Studies on the proteids of rye and barley and on the chemical nature of diastase.

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STUDIES ON THE PROTEIDS OF RYE AND BARLEY AND ON THE CHEMICAL NATURE OF DIASTASE.

By Thomas B. Osborne.

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THE PROTEIDS OF THE RYE KERNEL.

By Thomas B. Osborne.

The proteids of this seed have been but little studied and the statements published leave the subject in much confusion.

Einhof, who in 1805* undertook an analysis of rye, was the first to make observations on the proteids obtained therefrom. He found that an aqueous extract of rye meal contained two distinct proteid substances, one coagulating on boiling, and insoluble in alcohol, which he called albumin, and the other not coagulating, but soluble in alcohol, which he called gluten [kleber]. The latter he considered to be identical with the similar substance extracted from wheat gluten by alcohol. Treatment with alcohol yielded much more "kleber" than was extracted by water alone. It is interesting to note that Einhof in this investigation first discovered that characteristic differences exist between different kinds of vegetable proteid matter, it being thought at that time that gluten and albumin were simply modifications of the same body which under like conditions would show the same properties.

Heldt† in 1843 published a description of the proteid taken up by alcohol from rye meal. He prepared it by extracting the meal with hot alcohol, distilling off the alcohol and treating the residue with ether to remove fat, and with water to remove ether and sugar.

This preparation was analyzed with the following result:

Carbon	56.38
Hydrogen	7.87
Nitrogen	15.83
Sulphur, Oxygen	19.92
	100.00

Heldt remarked, "the same composition was found by Scherer and Jones for other nitrogenous constituents of plants, plant-casein, plant-albumin and plant-gelatin, to which last this body appears to stand nearest."

* Jour. d. Chem. v. Gehlen, V, 131.

† Ann. d. Chem. u. Phar., XLV, 195.

Jones* obtained albumin from rye by boiling the aqueous extract and treating the resulting coagulum with ether. He states that this albumin contained:

Carbon	54.74
Hydrogen	7.77
Nitrogen	15.85
Oxygen	21.64
	100.00

Verdeil,† contrary to the experience of Einhof, von Bibra, Ritthausen and the writer obtained gluten from rye meal by kneading and washing in a cloth until starch was removed. He states that there remained a tough, glutinous substance which could be easily drawn into threads. This gluten he says was not pure but was contaminated with a substance soluble in alcohol. When thoroughly extracted with alcohol he considered it to be pure. He determined sulphur in the gluten and found 0.989 and 0.972 per cent. Von Bibra‡ considered the proteid extracted from rye by alcohol to be the same as that similarly obtained from wheat. He gave the nitrogen content of this body as 15.73, 15.52 and 15.50 and the sulphur as 0.973 and 0.950 per cent. He also recognized the presence of "casein" which he did not analyze, and found from 1.565 to 2.799 per cent. of albumin which contained 15.53 and 15.42 per cent. of nitrogen. He considered the proteids of rye to be same as those of wheat.

Ritthausen§ described three proteid substances which he found in rye, albumin, soluble in water, mucedin, soluble in alcohol, and gluten-casein, soluble in dilute potash water but insoluble in water and cold alcohol.

Albumin, he says, is present in the aqueous extracts but he made no further examination of this body. The mucedin he considers to be the only proteid, soluble in alcohol, present in the rye kernel and this he regarded as similar to the mucedin which was believed to exist in the wheat kernel. He was unable to detect the presence of gliadin. Mucedin was prepared by extracting the meal with hot alcohol of 85 per cent. and concentrating and cooling the solution. The deposited substance was further

* Ann. d. Chem. u. Phar., XL, 66.

† Ann. d. Chem. u. Pharm., LVIII, 319.

‡ Die Getreidearten u. das Brod, Nuernberg, 1860, 291.

§ Jour. f. prakt. Chem., XCIX, 439, and Die Eiweisskoerper, etc., Bonn, 1872, p. 83.

extracted with alcohol and ether. It was then dissolved in acetic acid and fractionally precipitated with potash. He states the composition of the substance as:

Carbon	53.61
Hydrogen	6.79
Nitrogen	16.84
Sulphur	0.50
Oxygen	22.26
	100.00

The gluten-casein was obtained by extracting the rye meal directly with very dilute potash-water, precipitating with acetic acid and washing with water, alcohol and ether. Two preparations were made and gave as an average the following figures on analysis:

Carbon	52.14
Hydrogen	6.93
Nitrogen	16.38
Sulphur	1.06
Oxygen	23.49
	100.00

Sidney Martin* states that wheat, rye and barley contain a globulin substance similar in properties to animal myosin, being soluble in 10–15 per cent. sodium chloride solutions, precipitated therefrom by saturation with sodium chloride and with magnesium sulphate and coagulating at 55° to 60°. This globulin is precipitated by dialysis and thereby is converted into the albuminate form. It is very evident from the foregoing summary of previous work that we have no satisfactory knowledge of the rye-proteids.

In presenting the results of my investigation the subject may be most conveniently discussed under the following heads:

A, proteids soluble in water, B, proteids insoluble in water but soluble in saline solutions, C, proteids insoluble in water and in saline solutions and soluble in alcohol, and, D, proteid insoluble in water, saline solutions, or alcohol but soluble in dilute alkalies.

A, Proteids soluble in Water. Leucosin. Proteose.

The proteids soluble in water are best examined in extracts made in the first instance with 10 per cent. sodium chloride solution from which subsequently the soluble salts have been removed

* Jour. Physiol., VIII, viii.

by prolonged dialysis. When water is applied to the grain it becomes a weak saline solution which not only takes up globulins but also extracts gliadin whose presence greatly complicates the examination of the water soluble proteids. Rye meal* was accordingly exhausted with a solution containing 10-per cent. sodium chloride and the extract, after syphoning from the subsided insoluble matters, was freed from salts and globulin by dialysis in river water and filtration. The resulting solution yielded no more globulin by dialysis in distilled water and contained only those proteids extracted from the seed which were soluble in pure water. As the extract was bulky the proteids were precipitated by saturation with ammonium sulphate and thereupon dissolved in water. A comparatively concentrated solution was thus obtained which was very nearly freed from ammonium sulphate by dialysis. It then had the following properties. When heated slowly it became turbid at 52° and particulate at 63°. After filtering from this coagulum nothing more separated even on boiling. Saturation of the dialyzed solution with sodium chloride gave a precipitate that dissolved readily in water to a solution, which, heated to 63°, yielded a coagulum of albumin. The filtrate from this coagulum was again saturated with salt and a considerable precipitate obtained showing that with the albumin some proteose-like body was thrown down. Nitric acid added to the solution of this precipitate in water gave a precipitate which dissolved on warming and reappeared on cooling. The solution after filtering out the first precipitate of proteose and albumin, produced by saturation with sodium chloride, gave more precipitate on adding acetic acid, showing the presence of a further quantity of proteose. The coagulum above described, which separated on heating its solution to 65°, was washed thoroughly with water, alcohol, absolute alcohol and ether and dried over sulphuric acid. When dried at 110° it had the following composition:

COAGULATED RYE ALBUMIN, LEUCOSIN, *Preparation* 1.

	I.	II.	Average.	Ash-free.
Carbon	52.31	----	52.31	52.57
Hydrogen	6.78	----	6.78	6.81
Nitrogen	16.14	16.11	16.13	16.22
Sulphur } Oxygen }	----	----	----	24.40
				100.00
Ash	0.51			

* The rye meal used throughout this work was obtained by grinding, in the laboratory, portions of clean and fresh winter rye, as needed for each extraction.

Another extract was examined in a slightly different way. 1000 grams of rye meal were extracted with 11 liters of 10 per cent. sodium chloride solution and, in order to get rid of the large amount of gum taken up, the solution, after filtering, was dialyzed and then saturated with ammonium sulphate. The precipitate thus produced was dissolved, as far as possible in 10-per cent. sodium chloride brine, filtered clear and dialyzed until chlorides were removed. The solution after filtering clear was then heated to 65° and the albumin that separated was filtered out, washed thoroughly with hot water, with alcohol and with ether and dried over sulphuric acid. This preparation, **2**, weighed 1.21 grams and had the following composition:

COAGULATED RYE ALBUMIN, LEUCOSIN, *Preparation* **2**.

		Ash-free.
Carbon	53.04	53.29
Hydrogen	6.70	6.74
Nitrogen	16.57	16.65
Sulphur } Oxygen }	----	23.32
		100.00
Ash	0.50	

The solution containing the proteoses, filtered from preparation **2**, was then treated with 20 per cent. of its weight of dry sodium chloride and a little two-tenths per cent. hydrochloric acid was added which gave a considerable precipitate. This was filtered out, dissolved in distilled water and the solution dialyzed till free from chlorides. This solution then gave a precipitate with nitric acid, which dissolved on warming and precipitated again on cooling. The solution concentrated to a syrup on a water bath was precipitated by pouring into absolute alcohol. The precipitate when dried over sulphuric acid weighed 0.41 gram or one-third as much as the albumin. The filtrate, from the precipitation of this substance [with 20 per cent. of sodium chloride and acid], was saturated with ammonium sulphate and the precipitate thus produced filtered out and dissolved in distilled water. With copper sulphate and potash this substance gave a clear pink color. Its solution gave no precipitate on adding nitric acid until it had been saturated with sodium chloride, when a slight precipitate fell. It yielded no precipitate with copper sulphate. These reactions indicate that besides albumin the aqueous extract contains small quantities of proto- and deutero-proteose.

Again, 2000 grams of rye meal were treated with 10-per cent. sodium chloride solution and the extract filtered and saturated with ammonium sulphate. The precipitate produced was dissolved in 10-per cent. sodium chloride solution, filtered and dialyzed until all chlorides were removed. After filtering clear the solution was heated to 65° for some time and the coagulum filtered out, washed with hot water, alcohol and ether and dried for analysis, preparation **3.** The filtrate from **3,** was then concentrated by boiling, during which a coagulum developed. This was filtered out, washed as usual and dried for analysis, preparation **4.**

COAGULATED RYE ALBUMIN, LEUCOSIN, *Preparation* **3.**

	I.	II.	Average.	Ash-free.
Carbon	53.41	53.32	53.37	53.52
Hydrogen	6.90	6.82	6.86	6.88
Nitrogen	16.73	....	16.73	16.78
Sulphur } Oxygen }	....	....	....	22.82
				100.00
Ash	0.30			

COAGULATED RYE ALBUMIN, LEUCOSIN, *Preparation* **4.**

	I.	II.	Average.	Ash-free.
Carbon	52.64	52.53	52.58	52.86
Hydrogen	6.76	6.73	6.75	6.79
Nitrogen	16.86	....	16.86	16.95
Sulphur } Oxygen }	....	....	....	23.40
				100.00
Ash	0.56			

Another extract was made by treating 1700 grams of rye meal with 16 liters of water. After standing over night the solution was filtered off and saturated with ammonium sulphate. The meal residue was treated again in the same way and the filtered extract, after saturating with ammonium sulphate, was added to that first obtained. The precipitated proteids were then dissolved in water yielding a very gummy solution. As this solution was bulky the proteids were again precipitated with ammonium sulphate and the precipitate after filtering out was treated with 3 liters of 10-per cent. sodium chloride solution. The whole was then dialyzed, it having been found that these viscid solutions lost their gummy character on dialysis. After 8 days all the gum had disappeared. The solution was then readily filtered clear. In order to reduce the volume of the solution it was again

saturated with ammonium sulphate and the large precipitate treated with about a liter of 10-per cent. sodium chloride solution. A turbid liquid resulted which was not cleared by passing through filter paper but on standing became clear and the sediment was found to consist of gliadin, which is to be noticed later. Gliadin is soluble to a considerable extent in pure water and in water containing but a very small amount of dissolved salts, but the addition of a little sodium chloride to its solution precipitates it completely. After the solution had entirely cleared by subsidence it was dialyzed free from chlorides and heated to 65°. The resulting coagulum was then filtered out, washed and dried in the usual manner and found to weigh 1.55 gram. The composition of this preparation was as follows:

COAGULATED RYE ALBUMIN, LEUCOSIN, *Preparation* **5.**

		Ash-free.
Carbon	52.67	52.91
Hydrogen	6.78	6.81
Nitrogen	16.57	16.65
Sulphur	1.40	1.40
Oxygen	----	22.23
		100.00
Ash	0.47	

The meal residue, from the water extract that yielded **5**, was then treated with 10-per cent. sodium chloride solution and the filtered extract saturated with ammonium sulphate, the precipitate so obtained was filtered out, dissolved in water and sodium chloride solution and dialyzed till free from chlorides. The liquid was then filtered clear and heated to 65°. The coagulum washed and dried weighed 1.92 grams, being therefore considerably greater than the preparation obtained from the aqueous extract. It would appear therefore probable that the albumin forms a constituent of the aleurone grains which may not be broken up until treated with saline solutions. The following figures show the composition of this preparation:

COAGULATED RYE ALBUMIN, LEUCOSIN, *Preparation* **6.**

		Ash-free.
Carbon	52.55	52.77
Hydrogen	6.67	6.70
Nitrogen	16.61	16.68
Sulphur	1.29	1.29
Oxygen	----	22.56
		100.00
Ash	0.42	

SUMMARY OF ANALYSES OF COAGULATED RYE ALBUMIN, LEUCOSIN.

