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THE CHEMISTRY OF WHEAT GLUTEN.

BY

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By George G. Nasmith, B.A.

UNIVERSITY OF TORONTO, June 1st, 1903.

To the Registrar,
University of Toronto

SIR,

I beg to report that Mr. George G. Nasmith has passed satisfactorily the examination in Physiological Chemistry, his major subject for the degree of Doctor of Philosophy.

I beg to report also that Mr. Nasmith's dissertation, "The Chemistry of Wheat Gluten," is of distinction as a contribution to the knowledge of the subject and I recommend that it be accepted for the degree of Doctor of Philosophy.

A. B. MACALLUM,
Professor of Physiology.

I HEREBY certify that the thesis above mentioned has been accepted by the Senate of the University of Toronto, for the degree of Doctor of Philosophy in accordance with the terms of the Statute in that behalf.

JAS. BREBNER,
Registrar.

UNIVERSITY OF TORONTO, June 5th, 1903.

THE CHEMISTRY OF WHEAT GLUTEN.

BY GEO. G. NASMITH, B.A.

I.—HISTORICAL.

THE first preparation of gluten from wheat flour by washing away the starch from dough seems to have been made by Becari,[1] but Einhof[2] was the first to give special attention to its composition. He extracted wheat gluten with dilute alcohol, and he found that the substance which precipitated on cooling, diluting or concentrating the solution was practically identical with gluten itself.

Taddei[3] named the portion soluble in alcohol gliadin, the residue zymom.

Berzelius[4] thought that he found a second constituent in the part of the gluten soluble in alcohol, which he called mucin, and which was precipitated by acetic acid. He[5] regarded Taddei's gliadin as identical with the substance obtained by Einhof from wheat, barley and rye. The insoluble residue Berzelius called plant albumin, from its great similarity to animal albumin.

De Saussure[6] found that wheat gluten contained about 20 per cent. plant gelatin, or glutin, as he proposed to call it, 72 per cent. insoluble plant albumin, and 1 per cent. mucin; the latter, although differently prepared, he considered to be similar to the mucin of Berzelius, and it had, as he thought, the power of transforming starch into sugar.

Boussingault,[7] like Einhof, considered that part of the gluten soluble in alcohol to be identical with the entire gluten proteid.

Liebig[8] named the portion of the gluten insoluble in alcohol plant

fibrin ; he rejected the term zymom given by Taddei, and also that of plant albumin of Berzelius, in the latter case because solubility in water is a characteristic of albumins. The portion soluble in alcohol he called plant gelatin, and considered it to be a casein-like compound of a proteid with an undetermined organic acid.

Bouchardat[9] found in gluten a substance soluble in extremely dilute acid, which he named albumin, since he regarded it as forming the chief constituent of egg albumin, blood fibrin, casein and gluten.

Dumas and Cahours[10] found four proteids in flour, namely, an albumin which was obtained from the water used in washing out the gluten ; plant fibrin left as a residue on extracting gluten with alcohol ; a proteid from this alcohol which separated on cooling, and finally a second proteid which precipitates from the same alcohol on concentration and cooling. This latter he called glutin.

Mulder[11] prepared plant gelatin by extracting gluten with alcohol, filtering hot, allowing to cool and redissolving the white precipitate which settled out twice. This he considered to be a compound of sulphur with protein, and he found that it did not contain phosphorus.

Von Bibra[12] stated that on exhausting gluten with hot alcohol insoluble plant fibrin remained behind, while plant gelatin and plant casein dissolved ; the plant casein separated on cooling. These bodies he thought had the same elementary composition, and were in fact isomers.

Günsberg[13] held that gluten was composed of three proteids, gliadin being a mixture of two. These were, (a) gluten fibrin, soluble neither in alcohol nor warm water ; (b) gluten casein, insoluble in hot water but soluble in alcohol ; (c) gluten gelatin, soluble in alcohol and hot water.

Ritthausen[14] found four proteids in gluten, namely, gluten casein, gluten fibrin, plant gelatin or gliadin, and mucedin, of which the last three are soluble in dilute alcohol. His casein was prepared by extracting gluten with boiling alcohol, cooling, exhausting the casein which settled out with absolute alcohol, then with acetic acid, and finally neutralizing the clear filtrate from this with ammonia. The decanted alcoholic fluid from the casein contained the gelatin, which separated on evaporation.

Scherer[15] digested gluten with artificial gastric juice and observed that the greater part went into solution in about fourteen hours.

Martin[16] found that only one proteid was extracted from gluten by

dilute alcohol or hot water, which gave the reddish violet reaction of proteoses and peptones. Because of this reaction and its comparative insolubility he called it insoluble phytalbumose. The residue was coagulated by boiling water, and was soluble only in acids and alkalies. He claimed that dilute alcohol extracted only fat from dry flour, and came to the conclusion that insoluble phytalbumose was produced from a soluble albumose, and gluten fibrin from a globulin by pre-existing ferments.

