phides, left after washing with hydrochloric acid, in aqua regia, expel nearly all the acid, dilute, and add slight excess of tartaric acid, and then very great excess of sodium hydroxide. Boil and pass hydrogen sulphide through till no further precipitation occurs, filter immediately. Test the precipitate for cobalt by borax bead. The presence of nickel in the filtrate is indicated by its deep brown or black color. If nickel be absent the filtrate will be yellow or colorless.

If nickel be present add dilute hydrochloric acid to filtrate filter, and test the precipitate for nickel by borax bead.

The ammonium sulphide should be prepared as recently as possible, as an excess of sulphur in solution causes the solution of a portion of the nickel sulphide.

THE PROTEIDS OF BARLEY.[1]

By Thomas B. Osborne.

Received April 2, 1895.

THE proteids of barley have received little attention on the part of chemists. Mulder[2] 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[3] 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

[1] From the Report of the Connecticut Agricultural Experiment Station for 1894.

[2] Phys. Chem., I, 306–308.

[3] Die Getreidearten u. das Brod. Nürnberg, *1860*, p. 304.

results of which are given by Ritthausen.[1] 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

The extract made with seventy-five 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.

[1] Die Eiweisskoerper, etc., Bonn, 1872, p. 103.

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 ten 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 ten 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 ten 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 two and three-tenths 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 ten 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 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 four-tenths 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 twenty-eight liters of ten per cent. salt solution and seventeen liters of clear filtrate obtained, which was saturated with ammonium sulphate. The precipitate produced was dissolved as far as possible in ten 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 cc. 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 four and one-tenth 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.[1]	4.[1]	5.[2]	6.[3]	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 be compared with the leucosin[4] obtained from the wheat and rye kernels, it will be seen that the three are almost identical in composition.

[1] Not corrected for ash.
[2] Not corrected for ash; coagulated by alcohol.
[3] Coagulated by alcohol.
[4] Report of the Connecticut Agricultural Experiment Station, *1893*, p. 179.

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 ot 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 ten per cent. sodium chloride solution and heated, turbidity occurred at 90°, but no 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 ten per cent. salt solution, the filtered extract saturated with ammonium sulphate, and the resulting precipitate filtered out, dissolved in ten 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 ten 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 ten 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 ten per cent. salt solution and precipitated a second time by dialysis. One and nine-tenths 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.[1] 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

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

[1] Report Connecticut Agricultural Experiment Station, *1893*, pp. 179 and 216.

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 seventy-five per cent. After digesting a short time, the meal was squeezed out and again treated with seventy-five 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 nine-tenths 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 ten 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 nine-tenths specific gravity, 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 thirty 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 thirty 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 thirty-six 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 seven 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 ten 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 forty per cent. After squeezing out the liquid, alcohol was again added to the residual meal sufficient to increase the strength of the solvent to seventy-five 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 twenty-two 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 eighteen 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 some time 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 seventy-five per cent. alcohol and absolute alcohol was added until the precipitate began to reappear. The whole was heated until the precipitate again dissolved whereupon the solu-

tion 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 seventy-five per cent. alcohol, treated with absolute alcohol, 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, 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 seventy-five 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 seventy-five per cent. alcohol and allow 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 seventy-five per cent. alcohol, was treated in the usual manner and yielded preparation 22, weighing 1.56 grams 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 seventy-five 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 supernatent 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 two and

two-tenths 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 seventy-five 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 variations 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 seventy-five 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 eight 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 seventy-five 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 seventy-eight grams of a pure white preparation were obtained. Twenty-five grams of this were then dissolved in seventy-five 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 seventy-five 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 substance 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 seventy-five per cent. alcohol, and after standing some time the extract was filtered off and six 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 cc. of distilled water, the washings were poured off, and the mass of proteid dissolved in 500 cc. of seventy-five 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 seventy-five 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 cc. of absolute ether. Three or four cc. 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, ninety-three 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 cc. of alcohol of 0.865 sp. gr. by heating on a water-bath, and the solution was quickly cooled. After adding a few drops of ten 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 sp. gr., 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.

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 1870 by Proust[1] and ten years later by Hermbstädt[2] to certain products of their attempts to isolate the proximate principles of this cereal.

Hordein appears to have been obtained nearly pure from bar-

[1] Ann. chim. phys., 5, 337.
[2] J. tech. Chem., 12, 46.

ley 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 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 seventy-five 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 and a 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 six 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 ninety-three 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 two liters of hot seventy-five 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. Twenty and two-tenths 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 four 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 five per cent. salt solution and then, repeatedly, with hot seventy-five per cent. alcohol. The residue, washed with absolute alcohol and thoroughly air-dried, weighed seventy-one 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 seventeen 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 three-tenths 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 solution 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 seventy-five per cent. This is the barley proteid described by Ritthausen as mucedin. It has almost 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 four 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 forty-two per cent. of the total nitrogen, corresponding to proteid-matter equal to about four and five-tenths per cent. of the flour. It was not possible to extract more than a very small amount of this residual pro-

teid 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 be assumed that this all belonged to proteid-matter with seventeen per cent. of nitrogen, the flour would contain 10.75 per cent. of proteids. The barley accordingly contained about four and a half per cent. of insoluble proteid, four per cent. of hordein soluble in dilute alcohol, three-tenths per cent. albumin, and 1.95 per cent. of globulin and proteose.

THE DETERMINATION OF NITROGEN IN FERTILIZERS CONTAINING NITRATES.[1]

BY H. C. SHERMAN.

Received April 13, 1895.

AS soon as the accuracy of the Kjeldahl method for the determination of nitrogen was generally recognized, attention was turned toward the discovery of some simple modification by which it could be made applicable in the presence of nitrates.

Asboth (*Chem. Centrbl., 1886*) recommended the simple addition of benzoic acid to the decomposing mixture. It was soon found that this method was not sufficient.

The following year, two methods were brought before the Association of Official Agricultural Chemists, one by Mr. Scovell, the other by Mr. Farrington. The principal difference consisted in the use of salicylic acid with the sulphuric acid to fix the nitrogen oxides by the former, while phenol was used for the same purpose by the latter. Both used zinc dust as the reducing agent. The Scovell method was adopted and remains practically unchanged.

In 1890, the Association sanctioned the use of zinc sulphide as a reducing agent. In case of its use the acid mixture was to contain one gram of salicylic acid instead of two. The use of zinc dust with two grams salicylic acid was retained.

In 1892, sodium thiosulphate, which had been used for reducing nitrates in the Ruffle method, was adopted as a third reducing agent, and it was directed that five grams of the crystalized salt should be added "direct."

[1] Read before the Washington Section.