	1.	2.	3.	4.	5.	6.	Average.
Carbon	52.57	53.29	53.52	52.86	52.91	52.77	52.97
Hydrogen	6.81	6.74	6.88	6.79	6.81	6.70	6.79
Nitrogen	16.22	16.65	16 78	16.95	16.65	16.68	16.66
Sulphur	24.40	23.32	22.82	23.40	1.40	1.29	1.35
Oxygen					22.23	22.56	22.23
	100.00	100.00	100.00	100.00	100.00	100.00	100.00

Five hundred grams of rye flour* were extracted with 2000 c. c. of 5-per cent. sodium chloride solution and 1000 c. c. of the clear filtered extract were dialyzed till free from chlorides. The solution was then filtered and heated for 24 hours in a water bath of 70°. The coagulated albumin was filtered out, washed with water, alcohol and ether, and dried over sulphuric acid, and found to weigh 1.08 grams, equivalent to 0.43 per cent. of the flour.

So far as tested this albumin agrees in all respects with that obtained from wheat.† The variations in composition of these preparations are considerable but perhaps not greater than might be expected.

The aqueous and saline extracts of the rye meal contain much gum and coloring matters which render the isolation of pure proteids very difficult. It will be seen, however, that the preparations of wheat albumin and rye albumin have very nearly the same average composition and that both proteids show the same reactions and coagulate at the same temperature. They are unquestionably the same substance, for which I have adopted the name *leucosin*.

COAGULATED LEUCOSIN.

	Wheat. Av. of 5 analyses.	Rye. Av. of 6 analyses.
Carbon	53.02	52.97
Hydrogen	6.84	6.79
Nitrogen	16.80	16.66
Sulphur	1.28	1.35
Oxygen	22.06	22.23
	100.00	100.00

The proteoses of the rye also show the same reactions as those of the wheat kernel, and so far as it is possible to determine they are identical.

* Made by the Health Food Company of New York.

† Am. Chem. Jour., XV, p. 408; also Ann. Report of this Station for 1893, p. 179.

B. Proteid soluble in Saline Solutions. Edestin.

Owing to the large amount of gum extracted from the rye meal, the preparation of the globulin in a pure state was found to be extremely difficult. Such preparations as were made disagreed in composition and in only one case was a substance obtained which appeared to be pure enough to warrant the publication of its analysis. So far as could be detected the globulin which separated on dialysis had the same properties as that similarly derived from wheat. One preparation of this globulin, which had nearly the same composition as the wheat globulin and appeared to be free from gum and other impurities, was obtained as follows:—5000 grams of rye flour, made by the Health Food Co. of New York, were extracted with 15 liters of 5-per cent. sodium chloride solution and the extract filtered clear. 9 liters of extract were thus obtained, being approximately equal to a complete extraction of 3 kilos of rye flour.

The entire solution was dialyzed for four days in order to remove the greater part of the gum. The extract was saturated with ammonium sulphate, the precipitate filtered out, suspended in water and dialyzed for three days. Most of the substance was now dissolved and the insoluble matter was filtered out, washed with sodium chloride solution and the filtrate and washings returned to the dialyzer. When free from chlorides the solution was filtered from a small precipitate and this latter washed with water, alcohol and ether and dried over sulphuric acid. Only 1.21 grams of globulin were obtained, which when dried at 110° had the following composition:

Rye Globulin, Edestin, *Preparation* 7.		Wheat Globulin, Edestin.	
		Ash-free.	Av. of 5 analyses.
Carbon	51.03	51.19	51.03
Hydrogen	6.72	6.74	6.85
Nitrogen	18.14	18.19	18.39
Sulphur			0.69
Oxygen	----	23.88	23.04
Ash	0.33		
		100.00	100.00

The writer has no doubt that this globulin is identical with the *edestin* found in the wheat kernel and other seeds,* but owing to the difficulties encountered in preparing it from rye, further evidence on this point was not obtained.

* Annual Report of this Station for 1893, pp. 179 and 216.

With reference to Martin's statement concerning the presence of myosin—what is written on that point in the paper by Osborne and Voorhees on the Proteids of the Wheat Kernel applies equally to rye.*

C. Proteid soluble in Alcohol. Gliadin.

After extraction with sodium chloride solution, alcohol of 75 to 80 per cent. takes up a considerable quantity of proteid. 100 grams of rye meal were extracted thoroughly with 10-per cent. sodium chloride solution and then with 75-per cent. alcohol. The alcoholic extract was evaporated to very small volume and the separated proteid washed with water and ether and then dried. It weighed 3.93 grams, being therefore nearly 4. per cent. of the meal. 2000 grams of rye meal were then extracted with alcohol of 0.9 specific gravity, four successive times. Each extract, after filtering clear, was concentrated by distillation on a water bath. The first three extracts yielded on cooling a deposit of proteid but the fourth contained almost none. Each residue was then washed with water and dissolved in 75-per cent. alcohol. The substance from the first extract yielded an insoluble residue which when washed with dilute alcohol, absolute alcohol and ether, gave preparation **8.** This dried at 110° contained 17.00 per cent. of nitrogen. The solutions of the substances from the three extracts in dilute alcohol, were concentrated to about one-fourth their original volume and cooled, when the dissolved proteid separated. The substance from the first extract was digested with absolute alcohol which dissolved a part of it, then with ether and dried, giving preparation **9.** The residue from the second extract was washed superficially with water and then treated in a divided state with distilled water until dissolved. A little saturated sodium chloride solution was then added and the proteid wholly precipitated. The precipitate was then thoroughly dehydrated with absolute alcohol, digested with ether, and dried. This formed preparation **10.** The proteid separated from the third extract was digested with absolute alcohol and with ether, and yielded a small quantity of proteid, preparation **11,** which when dried contained, ash-free, 16.89 per cent. of nitrogen. The absolute alcohol used in dehydrating preparation **9,** with help of the water which it extracted, dissolved a considerable quantity of proteid. This was precipitated by adding a few drops

* Am. Chem. Jour., XV, p. 415.

of sodium chloride solution. The precipitate produced was then digested with absolute alcohol and ether and when dried was found to contain, ash-free, 16.02 per cent. of nitrogen. The preparation was therefore redissolved in dilute alcohol, filtered perfectly clear, concentrated to small volume and cooled. The proteid separating was then treated as before with absolute alcohol and ether and yielded preparation **12.** The proteid thus extracted showed in all respects the properties of wheat gliadin, and it will be seen that it has nearly the same composition.

RYE GLIADIN, *Preparation* **9.**

	I.	II.	Average.	Ash-free.
Carbon	52.76	----	52.76	52.84
Hydrogen	6.81	----	6.81	6.82
Nitrogen	17.14	17.23	17.19	17.22
Sulphur, Oxygen	----	----	----	23.12
Ash	0.16			100.00

RYE GLIADIN, *Preparation* **10.**

	I.	II.	Average.	Ash-free.
Carbon	53.06	52.90	52.98	53.23
Hydrogen	6.83	7.11	6.97	7.00
Nitrogen	17.13	17.17	17.15	17.23
Sulphur, Oxygen	----	----	----	22.54
Ash	0.48			100.00

RYE GLIADIN, *Preparation* **12.**

	I.	II.	Average.	Ash-free.
Carbon	52.99	53.11	53.05	53.11
Hydrogen	6.73	6.83	6.78	6.79
Nitrogen	17.57	----	17.57	17.59
Sulphur	1.44	----	1.44	1.44
Oxygen	----	----	----	21.07
Ash	0.12			100.00

1000 grams of rye meal were thoroughly extracted with 10-per cent. sodium chloride solution and drained as dry as possible on filters. The extracted residue was then treated with alcohol of 0.860 specific gravity four consecutive times. The four red-brown extracts were filtered clear, concentrated till most of the alcohol was removed and then cooled. The precipitates thus obtained were united and treated at first with stronger and afterwards with

75-per cent. alcohol until all soluble was dissolved. A considerable residue remained which appeared to be coagulated gliadin. This was washed thoroughly with absolute alcohol and ether, and when dried weighed 5.62 grams, preparation **13.** The dissolved proteid, after filtering its solution perfectly clear, was separated by concentrating to small volume and cooling. The deposit was then treated with absolute alcohol, dissolved again in a little dilute alcohol and precipitated by pouring into absolute alcohol. The proteid, preparation **14,** separated perfectly colorless, in a finely divided state. When dried it weighed 11.66 grams.

RYE GLIADIN, *Preparation* **13.**

	I.	II.	Average.	Ash free.
Carbon	52.36	----	52.36	52.62
Hydrogen	6.73	----	6.73	6.76
Nitrogen	17.75	17.59	17.67	17.75
Sulphur	1.18	----	1.19	1.19
Oxygen	----	----	----	21.68
				100.00
Ash	0.51			

RYE GLIADIN, *Preparation* **14.**

	I.	II.	Average.	Ash-free.
Carbon	52.74	----	52.74	52.93
Hydrogen	6.73	----	6.73	6.75
Nitrogen	17.32	17.52	17.42	17.48
Sulphur	1.23	----	1.23	1.23
Oxygen	----	----	----	21.61
				100.00
Ash	0.37			

These two preparations formed together 1.73 per cent. of the rye meal and have the composition of wheat gliadin. In order to prevent contamination of this proteid with the gum contained in rye meal, which Ritthausen* states to be freely soluble in 50-per cent. alcohol, the following method was tried.

After extracting rye meal with 10-per cent. sodium chloride brine the residue was treated with alcohol so strong that with the water retained by the meal, a mixture resulted containing about 75 per cent. of alcohol. After standing over night the extract was syphoned from the residue and greatly diluted with water. The proteid separated on standing and was filtered out and dis-

* Die Eiweisskoerper, etc., Bonn, 1872, p. 96, and Jour. f. prakt. Chem., XCIX, p. 454, and CII, p. 321.

solved in 75-per cent. alcohol. This solution was filtered perfectly clear, concentrated, cooled and the separated proteid treated with absolute alcohol and ether and dried. The resulting preparation **15,** was perfectly white. The residual meal was again extracted with 75-per cent. alcohol and the extract filtered clear, concentrated to one-fourth its volume, cooled, the precipitated proteid again dissolved in 75-per cent. alcohol, filtered clear, concentrated, cooled, and the separated proteid washed repeatedly with water. The substance was again dissolved in dilute alcohol and the clear solution precipitated by pouring into absolute alcohol. The precipitate produced was still again dissolved in dilute alcohol and a second time precipitated by pouring into absolute alcohol. The precipitate thus resulting was dissolved in dilute alcohol and precipitated by pouring into water and adding a little salt. The final pure white precipitate was digested with absolute alcohol and ether and dried, giving preparation **16.**

RYE GLIADIN, *Preparation* **15.**

	I.	II.	Average.	Ash-free.
Carbon	52.03	52.09	52.06	52.40
Hydrogen	6.78	6.91	6.85	6.89
Nitrogen	17.80	....	17.80	17.91
Sulphur	1.23	....	1.23	1.24
Oxygen	....	....	....	21.56
				100.00
Ash	0.68			

RYE GLIADIN, *Preparation* **16.**

	I.	II.	Average.	Ash-free.
Carbon	52.74	52.65	52.70	53.03
Hydrogen	6.90	6.96	6.93	6.97
Nitrogen	17.39	....	17.39	17.50
Sulphur	1.29	....	1.29	1.30
Oxygen	....	....	....	21.20
				100.00
Ash	0.65			

Another preparation of this substance was made by extracting 3000 grams of rye flour directly with 75 per cent. alcohol. The extract was concentrated to one-fourth its volume and the proteid which separated on cooling was washed many times with distilled water and dissolved in dilute alcohol, yielding a clear solution. This was then poured into three times its volume of absolute alcohol and an opalescent mixture obtained which depos-

ited a curdy precipitate after adding a little sodium chloride solution. The strong alcoholic solution from which this separated was clear and of a deep yellow color. The precipitate was treated with absolute alcohol as long as this was colored. During the process the substance was rubbed up to a fine powder. It was finally digested with ether for 24 hours and dried over sulphuric acid. This preparation, **17,** weighed 58 grams and was perfectly white. It formed very nearly 2 per cent. of the meal.