Chittenden and Smith[17] made preparations of gluten casein according to Ritthausen's method, which averaged 15.86 per cent. of nitrogen.

Osborne and Voorhees[18] in an exhaustive research brought many opposing views into harmony. Like Martin they found only one proteid in gluten that was soluble in alcohol, and considered that the various proteids claimed by previous investigators to have been soluble in alcohol were impure preparations, perhaps mixtures with fat. Martin's gluten fibrin they termed glutenin, and found its composition to be practically identical with that of gliadin, a conclusion that had not hitherto been suggested. The high percentage of nitrogen they thought due to their improved method of preparation by which all starch, etc., had been removed. Contrary to Martin's experience they found that dilute alcohol extracted gliadin directly from flour.

Osborne and Voorhees further arrived at the conclusion that gluten is made up of two forms of the same proteid, one being soluble in cold dilute alcohol and the other not. They found that flour exhausted with sodium chloride solution yielded the same amount of gliadin as was obtained from the gluten made from an equal quantity of flour, or by direct extraction of the flour with 70 per cent. alcohol. They, therefore, held that gliadin exists as such in the seed.

Teller[19] noted again the fact that gliadin possessed proteose-like characters, as previously stated by Martin. Gliadin he found to be slightly soluble in dilute salt solution, and he regarded it as identical with that body classified by Osborne and Voorhees as proteose.

O'Brien[20] found himself in agreement with Osborne and Voorhees in considering that gluten pre-existed as such in flour in the same proportions as in gluten, and that there was but one mother substance in flour which gave rise by a process of hydration to gluten. His conclusions were, (*a*) that the differently described derivatives of gluten soluble in alcohol merge into one another; (*b*) that the portion soluble in alcohol may be made to pass into the insoluble stage; (*c*) that a proteose is readily formed as a secondary product from gluten.

Fleurent,[21] whose work, like that of O'Brien, was unfinished when Osborne and Voorhees' results were published, found himself also in agreement with the latter in concluding that only one body soluble in alcohol was present in gluten. Besides gliadin and glutenin he found a very small quantity of a third body which he called conglutin.

Morishima[22] found only one proteid in gluten (that he named artolin) which he prepared by kneading gluten with dilute alkali and treating the decanted fluid with hydrochloric acid until it contained one per cent. of the acid in excess. The precipitate washed with one per cent. hydrochloric acid and made up with alcohol to a solution containing 70-80 per cent. of alcohol, was filtered and saturated with absolute alcohol and ether.

Its composition he found to be:

ARTOLIN (MORISHIMA).

C	52.29
H	7.02
N	16.51
S	.74
O	
Cl	1.57

Mayer[23] held that the old name of plant gelatin was inappropriate, since it was chemically unlike gelatin; the latter does not contain sulphur, and is insoluble in dilute alcohol.

Ritthausen[24] commented on Fleurent's and Osborne and Voorhees' careful work, but, nevertheless, clung to the views propounded in his early paper. In criticizing the work of Morishima, he pointed out the fact that proteids are soluble in dilute acids, and concluded that artolin was glutenin linked with hydrochloric acid.

The subjoined analyses, taken from the paper of Osborne and Voorhees were found by reference to the original papers to be accurate:

	BOUSSINGAULT.	JONES.	DUMAS and CAHOURS.		MULDER.	VON BIBRA.
	Gliadin.	Plant. Gelatin.	Casein.	Glutin.	Plant. Gelatin.	Plant. Gelatin.
C	52.30	54.44	53.46	53.27	54.85	53.57
H	6.50	7.42	7.13	7.17	7.05	7.12
N	18.90	15.98	16.04	15.94	15.71	15.57
S	....	....	....	....	.60	.88
O	22.30	22.16	23.37	23.62	21.79	22.86
	100.00	100.00	100.00	100.00	100.00	100.00

	Gunsberg.	Ritthausen			Osborne and Voorhees.
	Plant. Gelatin.	Gluten fibrin.	Plant. Gelatin.	Mucedin.	Gliadin.
C..... ..	52.68—52.65	54.31	52.76	54.11	52.72
H.....	6.77— 6.88	7.18	7.10	6.90	6.86
S.....	.....	1.01	.85	.88	1.14
N.....	17.76—17.45	16.89	18.01	16.83	17.66
O...	22.79—23.02	20.61	21.08	21.48	21.62
	100.00–100.00	100.00	100.00	100.00	100.00

GLUTENIN.

	Jones.	Scherer.	Dumas and Cahours.	Von Bibra.	Ritthauseen.
C.....	52.79	54.60—52.34	53.37—53.23	55.57	52.94
H	7.02	7.45— 7.13	7.02— 7.01	6.95	7.04
N	15.59	15.81—15.36	16.00—16.41	15.70	17.14
S.....	....	.....	.....	1.02	.96
O	24.62	22.14—25.17	23.64—23.35	22.76	21.92
	100.00	100.00–100.00	100.00–100.00	100.00	100.00

	Chittenden and Smith.	Osborne and Voorhees.
C.....	52.87	52.34
H.....	6.99	6.83
N.....	15.86	17.49
S	1.17	1.08
O.....	23.11	22.26
	100.00	100.00

II.—OBSERVATIONS.

