The nucleic acid of the embryo of wheat and its protein compounds.

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THE NUCLEIC ACID OF THE EMBRYO OF WHEAT AND ITS PROTEIN COMPOUNDS.

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Frankfurt (Versuchs-Stationen, **47**, 449) has estimated the proportion of various constituents of the embryo of wheat and found globulin, 21.62 p. c. and albumose 13.62 p. c.

O'Brien (Annals of Botany IX, 543) states that the proteids of wheat germs consist of globulins of the myosin-type, coagulating at 55°, soluble in dilute solutions of sodium chloride or magnesium sulphate and precipitated by excess of these salts; globulins of the vitellin-type, coagulating at 75°-78° and soluble in dilute solution of sodium chloride, but not precipitated by an excess; proteose; and albumin not coagulating below 80°, soluble in sodium chloride solution, not precipitated by an excess, nor by dialysis, nor by carbonic acid.

As neither of these investigators has given more than a superficial account of the protein* substances found by him in the embryo of wheat, we determined to investigate this subject, in order to learn definitely which of the proteins contained in the wheat kernel are predominant in the embryo, and whether there is any special difference in character between the proteins of the more physiologically active germ and those of the dormant endosperm.

As a result of this investigation we have found that, of the proteids described by one of us† as constituents of the wheat kernel, the albumin (leucosin), the globulin and the proteose, which in the whole seed are present in small proportions, together constitute a large part of the embryo, and further, that gliadin and glutenin, the principal proteid constituents of the endosperm, are not present in the embryo.

Although the globulin and leucosin obtained from the whole seed were free from phosphorus, all of our preparations

* In this paper the term protein denotes the basic molecules which, combined with simple acids, form the "simple proteids," and combined with other more complex groups, form the "compound proteids" (Hammarsten, Text-book of Physiology, translated by Mandel. Third Edition, 600). The term proteid in this paper includes both of these groups of protein compounds.

† Osborne & Voorhees, Am. Chem. Jour., XV, 1893, 392.

of the globulin, and many of those of the albumin, from the embryo contained various yet considerable quantities of this element, and when digested with pepsin, yielded insoluble products having the characteristics of nuclein. The elementary composition of those preparations of the embryo-albumin which contained no phosphorus, or only traces, was the same as that of leucosin prepared from the whole seed, while the composition of those embryo-albumin preparations which contained phosphorus differed from that of leucosin in proportion to the phosphorus present.

Analyses of different preparations of embryo-globulin showed no agreement, even when corrected for ash.

These facts led us to examine our extracts for nucleic acid, and having found this acid and determined its composition, it was seen that the differences just alluded to disappear if we assume the phosphorus to be present as nucleic acid and calculate the analyses free from this acid and from ash.

In a paper recently published by one of us on "Some Definite Compounds of Protein Bodies" (Jour. Am. Chem. Soc., XXI, 486), it was shown that many, if not most preparations of so-called native proteids are, in fact, compounds of protein bodies with acids, and it was suggested that nucleoalbumins might prove to be similar phosphoric acid compounds.

In that paper it was also shown that the crystallized globulin, edestin, obtained from hemp-seed, forms a compound with one, and another compound with two molecules of hydrochloric acid, both of which are crystalline, and that the preparations of this globulin as commonly obtained, are mixtures of these in various proportions. It was also shown that the protein molecule can combine with more than two molecules of acid, so that several multiple compounds of one acid with the same protein molecule can undoubtedly exist.

That our preparations from the wheat-embryo are mixtures of two or more compounds is shown by the variable proportions of phosphorus which they contain. That phosphorus is a chief factor in determining the proportion of nuclein that separates during pepsin digestion is shown by the fact that the amount of nuclein found is always in close relation to the amount of phosphorus contained in the preparation.

That the preparations are not mixtures of already formed nuclein with ordinary proteid matter is shown by the difference in solubility and behavior of the original preparation from that of the nuclein derived from it.

It would seem, then, that the nuclein obtained by pepsin digestion is not an original constituent of the extract nor of the cells of the embryo, but results through combination of several molecules of nucleic acid with one of protein.

Accordingly we conclude that these phosphorus-containing preparations from the wheat embryo are mixtures of different protein nucleates and that when subjected to pepsin digestion, in consequence of the conversion of a part of the protein substance into proteose, the proportion of nucleic acid to unaltered protein is increased, so that higher acid nucleates are formed which are insoluble in the digestive fluid.