RYE GLIADIN, *Preparation* **17.**

Carbon	52.68
Hydrogen	6.71
Nitrogen	17.89
Sulphur	1.22
Oxygen	21.50
Ash	0.00
	100.00

In order to establish conclusively whether more than one alcohol-soluble proteid is contained in the rye-kernel, five preparations were made from the same portion of meal, by fractional precipitation. 4000 grams of rye meal were thoroughly extracted with 10-per cent. sodium chloride solution and the greater part of the bran removed by washing the meal through coarse cloth with the salt solution. The residue after decanting the salt solution was extracted with 75-per cent. alcohol, the extract was filtered clear and divided into two parts. The first part was concentrated to one-fourth and cooled, the second to one-half. The precipitated proteid from each was washed repeatedly with distilled water, dissolved in a small amount of 75-per cent. alcohol, filtered clear and precipitated by pouring into absolute alcohol. The proteid thus separated was digested with absolute alcohol and with ether. From the first portion of the alcoholic extract, preparation **18** was obtained, from the second, preparation **19.** These had the following composition:

RYE GLIADIN, *Preparation* **18.**

		Ash-free.
Carbon	51.90	52.67
Hydrogen	6.87	6.97
Nitrogen	17.50	17.76
Sulphur	1.26	1.27
Oxygen	----	21.33
		100.00
Ash	1.48	

RYE GLIADIN, *Preparation* **19.**

		Ash-free.
Carbon	52.04	52.40
Hydrogen	6.66	6.71
Nitrogen	17.77	17.89
Sulphur	1.15	1.16
Oxygen	----	21.84
		100.00
Ash	0.71	

The water washings from these two preparations were severally mixed with a little saturated sodium chloride solution which gave a considerable precipitate in each. These precipitates were then washed superficially with distilled water, dehydrated with absolute alcohol and treated with ether. The washings from **18** yielded preparation **20,** those from **19,** preparation **21.**

RYE GLIADIN, *Preparation* **20.**

	I.	II.	Average.	Ash-free.
Carbon	51.36	51.55	51.46	53.05
Hydrogen	7.07*	6.61	6.61	6.92
Nitrogen	17.64	17.61	17.63	18.17
Sulphur	1.14	----	1.14	1.17
Oxygen	----	----	----	20.69
				100.00
Ash	3.01			

RYE GLIADIN, *Preparation* **21.**

	I.	II.	Average.	Ash-free.
Carbon	52.01	----	52.01	52.37
Hydrogen	6.88	----	6.88	6.93
Nitrogen	17.99	17.97	17.98	18.10
Sulphur	1.04	----	1.04	1.05
Oxygen	----	----	----	21.55
				100.00
Ash	0.71			

The mother liquors from which **18** and **19** separated, after concentration, were united, further concentrated and cooled. The portion so separated was washed with water, dissolved in alcohol, the solution filtered clear and precipitated by pouring into absolute alcohol and after digesting with absolute alcohol and ether yielded preparation **22.**

* Omitted in average.

RYE GLIADIN, *Preparation* 22.

		Ash-free.
Carbon	48.44	52.46
Hydrogen	6.22	6.73
Nitrogen	16.68	17.94
Sulphur	0.91	0.99
Oxygen	----	21.88
		100.00
Ash	7.67	

SUMMARY OF ANALYSES OF RYE GLIADIN.

	9.	10.	12.	13.	14.	15.	16.
Carbon	52.84	53.23	53.11	52.62	52.93	52.40	53.03
Hydrogen	6.82	7.00	6.79	6.76	6.75	6.89	6.97
Nitrogen	17.22	17.23	17.59	17.75	17.48	17.91	17.50
Sulphur	23.12	22.54	1.44	1.19	1.23	1.24	1.30
Oxygen			21.07	21.68	21.61	21.56	21.20
	100.00	100.00	100.00	100.00	100.00	100.00	100.00

	17.	18.	19.	20.	21.	22.	Average.
Carbon	52.68	52.67	52.40	53.05	52.37	52.46	52.75
Hydrogen	6.71	6.97	6.71	6.92	6.93	6.73	6.84
Nitrogen	17.89	17.76	17.89	18.17	18.10	17.94	17.72
Sulphur	1.22	1.27	1.16	1.17	1.05	0.99	1.21
Oxygen	21.50	21.33	21.84	20.69	21.55	21.88	21.48
	100.00	100.00	100.00	100.00	100.00	100.00	100.00

Comparing these results with those obtained by Osborne and Voorhees in analyzing gliadin from wheat* it is seen that they agree very closely, similar variations between the analyses existing in both cases. The averages of the two series of analyses agree well, as shown by the following figures:

GLIADIN.

	Wheat.	Rye.
Carbon	52.72	52.75
Hydrogen	6.86	6.84
Nitrogen	17.66	17.72
Sulphur	1.14	1.21
Oxygen	21.62	21.48
	100.00	100.00

In all their properties wheat gliadin and rye gliadin resemble each other so exactly as to leave no doubt of their chemical identity. Ritthausen, as already stated, failed to find gliadin in rye

* Amer. Chem. Jour., XV, p. 436.

meal and described the proteid soluble in alcohol as mucedin, having a lower nitrogen and higher carbon content. This disagreement is doubtless due to impurities in Ritthausen's preparations, which, as he mentions, contained coloring matter that could not be removed. This color was probably a result of extracting with hot alcohol, which Ritthausen appears to have used in all cases, cold alcohol having given him a small yield of proteid. I had, however, no trouble in obtaining an abundant yield of gliadin with cold alcohol of 70 per cent., and thereby have extracted far less coloring matter than with hot alcohol.

D. Proteid soluble only in dilute Alkalies.

The sample of rye flour previously used is this work contained 1.52 per cent. of nitrogen. The amount of nitrogen soluble in salt solution and in dilute alcohol was determined in this flour by extracting 100 grams with a large quantity of 5-per cent. sodium chloride solution and then with 75-per cent. alcohol. The residue was then thoroughly air-dried and found to weigh 78 grams. This residue contained 0.55 per cent. of nitrogen. The 100 grams of flour therefore contained 1.52 grams of nitrogen of which 0.43 grams remained after extraction and 1.09 grams was soluble in dilute salt solution and alcohol, or in other words, 71.7 per cent. of the nitrogen was soluble in the reagents named and 28.3 per cent. was insoluble. In the wheat kernel a considerable part of the nitrogen was likewise found to be insoluble in salt solution and in dilute alcohol, but as this substance could be separated as a constituent of the gluten it was possible to prepare it in quantity and in a state of comparative purity. Since rye flour yielded no gluten on washing with water, the proteid remaining in the meal after extracting with salt solution and dilute alcohol, could be obtained only by extracting the residual meal directly with dilute potash water. All attempts, however, to thus prepare this substance resulted only in the production of small preparations of very variable composition. The gum present in the seed dissolved freely in the alkaline solution and made it impossible by any means yet discovered to thoroughly purify the preparations. For this reason nothing positive can now be said in relation to the nature or composition of this residual proteid. Since the other proteids are the same as those found in the wheat kernel it might be conjectured that this proteid is identical with glutenin. The fact that rye flour yields no gluten is, however, opposed to such

a conclusion. It is therefore more probable that the substance in question is, partly or wholly, other than glutenin.

Quantities of the different Proteids in the Rye-Kernel.

Owing to the gum already mentioned the filtration and treatment of the rye extracts was difficult and prolonged and the amounts of globulin, albumin and proteose could not be determined separately, as in the case of wheat. The rye flour contained 1.52 per cent. of nitrogen. If we assume that the proteids of rye contain on the average 17.6 per cent. of nitrogen, as was very nearly the case with those of wheat, and that all the nitrogen exists in proteid form, this sample of flour would contain 8.63 per cent. of proteid. We have, therefore, 2.44 per cent. of insoluble proteid and 6.19 per cent. soluble in salt solution and alcohol. We have already shown that the alcohol-soluble gliadin amounted to 4.0 per cent. of the flour and the leucosin to 0.43 per cent., there thus remains 1.76 per cent. to be divided between edestin and proteoses.

Insoluble in salt solution	2.44 per cent.
Gliadin, soluble in alcohol	4.00 "
Leucosin, " " water	0.43 "
Edestin and Proteose, soluble in salt solution	1.76 "
	8.63 "

THE PROTEIDS OF BARLEY.

By Thomas B. Osborne.

The proteids of barley have received little attention on the part of chemists. Mulder* states that this grain contains six per cent. of albumin and plant gelatin; the latter was obtained by extracting barley meal with hot alcohol, cooling the resulting solution and treating the deposited substance with ether. The composition of this body he gave as follows:

	1.	2.
Carbon	54.93	54.75
Hydrogen	7.11	6.99
Nitrogen	15.71	15.71
Sulphur	0.57	0.62
Oxygen	21.68	21.93
	100.00	100.00

v. Bibra† names albumin, plant-gelatin and casein as constituents of barley but gives no particulars concerning these bodies further than that they all contain on the average 15.5 to 15.6 per cent. of nitrogen.

Kreusler made an investigation of the proteids of barley, the results of which are given by Ritthausen.‡ Kreusler employed coarsely ground meal and finely ground flour, the latter yielding purer preparations, the results being otherwise the same.

He states that the aqueous extract of the ground seed contains an albumin coagulated by boiling and of the following composition:

Carbon	52.86
Hydrogen	7.23
Nitrogen	15.75
Sulphur	1.18
Oxygen	22.98
	100.00

* Physiolog. Chemie, I, 306–308.

† Die Getreidearten u. das Brod. Nürnberg, 1860, p. 304.

‡ Die Eiweisskoerper &c., Bonn, 1872, page 103.

The extract made with hot 75-per cent. alcohol contains, according to Kreusler, three proteids; gluten-casein, gluten-fibrin and mucedin.

The gluten-casein separates on cooling the hot alcoholic extract and when purified by boiling with dilute alcohol and fractionally precipitated from solution in acetic acid has the composition stated below.

1, is not corrected for ash and represents the first precipitation from a turbid solution.

2, is the second precipitation from a clear solution and is corrected for ash.

The cold alcoholic extract contains gluten-fibrin and mucedin.

The composition of these Kreusler gives as follows:

	Gluten-casein.		Gluten-fibrin.		Mucedin.	
	1.	2.	From meal.	From flour.	From meal.	From flour.
Carbon	53.84	53.25	55.23	54.55	53.19	53.97
Hydrogen	7.16	7.13	7.24	7.27	6.65	7.03
Nitrogen	16.63		15.49	15.70	16.14	16.98
Sulphur }			22.04	22.48	24.02	0.68
Oxygen }						21.34
						100.00

These proteids were supposed to be the same as those similarly named and described by Ritthausen as occurring in the wheat kernel.

So far as the writer has been able to learn the preceding summary includes all that has been published hitherto in regard to the proteids of the barley kernel that is now worthy of notice.

My preliminary examination of barley meal showed that the seeds contain proteid matters soluble in water, in sodium chloride solutions and in alcohol, and that after complete extraction with all these reagents there remains a considerable quantity of proteid which can be partly extracted by dilute potash solutions, but the greater part of which is insoluble in any reagent hitherto applied.

The material employed consisted of meal made from two-rowed barley and of a very white barley flour kindly furnished by the Health Food Co., of New York, both of which yielded proteids of the same composition and properties, the preparations derived from the flour, however, being less contaminated with coloring matter than those derived from the meal made from the entire grain, including the ground husk, which was so closely adherent, as to render its removal in the laboratory impossible.

Proteids Soluble in Water. Leucosin. Proteose.

As an aqueous extract of any seed, is in reality a dilute saline solution, owing to the salts extracted from the seed, and as the proteid matter soluble in alcohol dissolves to a slight extent in very dilute saline solutions the proteids properly soluble in water were obtained by extracting the meal with sodium chloride solutions, dialyzing away the salts and filtering off the proteid that thereby precipitated. In this way the proteid matter, soluble in pure water, which had been extracted from the meal was obtained in solution by itself. Three kilos of barley, ground to a fine meal, were treated with nine liters of 10-per cent. salt solution, applied in successive portions, the bran being removed by washing on a coarse cloth. The starch and other suspended matter was allowed to settle out and the extract was filtered clear. This solution was then saturated with ammonium sulphate and the precipitate produced, after filtering out, was treated with 10-per cent. salt solution. The resulting liquid was filtered clear and dialyzed for five days. The globulin that separated in this process was collected on a filter, the solution was returned to the dialyzer for three days longer, and the very small additional amount of substance that separated was filtered out. The clear solution was then heated in a water bath to 65°, the water of the bath not exceeding 70°. After an hour the coagulum was filtered out, washed with warm water, alcohol and ether, and dried over sulphuric acid. This preparation, **1**, weighed 4.15 grams and when dried at 110° had the following composition :

Coagulated Barley Albumin, Leucosin. *Preparation* **1.**

Carbon	53.04
Hydrogen	6.78
Nitrogen	16.84
Sulphur	1.42
Oxygen	21.92
	100.00
Ash	0.29

Another preparation was made by treating two kilos of barley meal with 10-per cent. sodium chloride solution, squeezing out in a press and repeating the process on the residue. The filtered extract was saturated with ammonium sulphate, the precipitate dissolved in dilute salt solution, subjected to dialysis and when freed from chlorides filtered and heated to 65° in a water bath of

70°. The coagulum produced was washed thoroughly with hot water, alcohol and ether and dried over sulphuric acid. This preparation, 2, weighed 2.3 grams and when dried at 110° had the following composition:

COAGULATED BARLEY ALBUMIN, LEUCOSIN. *Preparation* 2.

Carbon	52.67
Hydrogen	6.77
Nitrogen	16.41
Sulphur } Oxygen }	24.15
	100.00
Ash	0.31

As this body separated slowly when its solutions were heated to 65° it was thought possible that more than one albumin was present, which if a fact might be shown by analysis of preparations precipitated in successive fractions.