While working at the composition of wheat flour, Professor Macallum suggested that I should trace to its source the phosphorous which he found to be present in the cellular elements of the wheat grain. It was sought for and found in gluten, no matter how carefully prepared or how long it had been washed in tap or distilled water. Gluten was prepared in the usual manner by kneading dough in a stream of water until free from starch, dried at 110 C. until the weight was constant and the phosphorus estimated according to Neumann's[25] method, which was found by experiment on known solutions of phosphoric acid to be perfectly accurate. Two quantities of gluten yielded 0.11 and 0.12 per cent. of phosphorus respectively.

In order to determine next which of the constituents of gluten (*i.e.*, gliadin or glutenin) contained phosphorus, gliadin was prepared by

extracting starch-free gluten with 70 per cent. alcohol, filtering the solution repeatedly, and afterwards evaporating completely to dryness.

Average of five estimations 0.83 per cent. ash.
" two " 0.29 " phosphorus.

Gliadin was prepared by extracting gluten with 70 per cent. alcohol, filtering and diluting with twice its volume of 1 per cent. sodium chloride solution; the white precipitate, separating out, was collected, washed with distilled water, till free from chlorine, and dried at 110 C. The analyses gave :—

	I.	II.	III.	IV.
Phosphorus.....	0.19	0.19	0.18	.....
Ash.....	0.205	0.201	.....	.....
Nitrogen	17.705	17.435	17.64	17.555

The ash from these was dissolved with hydrochloric acid ; the solution evaporated almost to dryness in a platinum crucible, was diluted with distilled water, and treated with a quantity of dilute hydrochloric acid containing also potassium ferrocyanide. A blue colouration immediately indicated the presence of iron ; repeated trials invariably yielded the same result.

In order to determine whether the iron was organic or inorganic, a solution of gliadin in ammonia-free distilled water was added to a solution of hæmatoxylin. No darkening whatever occurred, showing that the iron must be organically combined. Inorganic iron salts with hæmatoxylin give an intense dark blue colour. Pieces of freshly-prepared gliadin, suspended in hæmatoxylin, gave no reaction in thirty hours. The iron, like the phosphorus, must be in organic combination.

Previously to this I had found that on digesting gluten with artificial gastric juice, and repeatedly renewing the fluid, a part remained insoluble even after two months. This residue, after extracting with absolute alcohol and ether, was dissolved in 0.2 per cent. sodium hydrate, and precipitated by 0.2 per cent. hydrochloric acid, the precipitate being insoluble in excess of the acid. Evidently this was a nuclein, and must have come from the gliadin or glutenin of the gluten.

A gram of gliadin, purified by precipitating, dissolving, reprecipitating, and extracting with absolute alcohol and ether, was digested with artificial gastric juice at 38°C. A residue remained which gave all the reactions for nuclein, and undoubted reactions also for organic iron and phosphorus.

A large amount of gliadin was now prepared by extracting gluten with 70 per cent. alcohol, filtering, concentrating to a small quantity, precipitating with 95 per cent. alcohol, extracting in the Soxhlet apparatus for sixteen hours with absolute alcohol to remove fat and lecithin, and finally drying for three hours at 110°C

Analyses gave the following :—

GLIADIN.

C.....	52.39	Av. 6.
H.....	6.84	Av. 6.
N.....	17.46	Av. 2.
S.....	1.12	Av. 2.
O.....	21.89	
P.....	0.267	Av. 2.
Fe	0.034	Av. 2.
	100.00	

The iron was determined gravimetrically since the amount was so small that only a few drops of 1/40 normal solution of potassium permanganate were necessary by the volumetric method, and the exact end point was consequently difficult to determine. Taking all necessary precautions to eliminate aluminium and calcium, results were obtained by extracting the iron from the ash, which were concordant with those obtained from the filtrate after precipitating the phosphorus as ammonium phospho-molybdate. The weight of ferric oxide seldom exceeded 0.6 milligram. The analyses in other respects agree very well with those of Osborne and Voorhees, except that the carbon and nitrogen contents are slightly lower, and that they obtained no phosphorus.

A large quantity of gliadin was prepared and digested at 38°C. with artificial gastric juice in litre flasks. Digestion was continued for three weeks, the flasks being frequently shaken, and the clear supernatant fluid renewed several times. The considerable residue was collected on filters, washed free from proteoses and peptones with water, then with 70–95 per cent. alcohol which removed some fat. The residue dissolved in 0.2 per cent. sodium hydrate solution, was filtered, and the solution precipitated with excess of dilute hydrochloric acid, the process of solution and precipitation being repeated several times; the precipitate was then collected on "hardened" filters and washed with distilled water till free from chlorides. Extracted with absolute alcohol in the Soxhlet apparatus for sixteen hours, dried at 110 C., and analyzed, the residue yielded the following results :—

GLIADIN NUCLEIN.

