histidine N	0.00	...	0.00
Lysine N	1.68	...	10.71
Mono amino N	9.49[a]	...	60.52
Total filtrate N	10.13[b]	...	....
Non amino N	0.64	...	4.08

[a] If the usual solubility correction is used this figure should read 9.31% corresponding to 59.37% of the total nitrogen. The non-amino N would then correspond to 0.82% = 5.23% of the nitrogen.

[b] Calculated.

Percentage of Basic Amino Acids and of Tryptophane.—Recalculating the results described above, the hexone bases found may be tabulated. We have furthermore determined the tyrosine present by the colorimetric method of Folin and Dennis.[1]

The absence of histidine in the proteoses and the albumin is so complete that it is impossible to detect it even with the diazo test in the base fraction.

Percentage of Amino Acids in the Ragweed Pollen Proteins.

	Proteose 5/10–10/10.	Proteose (entire) dialysed.	Glutelin.		Albumin.
Arginine	1.48	2.08	4.69	4.70	6.15
Histidine	absent	absent	1.69	2.30	absent
Lysine	3.70	4.48	7.66	6.6	8.76
Tyrosine	0.78	1.10	4.7		2.79 2.83
Tryptophane	absent	absent	absent		absent

The above table summarizes the results of this study.

KALAMAZOO, MICH.

[1] *J. Biol. Chem.*, **12**, 239 (1912).