Accordingly six kilos of barley meal were extracted with 10-per cent. salt solution and the clear filtered extract saturated with ammonium sulphate. The precipitate produced was dissolved in brine and the solution, after filtering clear, was dialyzed until all the globulin had precipitated. It was then again filtered clear and in order to obtain a concentrated solution the filtrate was saturated with ammonium sulphate, the precipitate formed was dissolved in water and this solution was filtered clear and dialyzed. After six days only a very little more globulin had separated, which was filtered out and a portion of the clear solution was tested carefully for its coagulation point. When slowly heated in a double water bath it became faintly turbid at 39° and but very little more so at 49°. The turbidity then rapidly increased, flocks appearing at 56°. After heating at 56° for twenty minutes the solution was filtered and again heated. Turbidity occurred at 50° and flocks formed at 60°. After heating to 65° and holding at this temperature for some time the solution was filtered and again heated. Thereupon the turbidity took place at 70° and a very few flocks formed at 74°. The solution still had a just detectable acid reaction. The entire solution was then heated with great care to precisely 56° in a large water bath, the temperature of which did not exceed 57°. After keeping at this temperature for an hour the coagulum was filtered out, washed with hot water, alcohol and ether and dried over sulphuric acid

This preparation, **3**, weighed 0.36 gram and contained, when dried, without correction for ash, 16.48 per cent. of nitrogen.

The filtrate from preparation, **3**, was then heated to just 60° for three hours and the second coagulum filtered off and treated as the first had been. This preparation, **4**, weighed 0.4 gram and contained, without correcting for ash, 16.74 per cent. of nitrogen. Another part of this same extract after freeing from globulin as above described was dialyzed into alcohol for three days whereby the solution was concentrated and the proteid partly precipitated. In order to separate the albumin from any proteose thrown down with it, the precipitate produced by alcohol-dialysis was digested with absolute alcohol for three days longer and then washed thoroughly with water. A considerable part of the albumin was thus rendered insoluble in water, and after being further washed with absolute alcohol and ether was dried over sulphuric acid and found to weigh 0.51 gram. This preparation, **5**, contained 16.30 per cent. of nitrogen without correcting for ash.

Another preparation of albumin was made in the same way, in order to obtain a larger quantity for complete analysis. Six kilos of barley flour were mixed with 28 liters of 10-per cent. salt solution and 17 liters of clear filtrate obtained, which was saturated with ammonium sulphate. The precipitate produced was dissolved as far as possible in 10-per cent. salt solution, filtered clear, and in order to reduce the volume of the solution it was saturated with ammonium sulphate, the precipitate dissolved in 1000 c. c. of water and dialyzed until entirely free from globulin. The solution was then filtered and dialyzed into alcohol. After being concentrated, absolute alcohol was added and the precipitate filtered off, washed with absolute alcohol and ether and dried over sulphuric acid. This preparation, **6**, weighed 4.1 grams. It was then digested with water and the insoluble matter washed thoroughly with water, alcohol and ether, dried over sulphuric acid, and had the following composition when dried at 110°:

Coagulated Barley Albumin, Leucosin. *Preparation* **6.**	
Carbon	52.71
Hydrogen	6.78
Nitrogen	16.93
Sulphur	1.51
Oxygen	22.07
	100.00
Ash	0.50

SUMMARY OF ANALYSES OF COAGULATED BARLEY ALBUMIN—LEUCOSIN.

	1.	2.	3.*	4.*	5.*†	6.†	Average.
Carbon	53.04	52.67	----	---	----	52.71	52.81
Hydrogen	6.78	6.77	----	---	----	6.78	6.78
Nitrogen	16.84	16.41	16.48	16.74	16.30	16.93	16.62
Sulphur	1.42	24.15	----	----	----	1.51	1.47
Oxygen	21.92					22.07	22.32
	100.00	100.00				100.00	100.00

If this proteid is compared with the leucosin‡ obtained from the wheat and rye kernels it will be seen that the three are almost identical in composition.

LEUCOSIN.

	Wheat.	Rye.	Barley.
Carbon	53.02	52.97	52.81
Hydrogen	6.84	6.79	6.78
Nitrogen	16.80	16.66	16.62
Sulphur	1.28	1.35	1.47
Oxygen	22.06	22.23	22.32
	100.00	100.00	100.00

The aqueous extract of the barley kernel contains also a small quantity of one or more proteoses, but owing to the great difficulties encountered in attempting to separate these no pure preparations have been obtained.

PROTEID SOLUBLE IN SODIUM CHLORIDE SOLUTION. EDESTIN.

The large amount of gum extracted from barley meal by salt solution renders it very difficult to prepare the globulin in anything like a pure state. This difficulty is further increased by the readiness with which the globulin passes into the insoluble or albuminate condition and is thus lost for further purification. In only three cases was it possible to redissolve and reprecipitate this proteid in sufficient quantity for analysis. In all the extracts made, a considerable amount of globulin was precipitated by dialysis in the form of minute spheroids. So far as noticed, this globulin resembled in all respects that found in wheat and rye. It was readily and completely precipitated from salt solution by dialysis and also by adding acid. When dissolved in 10-per cent. sodium chloride solution and heated, turbidity occurred at 90° but no

* Not corrected for ash. † Coagulated by alcohol.

‡ Report of the Conn. Agricultural Experiment Station, 1893, p. 179.

coagulum formed until the solution was boiled and then only a small part of the dissolved substance separated.

Three kilos of barley meal were extracted with 10-per cent. salt solution, the filtered extract saturated with ammonium sulphate and the resulting precipitate filtered out, dissolved in 10-per cent. brine and the insoluble matter removed by filtration after adding a *very* small quantity of two-tenths-per cent. potash water in order to neutralize the slight acid reaction of the extract. The solution was then filtered clear and dialyzed for four days. The proteid separated in the form of small spheroids which were filtered out, washed with water, alcohol and ether, and after drying over sulphuric acid found to weigh 4.02 grams. This preparation was dissolved in 10-per cent. salt solution and again submitted to dialysis. After the proteid had precipitated it was filtered out, washed with water, alcohol and ether, and the final preparation, **7**, when dried at 110°, had the following composition:

BARLEY GLOBULIN, EDESTIN, *Preparation* **7**.

Carbon	51.43
Hydrogen	6.71
Nitrogen	18.14
Sulphur, Oxygen	23.72
	100.00
Ash	0.48

Again six kilos of barley flour were extracted with 10-per cent. salt solution, the filtered extract saturated with ammonium sulphate, the resulting precipitate dissolved in salt solution and dialyzed. The precipitated globulin was again dissolved in 10-per cent. salt solution and precipitated a second time by dialysis. 1.9 grams of preparation **8** were obtained, having the following composition:

BARLEY GLOBULIN, EDESTIN, *Preparation* **8**.

Carbon	50.82
Hydrogen	6.76
Nitrogen	18.16
Sulphur, Oxygen	24.26
	100.00
Ash	0.37

Another preparation was made in the same way, save that after dissolving the ammonium sulphate precipitate in salt solution, the proteids were again precipitated by saturation with ammonium sulphate and redissolved in brine, thus yielding a solution of smaller volume which was then dialyzed. After five days' dialysis, the chlorides having been removed, the precipitated globulin was treated in the usual manner and found to weigh 1.85 grams. This preparation, **9**, had the following composition:

BARLEY GLOBULIN, EDESTIN, *Preparation* **9.**

Carbon	50.40
Hydrogen	6.48
Nitrogen	18.00
Sulphur, Oxygen	25.12
	100.00
Ash	0.44

The foregoing analyses, although not showing the agreement to be desired, are on the whole sufficiently alike to warrant their publication, and for the sake of comparison they are here tabulated.

BARLEY GLOBULIN, EDESTIN.

	7.	8.	9.	Average.
Carbon	51.43	50.82	50.40	50.88
Hydrogen	6.71	6.76	6.48	6.65
Nitrogen	18.14	18.16	18.00	18.10
Sulphur, Oxygen	23.72	24.26	25.12	24.37
	100.00	100.00	100.00	100.00

In view of the close resemblance in properties and similarity in composition it is the writer's opinion that this globulin is the same as that found in a large number of other seeds and previously described under the name edestin.* The following table affords a comparison of the composition of this proteid from its different sources.

EDESTIN.

	Wheat.	Maize.	Hemp-seed.	Castor-bean.	Squash-seed.	Flax-seed.	Cotton Seed.	Rye.	Barley.
C.	51.03	51.71	51.28	51.31	51.66	51.48	51.71	51.19	50.88
H.	6.85	6.85	6.84	6.97	6.89	6.94	6.86	6.74	6.65
N.	18.39	18.12	18.84	18.75	18.51	18.60	18.64	18.19	18.10
S.	0.69	0.86	0.87	0.76	0.88	0.81	0.62	23.88	24.37
O.	23.04	22.46	22.17	22.21	22.06	22.17	22.17		
	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

* Report Conn. Agricultural Experiment Station, 1893, pp. 179 and 216.

On comparing the above analyses it will be seen that the preparations obtained from the cereals show the greatest deviation from the average of these figures. This is unquestionably due to the fact that in these seeds this substance is present in small quantity and is associated with other bodies so that it has been impossible to prepare it from them in a state of perfect purity.

Proteid soluble in Dilute Alcohol. Hordein.

After extracting 500 grams of barley meal with brine the residue was treated with alcohol added in sufficient quantity to form with the water retained by the meal, an alcohol of approximately 75-per cent. After digesting a short time the meal was squeezed out and again treated with 75-per cent. alcohol, and pressed out. The united alcoholic extracts were then filtered clear, concentrated to small volume on a water bath, cooled and the proteid thus separated washed thoroughly by kneading with distilled water. The separated substance now presented every appearance of gliadin, the proteid similarly obtained from wheat and rye. It was dissolved in a little dilute alcohol in which it was very readily soluble with the exception of a slight residue of coagulated proteid which rendered filtration extremely difficult. The solution was then precipitated by pouring into absolute alcohol and the precipitate digested with absolute alcohol, rubbed to a powder while still moist with alcohol and treated with ether. When dried over sulphuric acid this preparation, **10,** weighed 4.54 grams and when dried at 110° gave on analysis the following results:

Barley Proteid, *Preparation* **10.**

	I.	II.	Average.
Carbon	53.83	53.93	53.88
Hydrogen	6.72	6.92	6.82
Nitrogen	17.32	----	17.32
Sulphur } Oxygen }	----	----	21.98
			100.00
Ash			0.22

Another extract was made by treating 500 grams of barley meal with three liters of alcohol of 0.9 specific gravity applied directly to the freshly ground meal. The extract which had a red-brown color was squeezed out in a press and concentrated to about one-eighth of its volume. After standing over night the mother

liquor was poured off from the proteid which had separated in a firm mass on the bottom of the dish.

This was then dissolved in dilute alcohol and precipitated by pouring into absolute alcohol. The resulting precipitate was washed with absolute alcohol, digested with ether, dried over sulphuric acid and found to weigh 12.3 grams. This preparation, **11,** when dried at 110°, had the following composition:

BARLEY PROTEID, *Preparation* **11.**

Carbon	53.78
Hydrogen	6.51
Nitrogen	17.27
Sulphur	0.95
Oxygen	21.49
	100.00
Ash	0.19

The remainder of preparation **11** was then dissolved in dilute alcohol and, after filtering clear, poured into distilled water and precipitated by adding a few drops of sodium chloride solution. This substance was again dissolved in dilute alcohol and precipitated by pouring into absolute alcohol. After treating with ether and drying at 110°, this preparation, **12,** was analyzed with the following results:

BARLEY PROTEID, *Preparation* **12.**

Carbon	53.78
Hydrogen	6.82
Nitrogen	17.16
Sulphur	0.93
Oxygen	21.31
	100.00
Ash	0.86

Three kilos of barley meal were treated with 10-per cent. salt solution and washed on a coarse cloth until only the bran and larger particles of meal remained. This residue was then extracted with alcohol of 0.9 sp. gr. yielding a deep red solution, which was filtered through animal charcoal, but only a part of the coloring matter was removed. The clear solution was next concentrated on a water bath, poured into absolute alcohol and the resulting precipitate digested with absolute alcohol and treated with ether, giving preparation **13,** weighing 30 grams and when dry having the following composition:

BARLEY PROTEID, *Preparation* **13.**

	I.	II.	Average.
Carbon	53.80	53.70	53.75
Hydrogen	----	6.78	6.78
Nitrogen	17.48	17.33	17.41
Sulphur	0.93	----	0.93
Oxygen	----	----	21.13
			100.00
Ash			0.25

A portion of the solution from which preparation **13** had been obtained was precipitated separately by pouring into strong alcohol and adding a few drops of salt solution. The precipitate was treated in the usual manner and gave a preparation, **14**, containing much less coloring matter than the preceding and having the following composition:

BARLEY PROTEID, *Preparation* **14.**

Carbon	54.32
Hydrogen	6.74
Nitrogen	17.13
Sulphur, Oxygen	21.81
	100.00
Ash	1.43

The starchy portion of the barley meal which had been washed through the cloth, as described, was thoroughly extracted with salt solution and then with dilute alcohol, the extraction being repeated until the proteid was completely removed. The united extracts were filtered and concentrated to two-thirds of their volume by distillation when the solution was poured into a dish and the evaporation continued. The proteid separated as a skin on the surface of the liquid and as a solid mass on the bottom of the dish. When reduced to about one-half the volume of the original liquid the hot mother liquor was decanted from the separated proteid which formed a tough mass of a pink color. This was washed with water and redissolved in dilute alcohol, giving a deep red solution which was poured into absolute alcohol and the mass of substance that separated was cut up with scissors into small pieces and digested with absolute alcohol and with ether. When dried over sulphuric acid this preparation, **15**, was pinkish in color and weighed 30 grams. Dried at 110° and analyzed the following results were obtained:

BARLEY PROTEID, *Preparation* **15.**

Carbon	54.00
Hydrogen	6.72
Nitrogen	17.49
Sulphur, Oxygen	21.79
	100.00
Ash	0.95

The mother liquor, decanted from preparation **15,** was still further concentrated and allowed to cool over night. Only a little substance separated which however was washed with water, redissolved in dilute alcohol and precipitated by pouring into much distilled water to which a little salt had been added. On standing about 36 hours the milky solution cleared and the proteid was found in a transparent layer at the bottom of the vessel. After treating with absolute alcohol and ether and drying over sulphuric acid, preparation **16** was obtained weighing 7 grams and having when dried, the following composition:

BARLEY PROTEID. *Preparation* **16.**

Carbon	53.90
Hydrogen	6.63
Nitrogen	17.08
Oxygen, Sulphur	22.39
	100.00
Ash	0.23

As this proteid resembled gliadin so closely in its physical and chemical properties it seemed important to subject it to very thorough fractional precipitation in order to determine whether it was a mixture of gliadin with another body or a new, distinct proteid. Another extract was made by treating three kilos of freshly ground barley meal with 10-per cent. salt solution, squeezing out in a press and treating the residue again in the same way. The meal residue was then mixed with alcohol in quantity sufficient to make with the water retained by the meal an alcohol of about 40-per cent. After squeezing out the liquid, alcohol was again added to the residual meal sufficient to increase the strength of the solvent to 75-per cent. After digesting for some time the extract was squeezed out and found less colored than the first dilute alcohol extract. This second extract was concentrated by

distillation to small volume and cooled giving a deposit of proteid much whiter than any previously made. The mother liquor from this precipitate was poured into absolute alcohol and a second precipitate obtained. The two precipitates, when united, dehydrated in the usual way, treated with ether, and dried over sulphuric acid weighed 22 grams. Dried at 110° this substance had the following composition :

BARLEY PROTEID. *Preparation* **17.**

Carbon	54.30
Hydrogen	6.67
Nitrogen	17.47
Sulphur	0.84
Oxygen	20.72
	100.00

About 18 grams of this preparation were dissolved in alcohol of 0.9 specific gravity and absolute alcohol was added until a considerable precipitate resulted, when the mixture was heated on a water bath until the precipitate dissolved. The solution was then cooled and after standing sometime the mother liquor was decanted from the separated substance. This precipitate was marked I. The solution decanted from I, was further treated with absolute alcohol and a second precipitate II, obtained in the same way. The mother liquor from II was mixed with a large quantity of absolute alcohol and, as the proteid did not separate, a few drops of salt solution were added and the resulting precipitate III filtered off and treated with absolute alcohol and ether in the usual manner.

In the first place, precipitate I was dissolved in a small quantity of 75-per cent. alcohol and absolute alcohol was added until the precipitate began to reappear. The whole was heated until this precipitate again dissolved whereupon the solution was cooled. The substance which separated settled out leaving the solution milky. The mother liquor was decanted from the small amount of deeply colored proteid which adhered to the bottom of the beaker, and this deposit was dissolved in a little 75-per cent. alcohol, treated with absolute alcoho and the opalescent solution so produced mixed with a little ether. This gave a very small precipitate, almost black in color and very sticky. The solution decanted from this small deposit was treated with a drop of potassium acetate solution

and the resulting precipitate after washing with absolute alcohol and ether was dried over sulphuric acid. It formed a light pink powder, preparation **18**, weighing 0.65 gram and when dry contained, ash free, 16.60 per cent. of nitrogen. Its ash content was 1.04 per cent. The mother liquor, decanted from the first precipitation of **18**, was treated with a drop of potassium acetate solution and the precipitate produced allowed to settle. After standing, the substance settled out and adhered to the bottom of the beaker in a solid mass, from which the clear supernatant solution was decanted. This solution after treatment with absolute alcohol yielded a precipitate which, washed with absolute alcohol and ether and dried, formed preparation **19** weighing 1.79 grams and having the following composition :

BARLEY PROTEID. *Preparation* **19.**

Carbon	53.85
Hydrogen	6.69
Nitrogen	17.22
Sulphur } Oxygen }	22.24
	100.00
Ash	0.40

The substance deposited after the addition of potassium acetate to the solution from which **19** was derived, was dissolved in 75-per cent. alcohol, absolute alcohol added to the solution and the resulting precipitate dissolved by heating. On cooling, a part of the proteid separated and after this had settled, the liquid was decanted and mixed with absolute alcohol, and on treating the precipitate in the usual manner preparation **20** was obtained, which when dried weighed 1.18 gram and gave the following results on analysis :

BARLEY PROTEID. *Preparation* **20.**

Carbon	54.33
Hydrogen	6.81
Nitrogen	16.93
Sulphur } Oxygen }	21.93
	100.00
Ash	0.58

The substance deposited by cooling the solution from which **20** was obtained was only partly soluble in dilute alcohol. It was

accordingly treated with 75-per cent. alcohol and allowed to stand until the insoluble matter had settled out. The clear liquid was then decanted and completely precipitated with absolute alcohol. The separated substance was washed with absolute alcohol and ether and when dry weighed 0.81 gram. This preparation, **21**, contained ash-free, 16.65 per cent. of nitrogen and 0.32 per cent. of ash. The insoluble matter just described, after washing by decantation with 75-per cent. alcohol, was treated in the usual manner and yielded preparation **22**, weighing 1.56 gram and having the following composition :

BARLEY PROTEID. *Preparation* **22.**

Carbon	53.91
Hydrogen	6.77
Nitrogen	17.00
Sulphur } Oxygen }	22.32
	100.00
Ash	0.71

Precipitate II was dissolved in a little 75-per cent. alcohol and the solution mixed with absolute alcohol. The resulting precipitate (*a*) was dissolved by heating and the solution cooled, whereupon a part (*b*) of the proteid was precipitated. The supernatant solution was poured off, mixed with absolute alcohol and this precipitate (*c*) which contained all the proteid remaining was dehydrated with absolute alcohol and washed with ether, giving preparation **23**, weighing 2.2 grams and having when dry the following composition :

BARLEY PROTEID. *Preparation* **23.**

	I.	II.	Average.
Carbon	54.63	54.68	54.65
Hydrogen	6.62	6.50	6.56
Nitrogen	17.16	----	17.16
Sulphur } Oxygen }	----	----	21.63
			100.00
Ash			0.32

The substance (*b*) deposited on cooling the solution as above described, was dissolved in 75-per cent. alcohol and partly precipitated by adding absolute alcohol. After redissolving the precipitate by the application of heat, the solution was cooled and

allowed to stand some time to deposit the precipitate which formed. The liquid was then decanted and the separated substance treated with absolute alcohol and ether, yielding preparation **24**, weighing 3.11 grams and having the following composition after drying at 110°.

BARLEY PROTEID. *Preparation* **24.**

Carbon	54.27
Hydrogen	6.67
Nitrogen	17.39
Sulphur, Oxygen	21.67
	100.00
Ash	0.32

To the solution from which **24** separated absolute alcohol was added in considerable quantity and the proteid thus thrown down was dehydrated with absolute alcohol and washed with ether. When dried this preparation, **25**, weighed 0.87 gram and, without correction for ash contained 17.28 per cent. of nitrogen.

Precipitate III was treated with absolute alcohol and with ether, and dried over sulphuric acid. It weighed 1.63 grams and its composition after complete drying was :

BARLEY PROTEID. *Preparation* **26.**

Carbon	53.39
Hydrogen	7.02
Nitrogen	17.49
Sulphur, Oxygen	22.10
	100.00
Ash	0.59

If these figures are compared it will be seen that no fractional separation has been effected, the variation in the results being no greater than in the preparations previously described. Preparation **18** is low in nitrogen, but this is doubtless due to its containing nearly all the impurities precipitable from the solution. Preparation **21** is also low in nitrogen but this was the most colored of all the preparations and as it was also small in quantity the accuracy of the analysis could not be confirmed. Excluding these two preparations the results agree fairly as shown by the following table.

SUMMARY OF THE PRECEDING FRACTIONAL PRECIPITATES.

	19.	20.	22.	23.	24.	25.	26.	Original substance 17
Carbon	53.85	54.33	53.91	54.65	54.27	....	53.39	54.30
Hydrogen	6.69	6.81	6.77	6.56	6.67	....	7.02	6.67
Nitrogen	17.22	16.93	17.00	17.16	17.39	17.28	17.49	17.47
Sulphur } Oxygen }	22.24	21.93	22.32	21.63	21.67	....	22.10	21.56
	100.00	100.00	100.00	100.00	100.00		100.00	100.00
Weight	1.79	1.18	1.56	2.2	3.11	0.87	1.63	18.00

As all the preceding preparations were made by extracting barley meal which contained a large quantity of bran, they were much contaminated with coloring matter.

In order to obtain products free from color 880 grams of fine ground "pearled barley" (a commercial preparation of barley made by rubbing off the outer coat of the grain), were treated with salt solution and after squeezing out the excess of liquid the residue was digested with 75-per cent. alcohol. The extract was then filtered, concentrated to small volume, cooled and the mother liquor decanted from the separated proteid. This was then dissolved in dilute alcohol, the solution poured into distilled water and the proteid thrown down by adding a little salt. The precipitate was again dissolved in a small amount of dilute alcohol and reprecipitated by pouring into absolute alcohol, digested with absolute alcohol for some time, then with ether, dried over sulphuric acid and found to weigh 8 grams. This preparation, **27**, was pure white and had the following composition when thoroughly dried:

BARLEY PROTEID, *Preparation* **27**.

Carbon	54.37
Hydrogen	6.81
Nitrogen	17.33
Sulphur	0.88
Oxygen	20.61
	100.00
Ash	0.48

Another preparation was made by extracting six kilos of barley flour with salt solution and then treating the residue with alcohol added in sufficient quantity to make with the water of the brine which still adhered to the meal as nearly as possible 75-per cent. alcohol. After standing over night the extract was filtered off,

concentrated to about one-third its original volume and cooled slightly. The proteid that now separated out from the hot solution was removed from the liquid, rinsed with water, dissolved in a very little dilute alcohol to a thick syrup, and reprecipitated by pouring into absolute alcohol. The substance was then cut up into small pieces and digested with absolute alcohol and also with ether. When dried over sulphuric acid 78 grams of a pure white preparation were obtained. Twenty-five grams of this were then dissolved in 75-per cent. alcohol and the clear solution poured into a large volume of distilled water. A part of the substance separated, leaving the liquid milky. The milky solution was decanted from the separated substance and the latter was washed with water in which some of it dissolved. The turbid liquid and washings were united and precipitated with a little salt solution. After standing over night the proteid separated as a transparent viscid liquid on the bottom of the vessel in the same way as gliadin does under similar conditions. After decanting the supernatant liquid the deposit was dissolved in dilute alcohol and precipitated by pouring its solution into absolute alcohol. The separated proteid was then digested with absolute alcohol and with ether and dried over sulphuric acid. A pure white preparation, **28**, resulted, which when dried at 110° had the following composition:

BARLEY PROTEID, *Preparation* **28.**

Carbon	54.02
Hydrogen	6.79
Nitrogen	17.38
Sulphur	0.84
Oxygen	20.97
	100.00
Ash	1.00

The mass which separated on pouring the alcoholic solution into water, as above described, was dissolved in 75-per cent. alcohol and, as it contained a little insoluble proteid which rendered filtration impossible, the solution was allowed to stand over night. The clear supernatant solution was then poured off and concentrated to about one-third of its volume and cooled. The proteid which separated was again dissolved in dilute alcohol and precipitated by pouring into absolute alcohol. After thorough dehydration with absolute alcohol and digestion with ether the sub-

stance was dried over sulphuric acid and yielded preparation **29**, which was white in color and weighed 5.46 grams. This substance when dried had the following composition:

BARLEY PROTEID, *Preparation* **29**.

	I.	II.	Average.
Carbon	54.48	54.54	54.51
Hydrogen	6.70	6.79	6.75
Nitrogen	17.22	17.18	17.20
Sulphur } Oxygen }	21.60	21.49	21.54
	100.00	100.00	100.00
Ash			0.32

Another preparation was made without heating, by pouring a part of the original extract from which preparations **28** and **29** were derived into a large amount of distilled water and allowing the separated substance to deposit. After some time this settled and the supernatant liquid was poured off, the precipitate washed with water, dissolved in cold dilute alcohol and the solution poured into absolute alcohol. The precipitate produced was digested with absolute alcohol and then with ether and dried over sulphuric acid yielding a pure white preparation having when dry the following composition:

BARLEY PROTEID, *Preparation* **30**.