C.....	49.47	per cent.	Av. 2.
H.....	6.98	"	Av. 2.
N	16.60	"	Av. 2.
S.....	0.80	"	One.
P.....	0.29	"	Av. 2.
Fe	0.04	"	Av. 2.
O.....	25.82		
	100.00		

Ash ..... 0.24 per cent. Av. 2.

The amount of phosphorus was very small, practically the same, in fact, as the gliadin from which it was prepared. The chemicals used were carefully tested in blank experiments, but no trace of phosphorus was found in any of them. Possibly the prolonged digestion with frequent renewals of hydrochloric acid solution had removed some of the phosphorus. The result was, however, quite unsatisfactory, since it was to be expected that the amount of phosphorus and iron would be much greater than in the substance from which it was derived. The analyses of the gliadin and the nuclein, derived from it, may be compared side by side :—

	Gliadin.	Gliadin Nuclein.
C.....	52.39	49.47
H	6.84	6.98
N.....	17.47	16.60
S	1.12	0.80
O.....	21.89	25.82
P.....	0.267	0.29
Fe	0.034	0.04
	100.00	100.00

From this it may be gathered that the two compounds are quite distinct chemically as well as physically.

Glutenin was prepared, as recommended by Osborne and Voorhees[18] by extracting all the gliadin from gluten by dilute alcohol, dissolving the residue in 0.2 per cent. potassic hydrate, and precipitating by exactly neutralizing with 0.2 per cent. hydrochlorie acid; the precipitate washed with 70–95 per cent. alcohol, was again dissolved in 0.2 per cent. potassic hydrate and filtered perfectly clear through heavy filter paper in an ice chest. Precipitated from the solution by exact neutralization with 0.2 per cent. hydrochloric acid, washed with distilled water till free from chlorides, then with 70-95 per cent. alcohol, extracted

in the Soxhlet apparatus for ten hours with absolute alcohol, and dried for three hours at 110 C., the glutenin so prepared gave on analysis the following :—

GLUTENIN.

	NASMITH.	OSBORNE.
C	52.75	52.34
H	7.22	6.83
N	16.15	17.49
S	1.06	1.08
O	22.58	22.26
P	0.215	....
Fe	0.026	....
	100.00	100.00

Ash 0.188 per cent.

Another preparation by Fleurent's method[21] which was also carefully filtered, yielded 16.55 per cent. nitrogen. The figures are not at all in agreement with those of Osborne and Voorhees for this compound. Mine are considerably higher in carbon and hydrogen, and much lower in nitrogen, a result which might be accounted for by carbohydrate impurity. Since, however, it was prepared exactly as described by him this seems unlikely. The fact that the amount of iron and phosphorus is practically the same as in gliadin at once suggested the possibility of these elements being derived from a certain amount of nuclein mechanically carried along with these compounds in the attempted purification process.

Impure glutenin was digested with pepsin and hydrochloric acid, but the insoluble residue was so difficult to separate from soluble starch, and was so evidently impure that the complete analysis was not made, though the presence of iron and phosphorus in it was demonstrated.

In repeating the work of Morishima[22] a copious precipitate as usual occurred at the neutral point, but when more acid was added nearly all went into solution ; after twenty-four hours only a trace of precipitate settled out. Glutenin has again and again been shown to be soluble in dilute acids. Artolin, as I found, is derived from another source than Morishina supposed. A 0.4 per cent. hydrochloric acid extract of flour was made, filtered perfectly clear and potassic hydrate added until neutral, when a precipitate was thrown down, which proved to be nearly all gliadin. If to this 0.4 per cent. hydrochloric acid extract more acid was added, a precipitate began to appear which increased with the acidity. This in large part separated on heating, and it proved entirely soluble in 70-80 per cent. alcohol, the result showing it to be gliadin.

This property of gliadin, of being precipitated with excess of acid, has not, I think, been hitherto noted. Since the compound of Morishima was prepared in practically the same way artolin is evidently gliadin in acid combination. Glutenin remains in solution. The body obtained under these circumstances by Morishima would perhaps correspond to a proteid salt,[35] *e.g.*, a chloride of gliadin. I obtained the substance called conglutin by Fleurent[21] but in quantity insufficient for analysis.

In order to decide whether the iron and phosphorus in gliadin and glutenin were actually in molecular combination in these compounds, resource was had to the microscope. Grains of Manitoba hard wheat were imbedded in celloidin and sectioned. Macallum's methods for determination of iron[26],[27] and phosphorus[28] were used.