The grounds for these conclusions are given in the following detailed account of our investigation.

The material at our command consisted of a quantity of wheat germs, a specially prepared product of the Pillsbury Mills, from which the bran and endosperm had been very thoroughly removed, which was kindly procured for us by Mr. David Chidlow of Chicago.

The germ meal, which was prepared and sent to us in cold weather, was immediately extracted with petroleum naphtha and ground to a flour.

I. The Nucleic Acid of the Wheat Embryo.

We shall later show that the precipitate produced by saturating the slightly acid aqueous extract of this wheat embryo meal with sodium chloride contains almost all the phosphorus of the extract. We accordingly extracted a large quantity of the meal with water, saturated the extract with sodium chloride and subjected the precipitate to a vigorous pepsin digestion. We thus obtained a considerable quantity of nuclein from which we prepared nucleic acid in the following manner:

The nuclein was thoroughly washed with water and then dilute potash solution was added until all the nuclein had dissolved and its solution become faintly alkaline to phenolphthalein.

This solution was cautiously treated with dilute hydrochloric acid until a precipitate was formed, which separated readily from the solution. This was filtered out and the clear filtrate found to yield no precipitate on adding a *little* more acid. A considerable quantity of strong hydrochloric acid was then added, causing a precipitate of nucleic acid, which separated in large flocks that rapidly settled to a coherent layer. The deposit continued to contract and soon became so dense and brittle that it could be ground to a powder even under water. The solution was decanted, the sediment was thoroughly washed, redissolved with alkali and again thrown down by adding acid. Since this last precipitate still contained much coloring matter, it was again dissolved with alkali and the solution poured into alcohol. This retained the basic coloring matter and threw down a voluminous precipitate of potassium nucleate, which was thoroughly washed with large quantities of alcohol, dried to remove the alcohol and then dissolved in water and the nucleic acid reprecipitated by an excess of hydrochloric acid. Since all the coloring matter had not been removed by the preceding treatment, the precipitate was twice dissolved in alkali and precipitated by pouring into a large volume of alcohol. The nucleic acid was then thrown down by gradually adding dilute hydrochloric acid to the solution of the potash salt; after thoroughly washing the precipitated acid with water and with alcohol it was dried over sulphuric acid and found to weigh 10.14 grams.

After drying to constant weight in hydrogen at 100°, its weight remained unchanged on further heating at 110° in air. When thus dried it had the following composition:

	I.	II.
Carbon	36.18	36.31
Hydrogen	4.48	4.42
Nitrogen	16.03	16.10
Phosphorus	8.95	8.86
Ash	3.52	
P_2O_5 in ash	2.88	
Difference	0.64	

The ash consisted, chiefly if not wholly, of potassium metaphosphate and therefore by subtracting from it the P_2O_5 which it contained,we obtain the amount of inorganic base which

had been precipitated as an acid salt together with the free nucleic acid. Calculating our analyses free from this base, we have the following figures:

COMPOSITION OF NUCLEIC ACID.

	Found.	Calculated for $C_{21}H_{31}N_8P_2O_{18}$.
Carbon	36.48	36.16
Hydrogen	4.48	4.45
Nitrogen	16.17	16.01
Phosphorus	8.96	8.89
Oxygen	33.91	34.49
	100.00	100.00

About 2 grams of this preparation were hydrolyzed by heating for an hour and a half in a boiling water bath with 2 p. c. hydrochloric acid.

On adding ammonia to this solution, a precipitate soon separated which was digested on the water bath with an excess of ammonia, filtered out, washed, dried over sulphuric acid and found to weigh 0.27 gram.

This substance gave the murexide reaction and was insoluble in hot ammonia, both of which properties are characteristic of guanin. On analysis this crude guanin was found to contain:

	Found.	Calculated for guanin $C_5H_5N_5O$.
Carbon	40.96	39.74
Hydrogen	3.67	3.31
Nitrogen	45.21	46.36
Oxygen	10.16	10.59
	100.00	100.00

The solution filtered from the guanin was precipitated by cold ammoniacal silver nitrate and the voluminous, gelatinous precipitate washed, pressed on filter paper, suspended in water and decomposed by hydrochloric acid. The solution, containing the hydrochloride of another base, was repeatedly evaporated with water to decompose the chloride and the free base found to weigh 0.2272 gram. This was again dissolved in water and the silver salt precipitated from a boiling solution, the silver compound was decomposed with hydrochloric acid and the solution of the hydrochloride was evaporated and crystallized.