Carbon	54.23
Hydrogen	6.83
Nitrogen	17.27
Sulphur	0.75
Oxygen	20.92
	100.00
Ash	0.17

In order to obtain a larger quantity of a colorless preparation, five kilos of barley flour were treated with 10.5 liters of 75-per cent. alcohol and after standing some time the extract was filtered off and 6 liters of clear solution obtained. This was then concentrated to one-third its volume and rapidly cooled. The proteid separated as a bulky plastic mass, which, after decanting the mother liquor, was macerated with about 500 c. c. of distilled water, the washings were poured off and the mass of proteid dissolved in 500 c. c. of 75-per cent. alcohol, yielding a solution of a pale yellowish brown tint. This solution was poured in a thin stream

into a quantity of distilled water and the separated proteid, after removal from the liquid, was again dissolved in 75-per cent. alcohol and the perfectly clear solution poured in a small stream into a large quantity of absolute alcohol. As the soluble salts had been almost completely removed the proteid did not separate even after admixture of 800 c. c. of absolute ether. Three or four c. c. of salt solution were therefore added to the milky liquid and an immediate precipitate resulted which rapidly settled leaving the solution clear and free from proteid. This mixture of absolute alcohol and ether retained all the fat present in the proteid before precipitation and also some coloring matter, the liquid being yellow. The solution was decanted and the voluminous precipitate treated with successive portions of absolute alcohol and obtained as a snow white granular substance, weighing when dried over sulphuric acid 93 grams. This preparation, **31,** had the following composition when dried at 110°:

BARLEY PROTEID. *Preparation* **31.**

	I.	II.	Average.
Carbon	54.18	54.31	54.25
Hydrogen	6.98	6.65	6.82
Nitrogen	17.20	17.30	17.25
Sulphur	0.84	----	0.84
Oxygen	----	----	20.84
			100.00
Ash	----	----	0.09

In order to make certain that this proteid, which so closely resembled gliadin in every respect but composition, was not that substance contaminated with fat, a portion of this preparation was ground to a very fine powder and washed for a long time with hot ether in an extraction apparatus. Only a trace of substance was removed by this treatment and the proteid after drying had the same composition as before as the following figures show:

BARLEY PROTEID. *Preparation* **32.**

Carbon	54.20
Hydrogen	6.58
Nitrogen	17.07
Sulphur	0.91
Oxygen	21.24
	100.00
Ash	0.25

Another portion of **31** was dissolved in two-tenths per cent. potash water yielding a clear solution which was precipitated by neutralization with two-tenths per cent. hydrochloric acid. The precipitate was washed with water, dehydrated with absolute alcohol, washed with ether and analyzed with the following results:

BARLEY PROTEID. *Preparation* **33.**

Carbon	54.21
Hydrogen	6.87
Nitrogen	17.12
Sulphur	0.76
Oxygen	21.04
	100.00
Ash	0.25

Preparation **31** was then subjected to fractional precipitation in order to make sure that it was not a mixture of two or more proteids. Twenty-five grams were dissolved in 300 c. c. of alcohol of 0.865 specific gravity by heating on a water bath and the solution was quickly cooled. After adding a few drops of 10-per cent. salt solution the most of the proteid separated in a coherent mass leaving the liquid clear. After decantation the residue was treated in the same way again, the decanted solutions being united. The residue was again dissolved and absolute alcohol added to the hot solution until a considerable precipitate resulted, when it was heated until clear and then cooled. A few drops of salt solution were then added and the proteid precipitated leaving the solution slightly milky. This liquid was joined to the two solutions from which the proteid had been previously separated and a little more salt solution added to the mixture thereby precipitating the remainder of the dissolved proteid. After decanting the liquid from the separated substance the latter was treated with absolute alcohol and gave preparation **34,** representing the fraction soluble in the strongest alcohol and having when dry the following composition:

BARLEY PROTEID. *Preparation* **34.**

Carbon	54.32
Hydrogen	6.78
Nitrogen	17.02
Sulphur	0.94
Oxygen	20.94
	100.00
Ash	0.21

The proteid which had been precipitated during the preparation of this substance as just described was dissolved in alcohol of 0.865 specific gravity and the solution cooled rapidly by immersing in cold water. When a part of the substance had separated the solution was decanted and the separated substance treated with absolute alcohol. Preparation **35** was thus obtained which gave the following figures on analysis:

BARLEY PROTEID. *Preparation* **35.**

Carbon	54.47
Hydrogen	7.01
Nitrogen	17.15
Sulphur	0.74
Oxygen	20.63
	100.00
Ash	0.43

The above preparation represented the portion least soluble in strong alcohol. The solution decanted from this preparation was precipitated with absolute alcohol and a few drops of salt solution and the resulting precipitate after the usual treatment yielded preparation **36** having the following composition:

BARLEY PROTEID. *Preparation* **36.**

Carbon	54.37
Hydrogen	6.81
Nitrogen	17.30
Sulphur	0.84
Oxygen	20.68
	100.00
Ash	0.38

The following table includes all analyses of the preparations which were free from coloring matter. (See also the next page.)

HORDEIN. BARLEY PROTEID SOLUBLE IN DILUTE ALCOHOL.

	27.	**28.**	**29.**	**30.**	**31.**	**32.**
Carbon	54.37	54.02	54.51	54.23	54.25	54.20
Hydrogen	6.81	6.79	6.75	6.83	6.82	6.58
Nitrogen	17.33	17.38	17.20	17.27	17.25	17.07
Sulphur	0.88	0.84	21.54	0.75	0.84	0.91
Oxygen	20.61	20.97		20.92	20.84	21.24
	100.00	100.00	100.00	100.00	100.00	100.00

	33.	34.	35.	36.	Average.
Carbon	54.21	54.32	54.47	54.37	54.29
Hydrogen	6.87	6.78	7.01	6.81	6.80
Nitrogen	17.12	17.02	17.15	17.30	17.21
Sulphur	0.76	0.94	0.74	0.84	0.83
Oxygen	21.04	20.94	20.63	20.68	20.87
	100.00	100.00	100.00	100.00	100.00

This body differs essentially from all the well defined plant-proteids now known. As it appears to be characteristic of barley, I propose to adopt for it the latterly disused name *hordein*, which was first applied about 1820 by Proust* and ten years later by Hermbstädt † to certain products of their attempts to isolate the proximate principles of this cereal.

Hordein appears to have been obtained nearly pure from barley flour by Kreusler, as shown by the following comparison of his analysis with the average above given.

BARLEY PROTEID SOLUBLE IN DILUTE ALCOHOL.

	Kreusler.	Osborne.
Carbon	53.97	54.29
Hydrogen	7.03	6.80
Nitrogen	16.98	17.21
Sulphur	0.68	0.83
Oxygen	21.34	20.87
	100.00	100.00

Ritthausen regarded this proteid as identical with the mucedin believed by him to occur in wheat and rye, but which, as my investigations prove, does not exist in those grains.

Toward water my different preparations of hordein behave somewhat differently. Preparations dried over sulphuric acid and still retaining a little alcohol dissolve in cold water to a greater or less extent according to the amount of alcohol present. When dried completely at 110°, so that all the alcohol is removed, very little hordein dissolves in cold water and slightly more on raising the temperature. Solutions thus made with hot water do not precipitate on cooling or coagulate on boiling although they give no inconsiderable precipitates on adding salt. A large number of preparations of this proteid and of wheat gliadin were thus tested and compared under similar conditions. The gliadin

* Ann. Chim. phys., V, 337.

† Jour. of Techn. Chem., XII, 46.

showed variations in solubility in the same way as the barley proteids, but throughout was much more soluble than the latter, yielding solutions with warm water which were precipitated by cooling. As drying at 110° tends to render more or less of these proteids insoluble in 75-per cent. alcohol it is not possible to say definitely whether the difference was due to original difference in properties of the two proteids tested or to the drying. It is the opinion of the writer that the hordein of barley is decidedly less soluble in water than the gliadin of wheat.

Toward alcohol the hordein behaves, so far as could be detected, exactly like gliadin. In very dilute acids and alkalies it is readily soluble and is precipitated by neutralization. Dissolved in concentrated hydrochloric acid a beautiful crimson color is produced similar to that given by gliadin under like conditions. With a warm mixture of equal volumes of water and concentrated sulphuric acid a red color is given by hordein, not a purple red as by gliadin.

The most marked difference between hordein and gliadin is in composition, since hordein contains one and a half per cent. more carbon, one-half per cent. less nitrogen, and three-tenths per cent. less sulphur than gliadin.

In the extraction last described, 5000 grams of barley flour were treated with 10.5 liters of alcohol, and the extract obtained measured 6 liters, which was equivalent to 57.1 per cent. of the whole solution employed. If we assume, as is very nearly true, that this was equal to a complete extraction of 57.1 per cent. of the flour, the proteid obtained was equivalent to all the alcohol soluble proteid contained in 2855 grams of flour. In addition to the 93 grams of proteid above described, there was obtained a further quantity weighing when thoroughly dried over sulphuric acid 17.5 grams, thus making in all 110.5 grams. This quantity is 3.87 per cent. of the 2855 grams extracted. In order to confirm these figures 500 grams of barley flour were extracted with 2 liters of hot 75-per cent. alcohol, squeezed out in a press and the residual meal treated again in the same way with another liter of alcohol and the united extracts filtered clear and concentrated by evaporation. All the proteid contained in the solution separated on cooling and was washed with ether, then dehydrated with absolute alcohol, again digested with ether and dried completely over sulphuric acid. 20.2 grams of proteid were thus obtained equal to 4.04 per cent. of the flour. We may therefore assume that this barley flour contained about 4 per cent. of the alcohol-soluble proteid, hordein.

Proteid insoluble in Water, Saline Solutions and Alcohol.

The proteids thus far described form only a part of the total proteids of the seed. One hundred grams of barley flour were extracted, first, with a large excess of 5-per cent. salt solution and then, repeatedly, with hot 75-per cent. alcohol. The residue, washed with absolute alcohol and thoroughly air dried, weighed 71 grams and contained 1.07 per cent. of nitrogen. The air dry flour before extraction contained 1.83 per cent. of nitrogen. The 100 grams of flour therefore contained 1.83 grams of nitrogen and the residue after extraction contained 0.76 grams. The nitrogen removed by the solvents therefore amounted to 58.3 per cent. of the whole.

If we assume that the nitrogen all belonged to proteid matter containing 17 per cent. of nitrogen, the flour included 10.76 per cent. of proteids, of which 58.3 per cent. was soluble in the reagents used in extracting the proteids already described. We have therefore $10.76-6.28=4.48$ per cent. of proteid unextracted. It was only possible to obtain this proteid by treating the residue with potash water. All attempts, however, to thus prepare it in quantity sufficient to yield preparations of even approximate purity resulted in complete failure.

The previous extraction of the flour to remove the proteids already described seemed to render to a great extent the remaining proteid insoluble in potash water and only insignificant precipitates resulted on neutralizing the extracts. The barley flour also contained a large quantity of gum which rendered the filtration of the alkaline extract very difficult, as this gum dissolved freely in potash water. As the proteids prepared from the barley flour are all so similar to those obtained from wheat flour it is most probable that this seed also contains a considerable quantity of proteid soluble only in dilute alkaline solutions, but, as in the case of rye, the writer was unable to obtain results of any value whatever in regard to it.

Conclusion.

The barley kernel contains:

I. Leucosin coagulating at 52°, which is the same as the albumin found in the wheat and rye kernels. Its composition, as shown by the average of six analyses, is:

Carbon	52.81
Hydrogen	6.78
Nitrogen	16.62
Sulphur	1.47
Oxygen	22.32
	100.00

This substance forms about 0.3 per cent. of the seed.

II. A small quantity of proteose, the reactions and composition of which could not be definitely ascertained.

III. Edestin, a globulin which is the same as that found in the wheat and rye kernels and in a large number of other seeds. Its composition is approximately shown by the figures given below. Owing to the small amount of this body and the difficulty in preparing it, no perfectly pure preparations were obtained.

Carbon	50.88
Hydrogen	6.65
Nitrogen	18.10
Sulphur, Oxygen	24.37
	100.00

This is the proteid commonly known as vegetable vitellin. It is precipitated from saline solutions by dilution and by dialysis, is not coagulated by heating below 90°, and above that temperature only partially. It is not precipitated by saturating its solutions with sodium chloride, but is thrown down from saline solutions by adding acid.

IV. Hordein, a proteid insoluble in saline solutions, very slightly soluble in pure water and extremely soluble in alcohol of about 75-per cent. This is the barley-proteid described by Ritthausen as mucedin. It has almost exactly the same physical and chemical properties as gliadin obtained from wheat and rye kernels but a different composition.

Carbon	54.29
Hydrogen	6.80
Nitrogen	17.21
Sulphur	0.83
Oxygen	20.87
	100.00

About 4.0 per cent. of the seed consists of this substance.

V. After extracting the barley flour with salt solution and with alcohol the residue still contained 42 per cent. of the total nitrogen, corresponding to proteid matter equal to about 4.5 per cent. of the flour. It was not possible to extract more than a very small amount of this residual proteid with dilute potash water, as the treatment for removal of the other proteids rendered it insoluble, if it were not so already.

VI. The barley flour contained 1.83 per cent. of nitrogen and, if it is assumed that this all belonged to proteid matter with 17 per cent. of nitrogen, the flour would contain 10.75 per cent. of proteids. The barley accordingly contained about 4.5 per cent. of insoluble proteid, 4.0 per cent. of hordein soluble in dilute alcohol, 0.30 per cent. albumin and 1.95 per cent. of globulin and proteose.

THE CHEMICAL NATURE OF DIASTASE.

By THOMAS B. OSBORNE.