For iron the celloidin was removed by equal parts of alcohol and ether, the sections passed through absolute alcohol and inorganic iron salts removed by 2.5 per cent. hydrochloric acid in 95 per cent. alcohol Sections so treated showed no trace of colour with pure hæmatoxylin in aqueous solution (0.5 per cent.) after the lapse of thirty minutes. The sections now placed in sulphuric acid alcohol (4 vols. acid, 100 alcohol) at 40° C. were removed at intervals of half hours ; on washing out the acid, and placing in hæmatoxylin, the sections gave a marked reaction for iron, the organic iron combination having been broken up and the inorganic iron salt formed retained in sitû.

Sections unextracted by hydrochloric acid showed much inorganic iron in the aleuron layer and germ. When this had been removed by hydrochloric acid no colour whatever appeared after standing for twenty minutes in hæmatoxylin solution. After treatment with sulphuric acid alcohol the nuclei of the aleuron and large parenchymatous endosperm cells were stained with hæmatoxylin purplish blue-black. The aleuron cell contents gave no reaction, nor did the proteid matter of the endosperm, which constitutes gluten. Gliadin and glutenin, therefore, do not contain iron in their molecules, and that present must have been derived from the nuclei of the cells of the endosperm and aleuron layer, and possibly in small amounts from embryo cells.

The distribution of iron in the embryo, or germ, is a point of interest. The closely packed cells of the embryo each contained a large nucleus coloured with hæmatoxylin almost black. In the rapidly dividing cells of the radicle and plumule a diffused purplish blue-black reaction occurred, which under the highest power could not be identified with any definite granules or structures. Some of the cells, other than those in a

rapid state of division, gave a faint purplish reaction, perhaps from iron derived by diffusion from the nucleus.

In order to show the distribution of organic phosphorus the inorganic phosphates were first removed by soaking for half an hour in acetic acid alcohol. Sections removed at the end of this time, placed for a few minutes in the nitric-molybdate solution, and then in one per cent. solution of phenylhydrazine hydrochloride showed no trace of green colouration, this fact indicating that all inorganic phosphates had been removed.

Such extracted sections were now placed in nitric-molybdate solution at 35° C. and removed in series at intervals of half an hour. When placed in a solution of phenylhydrazine hydrochloride for a few minutes, they showed a green colour, which increased in depth with the times, during which the section remained in the molybdate solution. In twenty hours the aleuron layer and embryo were stained a bright green. Sections which had had the celloidin removed by alcohol and ether, and which were subsequently extracted with absolute alcohol in the Soxhlet apparatus for several hours, gave exactly the same reactions as those unextracted. Consequently lecithin could not have been present. The aleuron cells in such preparations showed a large nucleus of a much deeper green than the rest of the cells, and under the high power the colour was seen to be confined to the spaces between the aleuron grains, the coloured parts appearing in the form of a network. The network had a more or less punctated appearance, the grains themselves were perfectly colourless.

In the endosperm of such preparations the nuclei alone were coloured, though sometimes, after twenty-four hours, the proteid matter packed between the starch grains, and even the cellulose gave the phosphorus reaction. Possibly phosphorus had diffused from the nuclei. The manner in which the phosphorus is distributed in the different types of embryo cells is quite varied. The palisade-like absorption cells between the endosperm and embryo appeared finely granular and of a uniformly dark green tint. The cytoplasm of the radicle and plumule cells were of a finely granular character, and gave the phosphorus reaction. Around these tightly packed cells of the radicle and plumule were other cells much more loosely connected, whose contents appeared vesiculated. The intercellular material gave a faint phosphorus reaction, while the large granular nucleus was much darker and very prominent.

Between these vesiculated cells and the absorption tissue of the embryo were large cells loosely bound together. These cells, even under the low power, were very different from the others, containing large, well-separated granules, coloured a bright green. Under the high power these granules appeared round, angular, or often crescent-shaped. In very thin sections they were quite separated from one another and very brilliantly coloured; in thin sections the nucleus often was not apparent. In thicker sections these granules were seen to be connected, forming a loose kind of meshwork, the spaces between being filled with a finely granular substance, giving a faint but distinct phosphorus reaction. When treated for the iron reaction a very faint violet tinge appears in these cells, but only between the bodies which stain so brightly for phosphorus.

From this it seems that, with the exception of the rapidly dividing cells such as those of the radicle and plumule, iron is found in the nuclei only of the various cells of the wheat grain.

Phosphorus is more widely distributed, appearing between the aleuron grains; in fine grains in the radicle and plumule cells; in the foam-like mesh work of another type of embryo cell; in the very distinct large granules just described, and in the nuclei of all these cells. From the various ways in which these different cells stain, and the several methods of phosphorus distribution in them, one may conclude that there are probably several nucleins present.

Osborne and Campbell[29] extracted wheat germ with petroleum naphtha, ground the residue to a fine flour, extracted this with water, saturated the clear filtrate with sodium chloride, and subjected the resulting precipitate to a vigorous peptic digestion. The nuclein so prepared, they conclude, "is not an original constituent of the extract nor of the cells of the embryo, but results through several molecules of nucleic acid with one of Protein." To this nuclein, washed with water and dissolved in dilute potassic hydrate solution, was added hydrochloric acid until a precipitate formed, which readily separated. When this was filtered off a considerable excess of hydrochloric acid was further added to the filtrate, whereupon a precipitate of nucleic acid separated out which became so dense and brittle that it could be ground under water.