The substance separated wholly in four-sided prisms, most of which were truncated by planes at right angles.

This hydrochloride was then converted into the picrate by dissolving in water, adding a little ammonia, evaporating to dryness, dissolving in about 100 cc. of water and precipitating with a 1.1 p. c. solution of picric acid added cautiously. The very voluminous yellow precipitate was quickly filtered out with the help of a pump, washed thoroughly with water and dried over sulphuric acid. We thus obtained 0.3766 gram of a picrate, which lost nothing on drying at 110° and had the following composition:

	Found.	Calculated for Adenin Picrate, $C_{11}H_8N_8O_7$.
Carbon	36.07	36.27
Hydrogen	2.51	2.19
Nitrogen	30.28	30.77
Oxygen	31.14	30.77
	100.00	100.00

From the behavior of this base and the composition of its picrate, it is evidently adenin. Since this acid yields on hydrolysis the purin bases, guanin and adenin, as well as phosphoric acid, there can be no doubt that it is a true nucleic acid closely related to the guanylic acid recently described by Bang (Zeit. f. physiol. Chem. XXVI, 133). The facts that we obtained these two bases from the nucleic acid in nearly molecular proportion and that almost all the nitrogen of the acid was recovered in the guanin and adenin separated from it, lead us to believe that both these bases exist together in the acid molecule. If such is the case, our formula already given must be multiplied by 2.5, making it, $C_{53}H_{77}N_{20}P_5O_{37}$. This formula resembles that of guanylic acid, which calculated to the same basis, is $C_{55}H_{75}N_{25}P_5O_{42}$. The two acids are different, since Bang's guanylic acid yields a pentose on hydrolysis, whereas we have obtained no evidence that any sugar can be derived from our acid. As we are at present engaged in a study of the reactions and constitution of this acid, we will reserve further statements respecting it for a future paper, which we expect to be able to publish soon.

II. The Proteids of the Wheat Embryo.

The Aqueous Extract.

The germ flour, described on page 307, when treated with water, yields a gummy mass from which a clear extract is secured with difficulty. From 500 grams of meal an extract was obtained with 2,000 cc. of water, of which 1,400 cc. could be filtered clear. This extract was neutral to litmus, alkaline to lacmoid, and so acid to phenolphthalein that 19 cc. of decinormal alkali were required to neutralize 100 cc. of it to this indicator.

When a freshly prepared, dilute, aqueous extract of the recently ground wheat germs is heated in a water bath, no coagulation occurs, the solution becoming slightly opalescent. If a more concentrated extract, such as may be obtained by treating one part of meal with 5 parts of water, is thus heated, the entire solution solidifies to a firm, opaque jelly, free from visible particles. If, to either of these solutions a very little hydrochloric acid is added before heating, an abundant flocculent coagulum separates on heating.

After standing awhile, the aqueous extract becomes gradually acid to litmus, so that when heated slowly it becomes turbid at about 50° and a large flocculent coagulum separates at 55°. Heated to 65° for some time and filtered, a second coagulum may be obtained on raising the heat from 65° to 100°. The amount of this second coagulum is about one-third that of the first.

The coagulated proteid is dissolved by 0.5 p. c. KOH solution, but not perceptibly by 0.4 p. c. HCl solution, unless the latter is heated, when a clear transparent jelly is formed.

Freed from coagulable protein, the aqueous extract still contains a relatively large amount of substance which has the reactions of proteose.

When the concentrated aqueous extract is poured into a large volume of distilled water, a turbidity forms at first, which mostly disappears after shaking, indicating the absence of a notable quantity of globulin held in solution by the salts dissolved from the meal.

Saturation of the extracts with sodium chloride gives a considerable precipitate, only a small part of which can be

redissolved in dilute salt solution. When this dissolved part is precipitated by again saturating with salt, it also is converted, to a large extent, into an insoluble form; the part still remaining in solution is, like a globulin, precipitated by dialysis.

When the solution saturated with sodium chloride is filtered, and the diluted filtrate saturated with ammonium sulphate, a part of the precipitate produced, when redissolved in water, is thrown out of solution by saturating with sodium chloride, though before precipitation with ammonium sulphate it dissolved in saturated sodium chloride solution.

These reactions show that changes occur which involve the albumin coagulating at 55°, for after freeing the extract from all protein precipitable by saturating with salt or by dialysis, there remains in solution only a small proportion of this albumin.