Few substances are of more importance or of more interest than the enzymes or unorganized ferments, yet our knowledge relating to these bodies is almost wholly confined to the products of their activity and the conditions under which this is manifested. Although the existence of these ferments was recognized early in the present century, our information in respect to their true nature is exceedingly limited and unsatisfactory. It was for a long time supposed that the active substance causing a fermentative change is a soluble proteid, and the power of inducing such change seems by many to have been ascribed to soluble proteid matter in general. Later, this power was thought to be restricted to special forms of proteid, but no sufficient evidence was brought forward. Of late years investigators have undertaken to isolate ferments and prepare them in a state of purity. The results of these attempts have led to very conflicting conclusions respecting the character of these bodies. Some of the so-called pure preparations of ferments have had the properties of the proteids, and have more or less agreed with them in composition, while others have differed widely from the proteids in both respects.

It still seems to be the opinion of many that the enzymes are in fact true proteids and that the ferments thus far supposed to be obtained in a state of purity were simply somewhat contaminated with other substances. This opinion is based on the fact that all those changes which are ascribed to the action of enzymes occur only in solutions which contain proteid matter, and that the activity of the ferment is greatly influenced by conditions known to have a pronounced action on proteids, such as heat, the presence of acids and alkalies, salts of the heavy metals, etc.

The first discovered and one of the most carefully studied of these ferments is diastase. The practical application of the action of diastase in the manufacture of alcohol and of malt liquors has given rise to careful and extended studies of the conditions affecting the activity of this ferment, and the result of these studies has led some to the opinion that the active substance is the albumin present in the malt extracts. The conversion of

starch into maltose and dextrin by diastase increases in rate and extent as the temperature of the solution is raised, until the heat reaches the point at which the albumin begins to coagulate. The ferment then begins to lose power, and, when the heat is sufficient to completely coagulate the albumin, its amylolytic action ceases entirely. In 1883, C. Lintner, Jr., showed that the diastatic power of fifteen different samples of malt was very nearly proportional to the amount of coagulable albumin which they contained. In 1886, however, C. J. Lintner prepared diastase in, as he supposed, a state of purity, and came to the conclusion that the results of his analysis of diastase indicate that, in the ferments, we have a special class of proteid substances. The composition of Lintner's purest diastase differed much from that of the proteids, since it contained only two-thirds as much nitrogen and also less carbon. His diastase furthermore, failed to give the reaction with cupric sulphate and potassic hydrate which is characteristic of proteid matter. These results of Lintner's threw much doubt on the hypothesis that the vegetable albumin is identical with diastase.

In my investigations of the proteids of wheat, rye and barley, I found in all these grains the same albumin and was impressed with the close relation between the temperature at which this albumin coagulates and the temperature at which diastase begins to lose its activity. The aqueous extracts of these seeds, as is well known, possess considerable diastatic power, and it seemed to be more than probable that this was due to the albumin. I accordingly undertook an investigation of this subject, and I now offer the results thus far obtained, which are preliminary to a more extended study.

The usual method of preparing vegetable enzymes is to treat the aqueous or glycerin extract containing them with alcohol as long as a precipitate, having fermentative power appears, to purify this by repeated precipitation from its solution in water, by means of alcohol, and finally to subject the aqueous solution to dialysis to remove salts. This method is wholly unsuited to yield pure preparations, because the precipitate produced by alcohol contains not only a large amount of carbohydrates and salts, but also nearly all of the various forms of proteid matter present in the extract. Lintner employed this method, and there can be no doubt that he obtained a mixture of proteids with other substances which defied all attempts at further separation.

The most rational method (hitherto very little used) is first to separate the proteids from the carbohydrates and other soluble substances by saturating the extract with ammonium sulphate, thereby precipitating the ferment and proteids together, next to remove the proteid existing as globulin, by dialysis, and then, if possible, to separate the albumin and proteoses by fractional precipitation with alcohol. In following this method, a measured quantity of malt extract was saturated with ammonium sulphate, the precipitated proteid matter was filtered out, dissolved in water, and the clear filtered solution made up to the volume of the original extract. This solution was found to have the same diastatic power as before precipitation, thus showing that ammonium sulphate had not injured the diastase. Throughout my work diastatic power has been measured by Lintner's method, which gives a very ready means of accurately comparing different preparations. This method consists in adding to each of a series of carefully measured volumes of the solution containing definite amounts of the diastatic preparation, ten c. c. of a two per cent. solution of soluble potato starch, and allowing the ferment to act upon the starch for one hour at the ordinary temperature of the room. At the end of this time five c. c. of Fehling's solution are added to each portion and the mixtures are heated for ten minutes in a boiling water bath. After the precipitated cuprous oxide has settled, where too little sugar has been formed to precipitate all the copper, the liquids will be blue; if sugar is in excess they will be yellow. The one colorless liquid that should result gives the measure of diastatic power. Lintner represented the value of his most active preparation by 100, and that of the other preparations by figures stating the amount of each necessary to give a complete reaction with Fehling's solution under the above conditions, in comparison with his most active preparation, of which, under the conditions of the test just described, twelve one-hundredths of a milligram completely reduced the five c. c. of Fehling's solution.

For the sake of comparison I have measured the activity of my preparations by the same standard, so that a preparation whose activity is given as 200 means that six one-hundredths of a milligram sufficed to give a complete reduction.

As Lintner recommended extracting the malt with water containing twenty per cent. of alcohol instead of pure water, since thereby less foreign matter was removed with the proteid, this

procedure was first tried. Fifteen hundred grams of ground air-dried malt, prepared in the laboratory, were treated with three liters of twenty per cent. alcohol, the extract squeezed out in a press and the residue again treated with another liter of the same dilute alcohol. Three liters of extract were obtained which after being filtered clear were saturated with ammonium sulphate. Owing to the presence of the alcohol much less ammonium sulphate was dissolved than by a water extract, and the proteids were consequently incompletely precipitated. The precipitate obtained was treated with water and a considerable quantity of insoluble matter, consisting mostly of globulin rendered insoluble by contact with the reagents, was filtered out. The solution was saturated with ammonium sulphate, and the precipitate dissolved in water. This clear solution was then dialyzed in water for some days, and after filtering from a slight deposit was dialyzed in alcohol of 0.845 sp. gr. for 48 hours.

As the water passed out of the dialyzer faster than the alcohol entered, the solution became concentrated and a considerable precipitate formed. This was filtered out and washed, first with dilute alcohol and afterwards with absolute alcohol, and dried over sulphuric acid. This preparation, **1**, when thus dried, dissolved in water with the exception of a not inconsiderable residue. When filtered clear, the solution, on heating, gave an abundant coagulum, and after boiling and filtering out the coagulum, the filtrate gave a strong pink color with cupric sulphate and potassic hydrate, showing the presence of proteose. The diastatic power of this preparation, in comparison with Lintner's best was 86, but, as it was afterward found to contain a comparatively small amount of ash, the test was repeated with the addition of a few milligrams of sodium chloride and then found to equal 150.

The composition of preparation **1** was as follows:

PREPARATION **1**.

	Ash-free.
Carbon	52.55
Hydrogen	6.48
Nitrogen	16.41
Sulphur } Oxygen }	24.56
	100.00
Ash	2.29

These figures indicate that this preparation consisted almost wholly of proteid matter, and the reactions proved the presence

of at least three forms, namely, coagulated proteid, albumin and proteose. This mixture was one and a half times more active than Lintner's most energetic preparation, and contained about six per cent. more nitrogen and one-half as much ash. The composition of the preparation is very similar to that of the coagulated albumin-like body obtained from wheat, rye and barley, and for which I have adopted the name leucosin. As this albumin has been found to have the same composition, whether coagulated by heat or by alcohol, and as most, if not all of the proteids have identical composition (so far as analysis can show), in the soluble and the coagulated states, it seems probable that preparation **1** consisted mostly of coagulated and soluble leucosin together with a little proteose.

The filtrate from this preparation on addition of absolute alcohol, yielded a small precipitate **2** which dissolved wholly in water and gave only a very slight coagulum on heating, but a strong pink biuret reaction, showing it to be mostly proteose. Its diastatic power was only 19.

As above stated, owing to the presence of alcohol, saturation of the original extract of malt with ammonium sulphate, precipitated only a part of the proteids. Accordingly the filtrate from this first precipitation was dialyzed for 24 hours, so as to remove most of the alcohol, and was again saturated with ammonium sulphate. The resulting precipitate was dissolved in water, filtered from a slight residue, and the clear solution dialyzed until nearly all the ammonium sulphate was removed. The dialyzer was then transferred to alcohol and left for 48 hours. The resulting precipitate was then filtered out and treated in the manner before described. After drying, this substance, preparation **3**, like preparation **1**, consisted of insoluble proteid, soluble leucosin and proteose. Its diastatic power was 133, and it had the following composition :

PREPARATION **3**.

	Ash-free. I.	II.	Average.
Carbon	52.34	----	52.34
Hydrogen	6.73	----	6.73
Nitrogen	15.90	15.92	15.91
Sulphur, Oxgen	----	----	25.02
			100.00
Ash			0.82

The filtrate from this preparation was next treated with a large quantity of absolute alcohol, and the contained proteid completely thrown down. This substance, preparation 4, dissolved entirely in water; its solution yielded but a trace of coagulum on heating, and when boiled and filtered gave a strong proteose reaction. It contained, ash-free, only 12.02 per cent. of nitrogen, and had a diastatic activity of 11.

These results prove: that extraction of the malt with twenty per cent. alcohol is not suited for a subsequent precipitation of the proteids with ammonium sulphate: that otherwise, the method is capable of yielding preparations of diastase of high fermentative power, which to a certain extent, can be separated into fractions containing the different forms of proteid matter: that the fractions including the greatest amount of soluble albumin have the greatest diastatic power: and that a part at least of the proteose is almost if not entirely free from this power.

Another extraction was made on a much larger scale, so that the fractional precipitations might be more numerous, and the fractions examined more closely.

Ten kilograms of malt were exhausted with water and the extract was saturated with pure and neutral ammonium sulphate. The very bulky precipitate was suspended in four liters of water and dialyzed until much of the sulphate had been removed and the precipitated proteid largely dissolved. The solution was then filtered from an insoluble residue consisting mostly of globulin, and the clear filtrate was saturated with ammonium sulphate. The precipitate thus obtained was suspended in 1500 c. c. of water and was dialyzed until nearly all the sulphate had been removed and the precipitate mostly dissolved. The globulin contained in the extract was thus largely separated and, after it had been filtered out, the clear solution was dialyzed into an equal volume of alcohol of .84 sp. gr. After 48 hours the precipitate, number I, which had separated was filtered out and set aside for further examination. The filtrate was again dialyzed into an equal volume of alcohol of 0.84 sp. gr., and after 48 hours another precipitate II obtained. The filtrate was further dialyzed into a rather larger quantity of somewhat stronger alcohol, and precipitate III separated, and by similarly treating the filtrate from this, precipitate IV was obtained, the filtrate from which, on adding a large quantity of absolute alcohol yielded precipitate V. All the proteid in the extract was thus separated. Precipitate I was much

contaminated with coloring matter, II less so, and III was nearly colorless, as were also IV and V.

The approximate weights of each of these precipitates was as follows: I, 13.0 grams; II, 8.0; III, 6.0; IV, 5.0; and V, 3.0, a total of 35.0 grams.

Precipitate I was treated with water and found to be very largely insoluble. The insoluble matter was filtered out and washed with water, and the clear solution was dialyzed for several days to remove all the salts. No proteid was thus precipitated, and the dialysis was continued in strong alcohol, thereby throwing down all but a trace of proteid. The precipitate, preparation **5**, weighed 2.11 grams. After drying, it dissolved in water with the exception of a small residue, and its solution when slowly heated became turbid at 65° and deposited flocks at 70°. After boiling and filtering out the slight coagulum, the solution gave a strong pink reaction with the biuret test. These tests show the preparation to consist largely of proteose. Its composition was as follows:

PREPARATION 5.

	Ash-free.
Carbon	53.16
Hydrogen	7.03
Nitrogen	16.50
Sulphur	1.50
Oxygen	21.81
	100.00
Ash	0.49

With Lintner's test this preparation showed a diastatic power of 30.

The insoluble residue, remaining after treating precipitate I with water, was thoroughly extracted with ten per cent. sodium chloride solution, what remained insoluble in this menstruum was filtered out and the clear solution dialyzed until free from chlorides. The precipitate thus formed, preparation **6**, weighed 1.20 grams, and after drying was not soluble in water, but dissolved readily and nearly completely in salt solution, having, as was to be expected, the properties of a globulin. This substance had a very slight diastatic power, and its sodium chloride solution when heated slowly became turbid at 60°, a few flocks appearing at 65°, due to a trace of albumin. Its composition was as follows:

PREPARATION 6.

	Ash-free.
Carbon	53.11
Hydrogen	6.45
Nitrogen	15.78
Sulphur } Oxygen }	24.66
	100.00
Ash	0.75

The filtrate from preparation 6 still contained proteid matter which was separated by dialysis in alcohol. Preparation 7 was so obtained, weighing 1.54 grams, having the same properties as 6, and the following similar composition:

PREPARATION 7.

	Ash-free.
Carbon	53.58
Hydrogen	6.70
Nitrogen	15.87
Sulphur } Oxygen }	23.85
	100.00
Ash	1.43

After extracting precipitate I with water and salt solution a very considerable part still remained undissolved. This was treated with water to remove all the salt, and then with alcohol, and was dried over sulphuric acid. This preparation, 8, weighed 8 grams and was quite dark in color. It had the properties of an insoluble form of globulin, being dissolved in one-half per cent. sodium carbonate solution and precipitated therefrom by neutralization. Its composition was nearly the same as that of the two last globulin-like preparations and is probably a so-called "albuminate" derived from that substance. The composition of preparation 8 was:

PREPARATION 8.