This operation, as described, I repeated, but a small quantity only of nucleic acid was obtained, which, however, did not become brittle under water. As I expected, the ash of this nucleic acid and of the

nuclein, also prepared, gave distinct reactions for iron, even after standing for several weeks under dilute hydrochloric acid, a fact unnoticed by Osborne, and showing that part at least of his nuclein had come from the nuclei of the cells. If this nuclein had been derived from the nuclei of the embryo cells, it must have contained iron, since, as above demonstrated, its presence is invariable in the nucleus. Probably his nuclein was derived both from nuclei and ground substance of the cells.

It may then probably be admitted that the phosphorus and iron invariably found in gliadin and glutenin, no matter how carefully they have been prepared, are present in the form of nuclein or nucleic acid, which have been derived from the nuclei of the parenchymatous endosperm cells chiefly, and carried with them in the purification process. Perhaps aleuron and embryo cells imperfctly separated in the milling process contribute part of them.

III.—Properties of Gliadin.

Gliadin extracted directly from raw flour by dilute alcohol is always contaminated with fat, which gives to its solution a yellow tinge. On diluting this solution with an equal volume of sodium chloride solution, a snow-white precipitate separates, which, if the dilution is sufficient, collects into brownish floculent masses, and either rises or sinks, according to the strength of the salt solutions. Prepared in this way gliadin is exceedingly viscid, adhering to everything with which it comes in contact. When precipitated by water alone, gliadin will not readily separate. Evaporation of the alcoholic solution and cooling cause a considerable gummy mass of gliadin to separate, while a few drops of sulphuric acid to the supernatant fluid throws down almost all of the gliadin left in solution.

A solution of gliadin evaporated to dryness forms a glue-like brittle, opalescent, yellow mass; hydrated gliadin, exhausted with absolute alcohol and ether, and dried over sulphuric acid, forms a pure white friable mass. Either variety will almost wholly go into solution on warming in dilute alcohol. Gliadin is slightly soluble in distilled water, and then gives the pink biuret reaction; it is not entirely insoluble in dilute salt solutions, as stated by Osborne and Voorhees. In dilute alkalies it readily dissolves, and the greater part of that dissolved separates on neutralizing. Its action with hydrochloric acid is peculiar; it may be extracted directly from flour by dilute acids, filtered perfectly clear, and yet an additional drop of acid throws down a cloudy precipitate which increases in quantity with further addition of acid,

but separates completely only on heating; as it cools, however, more or less of the precipitate goes back into solution. A drop of alkali to the acid solution only produces a faint opalescence, which does not increase with additional alkali until the neutral point is reached, when a sudden clouding occurs, and a precipitate settles out on heating.

A cold alcoholic solution of gliadin filtered clear, clouds slightly in twenty-four hours, depositing a small precipitate which increases in quantity with the length of time under alcohol. It is much more soluble in boiling than in cold alcohol, a saturated solution of the former depositing a heavy precipitate on cooling. Heating to 130° C. in the autoclave renders gliadin insoluble in alcohol. In artificial gastric juice at 38° C. it rapidly dissolves, depositing a small amount of nuclein, and yielding a considerable amount of true peptone, as evidenced by the deep red colouration with potassic hydrate and cupric sulphate in the filtrate after removal of proteoses by saturation with ammonium sulphate. It is a unique proteid, in that it gives this red biuret reaction before as well as after digestion. In this particular the name "insoluble phytalbumose" applied to it by Martin[16] does not appear appropriate. The proteid is entirely insoluble in absolute alcohol, and is precipitated by strong alcohol from solutions in weak. Addition of salt to a solution of gliadin in 70 per cent. alcohol does not produce precipitation until water is added. Millon's reagent, and nitric acid give the usual proteid reactions.

Gliadin is distributed throughout the endosperm, especially toward the periphery, where the small proteid granules are much thicker and the starch granules they enclose smaller. It is also contained in bran, and probably in aleuron cells as part of the packing between the aleuron grains, for both bran and shorts yield gliadin to dilute alcohol.

IV.—PROPERTIES OF GLUTENIN.

Glutenin is almost completely insoluble in salt solutions, water, and alcohol; readily soluble in dilute acids and alkalies, from which solution the proteid is precipitated unaltered when the solution is rendered neutral to litmus. It has a definite coagulation temperature which lies about 70° C. Gluten dehydrated with absolute alcohol and ether, is very slowly soluble in dilute acids and alkalies, more or less remaining undissolved. Experimental evidence seems to show that glutenin exists as such in the wheat grain. Its composition, according to Osborne, is practically identical with that of gliadin, results differing greatly from

those of previous investigators, who had only in one instance obtained from glutenin as much as 17 per cent. of nitrogen.

