THE PROTEIDS OF THE RYE KERNEL.[1]

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

Einhof, who in 1805[2] 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[3] 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

[1] From the report of the Connecticut Agricultural Experiment Station for 1894. Communicated by the author.

[2] *J. d. Chem. v. Gehlen*, 5, 131.

[3] *Ann. d. Chem. u. Pharm.*, 45, 195.

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."

Jones[1] 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,[2] 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[3] 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 the same as those of wheat.

Rittenhausen[4] 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

1 *Ann. d. Chem. u. Pharm.*, 40, 66.
2 *Ann. d. Chem. u. Pharm.*, 58, 319.
3 Die Getreidearten u. das Brod, Nuernberg, 1860, 291.
4 *J. prakt Chem.*, 99, 439, and Die Eiweisskoerper, etc., Bonn, 1872, p. 83.

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 eighty-five per cent. and concentrating and cooling the solution. The deposited substance was further 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[1] states that wheat, rye, and barley contain a globulin substance similar in properties to animal myosin, being soluble in ten to fifteen 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

[1] *Jour. Physiol.*, 8, viii.

but soluble in saline solutions; C, proteids insoluble in water and in saline solutions but soluble in alcohol; and, D, proteids 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 ten per cent. sodium chloride solution from which subsequently the soluble salts have been removed 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[1] was accordingly exhausted with a solution containing ten 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

[1] 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.

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			

Another extract was examined in a slightly different way. 1,000 grams of rye-meal were extracted with eleven liters of ten 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 ten 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 twenty 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 twenty 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, 2,000 grams of rye-meal were treated with ten per cent. sodium chloride solution and the extract filtered and saturated with ammonium sulphate. The precipitate produced was dissolved in ten 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 1,700 grams of rye-meal with sixteen 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 three liters of ten 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 eight 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 ten 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 grams. 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 ten 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 ANALYSIS 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, Oxygen	24.40	23.32	22.82	23.40	1.40 22.23	1.29 22.56	1.35 22.23
	100.00	100.00	100.00	100.00	100.00	100.00	100.00

Five hundred grams of rye flour[1] were extracted with 2,000 cc. of five per cent. sodium chloride solution and 1,000 cc. of the clear filtered extract were dialyzed till free from chlorides. The solution was then filtered and heated for twenty-four 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.[2] 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. Average of five analyses.	Rye. Averave of six 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.

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

[1] Made by the Health Food Company of New York.

[2] *Am. Chem. J.*, 15, 408; also Ann. Report Conn. Expt. Station for 1893, p. 179.

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: 5,000 grams of rye flour, made by the Health Food Co. of New York, were extracted with fifteen liters of five per cent. sodium chloride solution and the extract filtered clear. Nine liters of extract were thus obtained, being approximately equal to a complete extraction of three 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.	Average of five analyses.
Carbon	51.03	51.19	51.03
Hydrogen	6.72	6.74	6.85
Nitrogen	18.14	18.19	18.39
Sulphur		23.88	0.69
Oxygen			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,[1] but owing to the difficulties encountered in preparing it from rye, further evidence on this point was not obtained.

With reference to Martin's statement concerning the presence of myosin—what is written on that point in the paper by Osborne

[1] Annual Report Conn. Expt. Station for 1893, pp. 179 and 216.

and Voorhees on the "Proteids of the Wheat Kernel" applies equally to rye.[1]

C. PROTEID SOLUBLE IN ALCOHOL. GLIADIN.

After extraction with sodium chloride solution, alcohol of seventy-five to eighty per cent. takes up a considerable quantity of proteid. One hundred grams of rye-meal were extracted thoroughly with ten per cent. sodium chloride solution and then with seventy-five 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 four per cent. of the meal. Two thousand 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 seventy-five 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 seventeen 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 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 con-

[1] *Am. Chem. J.*, 15, 415.

siderable quantity of proteid. This was precipitated by adding a few drops 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
				100.00
Ash	0.12			

One thousand grams of rye-meal were thoroughly extracted with ten 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 seventy-five 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	1.19	....	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[1] states to be freely soluble in fifty per cent. alcohol, the following method was tried:

After extracting rye-meal with ten per cent. sodium chloride

[1] Die Eiweisskoerper, etc., Bonn, 1872, p. 96, and *J. prakt. Chem.*, 99, 454, and 102, 321.

brine, the residue was treated with alcohol so strong that with the water retained by the meal, a mixture resulted containing about seventy-five 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 dissolved in seventy-five 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 seventy-five per cent. alcohol and the extract filtered clear, concentrated to one-fourth its volume, cooled, the precipitated proteid again dissolved in seventy-five 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 three thousand grams of rye flour directly with seventy-five 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 deposited 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 twenty-four hours and dried over sulphuric acid. This preparation, 17, weighed fifty-eight grams, and was perfectly white. It formed very nearly two 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. Four thousand grams of rye-meal were thoroughly extracted with ten 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 seventy-five 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 seventy-five per cent. alcohol, filtered clear, and pre-

cipitated 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[1]	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			

[1] Omitted in average.

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.

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[1] 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-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 seventy 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 in 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 five per cent. sodium chloride solution and then with seventy-five per cent. alcohol. The residue was then thoroughly air-dried and found to weigh seventy-eight grams. This residue contained 0.55 per cent. of nitrogen. The 100 grams of flour therefore con-

[1] *Am. Chem. J.*, 15, 436.

tained 1.52 grams of nitrogen of which 0.43 gram remained after extraction and 1.09 grams were 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 comparitive 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 be said now 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 four 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, soluble in water	0.43	"
Edestin and Proteose, soluble in salt solution	1.76	"
	8.63	"

ZIRCONIUM SULPHITE.

BY F. P. VENABLE AND CHARLES BASKERVILLE.

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VERY little is recorded in the text-books on chemistry with regard to this compound of zirconium. Berthier is reported as having examined it and found it to be a white insoluble body, slightly soluble, however, in an aqueous solution of sulphurous acid, from which it is thrown down again upon boiling. Whether this was what is commonly known as the neutral, or the acid, or a basic sulphite, is not recorded. It is highly probable that with so weak an acid as sulphurous acid, zirconium would form under these circumstances only basic compounds. We may state with regard to our own work that we have been unable with one exception to form any sulphite corresponding to the acid or the neutral. Only very indefinite compounds or mixtures of the sulphite with the hydroxide have come into our hands, as a rule.

The subject was first brought to our attention by the study of the reaction utilized by Baskerville for the quantitative separation of zirconium from iron and aluminum.[1] It was also put into use by him for shortening the method of preparing the pure zirconium chlorides.[2] The reaction in question is that which takes place when a nearly neutral solution of zirconium chloride is boiled with sulphur dioxide in excess.

Several points of interest were observed as to this reaction. It was found that when a solution of the sulphate was used it was difficult to secure any precipitation by means of sulphur dioxide even with persistent boiling. The chloride was clearly

[1] THIS JOURNAL, 16, 475.

[2] J. Elisha Mitchell, Scientific Society, 11, 85.