	Ash-free.
Carbon	53.55
Hydrogen	7.01
Nitrogen	15.72
Sulphur	1.23
Oxygen	22.49
	100.00
Ash	1.09

Precipitate II was treated with water and the solution thus formed was dialyzed in water for several days and then in alcohol

for 24 hours. A quantity of absolute alcohol was finally added to the contents of the dialyzer, thus completely precipitating the proteid. This preparation after drying was almost wholly soluble in water, and when heated slowly its solution became turbid at 60° and deposited flocks at 66°. The amount of proteid thus coagulated was somewhat greater than was given by preparation **5,** and its diastatic power was likewise greater, being 75. Analysis showed its composition to be as follows:

PREPARATION **9.**

	Ash-free.
Carbon	53.19
Hydrogen	6.71
Nitrogen	16.74
Sulphur	1.38
Oxygen	21.98
	100.00
Ash	0.78

This preparation contained a slight amount of insoluble matter, some albumin and much proteose.

The residue of precipitate II, which was not dissolved by water, was treated with sodium chloride solution and the clear extract dialyzed till free from chlorides, but as no precipitate was produced, the dialyzer was transferred to alcohol when preparation **10** separated, weighing 0.49 gram, and containing, ash-free, 15.18 per cent. of nitrogen. It is probable that this is the same globulin obtained in larger quantity from precipitate I, but less pure. That part of precipitate II which remained undissolved after extracting with water and salt solution, was then washed thoroughly with water and with alcohol, yielding preparation **11,** which weighed 5.0 grams and had the following composition:

PREPARATION **11.**

	Ash-free.
Carbon	53.51
Hydrogen	6.75
Nitrogen	15.76
Sulphur	1.12
Oxygen	22.86
	100.00
Ash	0.66

These figures show that precipitate II contained less globulin and proportionately more leucosin and proteose than precipitate I and it was accordingly found to be more powerfully diastatic.

Precipitate III was in turn treated with water, the resulting extract filtered clear, dialyzed for several days in water and then in alcohol, absolute alcohol being finally added in quantity to the contents of the dialyzer. The resulting precipitate, preparation **12,** weighed 3.0 grams. It was almost completely soluble in water, and its solution when slowly heated became turbid at 55° and flocculent at 60°. The amount of this coagulum was much greater than that yielded by preparation **9.** The filtrate from the coagulum gave a strong proteose reaction. The diastatic power was 222, indicating the presence of much more diastase than any of the preceding preparations. Its composition was as follows:

PREPARATION **12.**

	Ash-free.
Carbon	52.80
Hydrogen	6.96
Nitrogen	16.09
Sulphur	1.45
Oxygen	22.70
	100.00
Ash	0.59

The residue of precipitate III was digested with salt solution, the filtered extract was dialyzed in water till free from chlorides, and then, as no proteids separated, the dialysis was continued in alcohol. Only 0.28 grams of proteid resulted, which, without correction for ash, contained 12.53 per cent. of nitrogen. This was marked preparation **13,** and considered to be impure globulin.

The part of precipitate III still undissolved was washed with water and with alcohol, yielding preparation **14,** which weighed 2.87 grams. This had the following composition:

PREPARATION **14.**

	Ash-free.
Carbon	53.25
Hydrogen	7.65
Nitrogen	16.12
Sulphur	1.38
Oxygen	21.60
	100.00
Ash	0.55

This preparation has a somewhat higher nitrogen and lower carbon content than preparations **8** and **11,** which is probably due to its being a mixture of the insoluble form of the globulin with

some insoluble albumin coagulated by the long contact with alcohol to which it had been subjected. This is to be expected, as precipitate III contained relatively more albumin than precipitates I and II.

Precipitate IV was next treated with water, the solution filtered clear, dialyzed for some days in water, and afterwards transferred to alcohol and the dialysis continued. Absolute alcohol was then added to the contents of the dialyzer, giving preparation **15**, weighing 4 grams. This substance dissolved in water to a nearly clear solution, which when filtered perfectly clear and heated carefully became turbid at 50° and gave a large coagulum at 56°. After heating the solution and filtering off the coagulum, a good reaction for proteose was obtained with the biuret test. This preparation had a diastatic power of 600. As this was a much more powerful ferment than any yet produced, its properties were carefully studied and will be described at length later. When analyzed this substance was found to have the following composition:

PREPARATION **15**.

	Ash-free.
Carbon	52.50
Hydrogen	6.72
Nitrogen	16.10
Sulphur	1.90
Oxygen	22.78
	100.00
Ash	0.66

It will be noticed that the sulphur in this preparation is a little higher than in the preceding preparations, which is probably due to its containing some sulphate.

The part of precipitate IV which did not dissolve in water was treated with salt solution, but no globulin was extracted. The residue was then washed with water, giving preparation **16**, which weighed 0.9 gram and had the following composition:

PREPARATION **16**.

	Ash-free.
Carbon	53.42
Hydrogen	7.15
Nitrogen	16.65
Sulphur, Oxygen	22.78
	100.00
Ash	0.24

The composition of this insoluble product shows it to be probably coagulated leucosin.

A portion of precipitate V, when treated with water, was found to dissolve completely. It was therefore washed with absolute alcohol, yielding preparation **17,** which weighed 2.87 grams. The clear solution of this substance when heated became turbid at 50°, and yielded a small coagulum at 58°. Boiled and filtered a strong pink coloration was given with the biuret test, thus showing it to consist mostly of proteose. The diastatic power of this substance was 60, only one-tenth that of preparation **15.** Its composition was:

PREPARATION **17.**

	Ash-free.
Carbon	51.21
Hydrogen	6.52
Nitrogen	15.40
Sulphur, Oxygen	26.87
	100.00
Ash	2.37

The lower nitrogen content of this preparation indicates that the strong alcohol had thrown down, together with the proteids, some non-nitrogenous substances.

Much is to be learned by studying these results which will be of service in future attempts to isolate pure diastase.

In the first place, it is plain that we have in our malt extract a globulin, an albumin and at least one, more probably, two, forms of proteose. I believe the substance soluble in salt solution to be a true globulin, since it so readily assumes an insoluble form, and also because a much larger quantity of the same body was obtained by extracting with ten per cent. salt solution, the malt-residue remaining after the extraction with water. I also think that at least two forms of proteose are present, for the water-soluble portion of precipitate I consisted chiefly of proteose, as did also precipitate V. The amount of proteose diminished from precipitate I to precipitate IV, which contained the least, while precipitate V, which, it will be remembered, was thrown down by adding to the solution a very large amount of absolute alcohol was mainly proteose. A part of the proteose was precipitated by alcohol more readily than the albumin, while another part was less readily precipitated. Beside the albumin, globulin and

proteoses, we have also to take account of the "albuminate" or insoluble forms of the albumin and globulin. The results of this extraction show that the globulin is rendered insoluble more rapidly than the albumin, so that precipitation with alcohol and solution in water, repeated a few times, may be depended upon to remove the former. Whether repeated fractional precipitation can be employed to completely separate the albumin from the proteoses is not so certain. The albumin is thrown down from the malt-extract by saturation with magnesium sulphate, and it is not unlikely that a complete separation can be accomplished by this reagent. It is, however, not to be forgotten that the diastase may be a substance which when heated to from 50°–60° splits apart into an albumin and a proteose, and that the proteose found in the solutions which have been heated is a decomposition product of the diastase. Kühne's attempts to produce pure trypsin led him to suspect that this ferment is a body which when heated yields a coagulable fraction and a proteose-like substance.

Now that we have some precise knowledge of the associated substances, it seems probable that we may succeed in obtaining diastase nearly if not quite pure, and arrive at a clearer and more positive knowledge of this ferment, and also have a guide in further study of other enzymes, which will lead to a more satisfactory understanding of this whole subject. It is probable that the ferments contained in seeds are much easier to prepare than those of animal origin, since the substances with which they are associated are largely non-proteid and comparatively easy to separate. It is also certain that the amylolytic ferments present an easier problem than the proteolytic, for the products of the activity of the latter are so similar, in their nature, to that which the ferment is supposed to possess, as to make it always a matter of great uncertainty whether the separated enzyme is free from those bodies or not.

As already stated, preparation **15** was a very energetic ferment, and on this account its properties were more fully studied, with the following results:

Dissolved in water this substance gave all the usual proteid reactions, and when heated slowly became turbid at 50° and gave a flocculent coagulum at 56°. This is exactly the temperature of coagulation of the albumin (leucosin) which I have prepared from wheat, rye, barley and malt, with identical composition and properties. The aqueous extracts of these grains have,

moreover, a strong diastatic action on starch. The amount of coagulable albumin in preparation **15** was determined and found to be 53.2 per cent. of the dry substance.

These facts point strongly to the albumin as being the diastatic substance, yet there are several facts, hard to explain if this is true, which cannot be overlooked. Although in general the diastatic power of my preparations was greater the larger the amount of coagulable albumin they contained, I have never yet been able to establish any numerical relations between the two. In no case have I found any diastatic action with solutions free from albumin. Furthermore, the activity of my preparation **15,** is such as to require a much greater diastatic power for malt than this shows if its coagulable albumin is the enzyme.

A malt extract corresponding to a solution of the diastase in five milligrams of malt had the same diastatic power as 0.02 milligram of preparation **15.** As the preparation contained but a little over 50 per cent. of coagulable albumin, this would correspond to only 0.01 milligram of albumin in the five milligrams of malt, or two-tenths per cent. The amount of albumin in malt is much greater than this, as it is also in wheat, rye and barley, whose diastatic power is greatly inferior to that of malt. It is not probable that the *separated* diastase is more active than that *in the seed*, especially in view of the experiments which follow, comparing the action of malt extract and preparation **15.** The only explanation of this that occurs to me, is that the active diastase is a combination of albumin with some other body, presumably the proteose, which breaks up on heating, yielding coagulated albumin, and that, besides this combined albumin, free albumin is also present, which has no diastatic power, but which is coagulated at the same time. There is no direct evidence, however, that this hypothesis is correct.

Compared with other so-called pure ferments, preparation **15** is very active. At 20° it was in a condition to produce, from soluble starch, over 2000 times its weight of maltose and a further undetermined quantity of dextrin, within one hour. After having been dried over sulphuric acid and kept for six months, its activity was reduced to one-half, but in this condition it produced in 17 hours, at 20°, 10,000 times its weight of maltose besides an unknown quantity of dextrin. At 45° the same quantity of maltose was produced in one hour as at 20°. At 50° much less and at 55° very little maltose was formed. These tests were made by using

an amount of diastase solution just sufficient to produce enough maltose at 20° to exactly reduce 5 c. c. of Fehling's solution.

Compared with malt extract of the same diastatic strength, as measured by the amount of maltose produced in one hour at 20°, the distilled water solutions of preparation **15** have a less powerful action in liquifying starch paste. Five c. c. of malt extract added to ten c. c. of a starch paste containing 2 per cent. of starch, dissolved the starch completely in eight minutes, while the solution of preparation **15** required thirty-seven minutes.

The malt extract is also more energetic in converting starch completely into bodies giving no color with iodine. Five c. c. of the same malt extract added to ten c. c. of soluble starch solution caused the blue reaction with iodine to disappear in thirteen minutes, while it required thirty-eight minutes to reach the same result with the solution of the separated diastase. When, however, the diastase was dissolved in malt extract, whose enzymes had been previously killed by heating, the difference between the separated diastase and that in the malt extract nearly disappeared.

Two test tubes were each charged with 10 c. c. of starch paste. To one tube was added 5 c. c. of fresh malt extract, and to the other the same amount of boiled and cooled malt extract in which had been dissolved a quantity of preparation **15**, sufficient to make a solution of the same sugar-producing power as the fresh malt extract itself.

The fresh malt extract liquefied the starch in seven minutes, the mixture of preparation **15** and boiled malt extract in fourteen minutes, while thirty-seven minutes were required to produce the same result with a distilled water solution of preparation **15**. In completely converting starch into bodies giving no color with iodine, the solution of preparation **15** in boiled malt extract gave exactly the same result as the fresh malt extract, showing that the difference first noticed was due to the conditions and not to the ferment.

In view of these results, it seems highly probable that diastase is a true proteid, for if we consider the extremely minute quantity of preparation **15** required to produce large amounts of maltose, it is hard to believe that this action is due to some substance adhering to the proteid to the extent of only three or four per cent. at the most. If such were the case it is also remarkable that the enzyme should adhere in so much greater quantity to

the particular precipitate represented by preparation **15** than to any of the other numerous fractions. If diastase, then, is to be considered as a true proteid, it is evidently either an albumin, a combination of an albumin with a proteose, or a proteose. We have seen that those fractional precipitates which consist largely or wholly of proteose have little or no diastatic action, amylolytic power being manifested most strongly in the fractions containing the most albumin, and least in those containing but little, though not in strict proportion to the amount of the albumin. It is to be concluded that the diastatic enzyme is most closely related to the albumin, named leucosin, and it is not improbable that further careful study will show more clearly what this relation is.