Osborne considered it an altered form of gliadin, but the fact that it has a definite coagulating point, while gliadin has none, would indicate that it is improbable. No one has yet succeeded in making gliadin assume a form at all resembling glutenin. In my opinion the two proteids are entirely distinct in origin as well as in properties. Osborne states that glutenin is slightly soluble in cold but much more in hot dilute alcohol, the dissolved proteid separating on cooling. Since glutenin is coagulated at about 70° C. the proteid dissolved must have either been due to gliadin imperfectly separated from the glutenin, or to part of the latter split off by heat. The trace soluble in cold alcohol, as Osborne himself hints, may have been gliadin, which is exceedingly difficult to separate from glutenin.

V.—THE FERMENT THEORY OF GLUTEN FORMATION.

The question whether gluten exists as such in flour, or whether it results by the activity of a ferment, is one on which there are considerable differences of opinion. Weyl and Bischoff[30] considered gluten to be formed from pre-existing globulins by a pre-existing ferment in flour. They held that flour extracted by 15 per cent. solution of sodium chloride, and heated to the coagulation point of globulin, gave no gluten. They were, however, unable to isolate the ferment.

Martin[16] thought that gluten did not pre-exist in flour as such, but that his gluten fibrin was derived from a precursor globulin, and his insoluble phytalbumose or gliadin, from a soluble albumose. He stated that gliadin was not extracted directly from flour by 70 per cent. alcohol.

Johannsen[31] advanced arguments against the ferment theory, and thought gluten existed as such in a finely divided state in the wheat grain. He stated that a temperature of 60°C. did not injure the gluten-forming power of flour, and that flour made by mixing dry starch and finely-powdered gluten behaved like ordinary flour.

Ballard[32] maintained that gluten pre-existed as such in flour. Osborne[29] arrived at the same conclusion. O'Brien[20] found that flour heated to 100° C. for thirteen hours gave practically the usual amounts of gluten; also that a paste made with boiling water yielded gluten in apparently normal quantities; that flour left twenty-four hours under absolute alcohol and ether, yielded gluten when these evaporated. He concluded that there is but one compound soluble in alcohol, that the

portion soluble in alcohol may be made to pass over into the insoluble stage, and that there exists but one mother substance of gluten in flour.

None of the proofs as to the existence or non-existence of a ferment appear at all conclusive. Dry heat at 100° C. or even 110° C. for several hours does not kill ferments, neither does alcohol for a short period. To prove the non-existence of a ferment presents in this case peculiar and apparently unsurmountable difficulties, but a few facts bearing on the point may be given here.

Seventy per cent. alcohol, cold or hot, applied directly, extracts gliadin from dry flour ; warm 95 per cent. alcohol does the same ; flour moistened with 95 per cent. alcohol and heated to 80° C. yields abundant gliadin, as does flour stirred into boiling water and then extracted with alcohol. When flour, however, is slowly sifted into boiling water, so that every particle comes into instant contact with water or steam at 100° C. it yields no gliadin to dilute alcohol.

Dough made from flour and boiling water does yield gluten on washing, as stated by O'Brien, but it is smaller in amount and is of irregular consistency. The temperature of the dough when mixed was found to be only 52.5° C. Now glutenin has a definite coagulation point. Martin[16] stated that the residue after extracting gluten with dilute alcohol was coagulated by boiling water. Before noticing his work I had found the coagulation point of glutenin to be about 70° C. When, therefore, a dough was made with boiling water, and only reached the temperature of 52° C. only a comparatively small amount of the flour must have been heated to 70° C., a temperature which coagulates glutenin. Consequently a quantity of gluten would be formed from the portion of the flour not heated to that point. A dough made in this way and gradually heated till it reached a temperature of 80° C. yielded no gluten, proving that its formation depended upon the glutenin not being coagulated.

A dry heat of 110° C. for ten hours does not coagulate proteid, and flour heated to this point still yields gluten ; but if flour is heated to 120° C., or even 100° C., for half an hour in the autoclave a dough of little coherence results, and no gluten is obtainable on washing even over silk. The glutenin had been coagulated. In other words any temperature or manipulation that would kill a ferment which might be present would coagulate the glutenin and therefore gluten could not be obtained. The fact that gluten has a definite coagulation point would seem to indicate that it is not derived from the same substance as

gliadin. I have never been able to transform one of these compounds into anything at all like the other. With the idea of finding out whether gluten changed into gliadin, I extracted all the latter from flour, let one half stand over night under water and the other under alcohol for twenty-four hours, but neither yielded anything to dilute alcohol.

The fact that ground, dried glutin mixed with starch yielded dough of normal properties, as stated by Johannsen[31] is no proof as to the non-existence of ferment action, since if ferment action were present the dried gluten itself would have been the resultant product of the ferment action.

Flour was slightly moistened with absolute alcohol and heated on a warm bath to 70° C., being stirred all the while with a stout thermometer in order to heat the mixture evenly throughout. Alcohol was used to prevent any possibility of ferment action. After drying in the air, one half was taken and made into a dough, from which, as I expected, gluten could not be obtained. A small quantity of raw flour was intimately mixed with the other half and this was also made into a dough. In this case also no gluten could be obtained. This proved that the formation of gluten depended altogether on whether glutenin was coagulated or not, since the ferment if existing should have been present in the added raw flour.

Now ground air-dried gluten mixed with starch and made into dough yields gluten of normal properties. Such a dough of ground gluten and starch warmed above 70° C. does not yield gluten since the glutenin has been coagulated. Therefore when glutenin which had been already made, as in the the second case, or glutenin, or even its predecessor in the raw flour in the first case, were coagulated, a similar result obtained. The probability, therefore, seems to be strong that glutenin is present in flour as such. And since gliadin is extracted directly from flour or bran with 70–95 per cent. alcohol, cold or boiling, and also by dilute acids or alkalies, it also apparently is present as such in flour, and not derived, as O'Brien[20] holds, from the same parent substance as gluten.

VI.—THE ALEURON LAYER OF WHEAT.

The outer endosperm layer of wheat was stated by Sachs[33] in 1862 to be rich in oil and nitrogenous compounds. Ten years later Pfeffer[34] pointed out the fact that gluten was not derived from the aleuron layer as was commonly believed. He maintained that the high

nitrogenous value of the latter was due to substance not proteid in nature, and to adhering endosperm rich in gluten.

Johannsen[31] in 1888 again emphasized the fact that aleuron cells do not contain gluten, he stated that these cells contained nitrogenous granules imbedded in a soft protoplasmic mass, rich in fatty matter.

According to O'Brien[20] the protoplasm of an aleuron cell is continuous with that of adjacent cells, aleuron as well as endosperm. He found oil present in considerable quantities. The individual aleuron grains on addition of water appeared to consist of a central core which was more or less soluble in water, salt solutions. dilute acids and alkalies, and not readily stainable. The layer surrounding this core he found to stain readily with iodine, hæmatoxylin and aniline stains, and to be insoluble in any of the above mentioned reagents.

From an aqueous extract of bran he obtained a coagulable proteid, probably a globulin, and proteose which, when evaporated to dryness, yielded a gelatinous semi-transparent substance, partly separating in small round spherules, regarded by him as artificial aleuron grains, since they gave all the reactions of those imbedded in cell protoplasm.

He also extracted from bran by means of dilute alcohol a proteid which corresponded to gliadin.

Dilute alcohol, I found, extracted gliadin from both bran and shorts. Aqueous extracts of bran gave a globulin coagulable by heat, and also a proteose-like body which was not gliadin. On evaporation of this proteose extract no granule corresponding to O'Brien's artificial aleuron grains could be obtained, although a granular material did separate ; the solution at the same time exerted a very strongly reducing action upon Fehling's fluid. I was unable to make out a double coat to the aleuren grains. The substance between the aleuron grains seems to be chiefly gliadin, and contains inorganic iron, calcium salts and phosphorus-holding compounds.

VII.—CONCLUSIONS.

Gliadin and glutenin do not come from the same parent substance, nor are they of the same composition. Gliadin has not a definite coagulation point, while glutenin has. Gliadin is obtained from rye, barley, and maize, and from the bran and shorts of wheat, while glutenin cannot be obtained from these. By chemical or other means one has as yet not been transformed into anything at all resembling the other.

Both gliadin and glutenin invariably give the reactions for organic iron and phosphorus, but are not nucleo-proteids. Under the microscope the gluten matrix in thin sections of wheat does not show any indication of iron or phosphorus, and it must, therefore, be concluded that the organic iron and phosphorus found in gluten is due to nucleins or nucleic acid derived from the nuclei of the large endosperm cells. Probably part is derived from nuclei of the aleuron cells, or of the embryo cells, or from the nucleins present in the cytoplasm of the embryo cells.

Gliadin exists as such in the wheat grain, and the theory of its formation by means of ferment action is not justifiable. Strong alcohol mixed with flour and then diluted with water to a 70 per cent. solution extracts gliadin from it; boiling alcohol also extracts gliadin from flour or bran.

Glutenin exists as such in the wheat grain; any manipulation that will destroy the hypothetical ferment will coagulate glutenin, thus making gluten formation impossible.

Gluten formation is not merely a mechanical mixture of gliadin with glutenin, but a definite physical state of the two mixing substances is necessary. Coagulated glutenin with gliadin does not form gluten.

There are probably several nucleins or nucleo-proteids in wheat, as shown in the various ways phosphorus is distributed in the different types of embryo cells. Organic iron is found only in the nuclei of the endosperm, aleuron, and embryo cells, and in the cytoplasm of the absorption layer, plumule and radicle cells. The proteid between the aleuron grains shows the presence of organic phosphorus only.

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