Thus, an aqueous extract corresponding to 666 grams of germ meal, when heated to 65°, yielded 62 grams of coagulum, or 9.3 p. c.; a similar extract on dialysis deposited 9.2 p. c.; only 0.87 p. c. of coagulable and 2.0 p. c. of uncoagulable protein remaining in solution. The precipitate, produced by dialysis, was but slightly soluble in salt solution, having become largely coagulated. From these facts it is clear that one and the same protein substance gives rise to these apparently different protein bodies, and consequently the substance which O'Brien considered to be a globulin of the myosin type and an albumin, coagulating at 80°, are in fact derivatives of the albumin which coagulates mostly at 65°.

The cause of these changes was not determined, though it seems most probable that they are the result of a slow development of acid in the extract, which by uniting to the protein, in increasing proportions, forms chemically different substances. Such a development of acid takes place rapidly in muscle plasma, under the influence of which quite similar changes in the proteins there present can be observed.

Why Frankfurt overlooked albumin, present in such large proportion in the aqueous extract, is not easily understood, unless, before heating his solutions, he either added no acid or too much, so that he converted this substance into an uncoagulable acid compound.

Hydrochloric acid added to the extract in very small quantity causes a flocculent coagulum to separate on heating,

while a slightly larger quantity, added before heating, entirely prevents the formation of this coagulum. Acetic acid and nitric acid give precipitates in the extracts which are not soluble in a reasonable excess of either of these acids.

In order to determine definitely the relations of these variously obtained substances, we have made a large number of fractional precipitations under quite different conditions, an account of which we now give:

An extract was made by treating 700 grams of germ meal with seven times its weight of water, straining through bolting cloth and filtering the fluid perfectly clear. This was slightly colored, perfectly neutral to litmus, alkaline to lacmoid and strongly acid to phenolphthalein. A portion of it was at once heated for one hour in a water bath at 60°, and the large coagulum produced was filtered out, washed thoroughly with hot water and with alcohol and dried over sulphuric acid, giving 24.0 grams of preparation **1.**

Another preparation was made by heating in a water bath at 65°, 2,000 cc. of a clear aqueous extract, obtained by treating 3,000 grams of the germ meal with 9,000 cc. of water. The coagulum produced, when washed with hot water and alcohol and dried over sulphuric acid, weighed 62 grams, forming more than 9 p. c. of the oil-free germ meal. This is preparation **2.**

Another aqueous extract was heated at 65°, until all the proteid coagulable at this temperature had separated. The coagulum produced, when washed with hot water and alcohol was dried over sulphuric acid and found to weigh 16.68 grams. The filtrate from this coagulum, heated in a boiling water bath, yielded a second coagulum which, when washed and dried, formed preparation **3,** weighing 4.9 grams.

A portion of the extract, which yielded preparation 1, was saturated with ammonium sulphate, the resulting precipitate was dissolved as far as possible in water, its solution filtered clear and dialyzed for four days. During this time a considerable precipitate formed, that, when filtered out, was found to be insoluble in salt solution. The solution, filtered from that substance and dialyzed in running water until nothing more separated, was filtered and heated at 60°, which caused a coagulum. This coagulum, washed with hot water and with alcohol

and dried over sulphuric acid, weighed 7.1 grams and made preparation **4.**

In a clear water extract of wheat germ meal, dialyzed four days, there appeared a dense turbidity, due apparently to a globulin, since it dissolved on adding sodium chloride. Passing carbon dioxid gas through the dialyzing solution seemed to increase the turbidity, but effected no definite separation. As it was found that 10 cc. of $N/_{10}$ HCl per 100 cc. of the extract caused a separable precipitate, this proportion was added and the resulting flocculent precipitate brought into solution again by adding salt. The clear extract was then dialyzed for two days in running water and filtered from an amorphous precipitate, which was treated as later described on p. 326.

The filtrate from this precipitate was further dialyzed for three days more in running water and then, as nothing separated, for four days more into alcohol. The precipitate which resulted was dried over sulphuric acid, exhausted with water, in order to remove all uncoagulated proteids, as well as other soluble substances, dehydrated with absolute alcohol, again dried and weighed, yielding 12.0 grams of preparation **5.**

Another aqueous extract was saturated with pure sodium chloride, the abundant precipitate filtered out, treated with dilute brine and the resulting solution filtered from a relatively considerable quantity of insoluble matter. This filtrate was saturated with sodium chloride, a second precipitate filtered out and likewise treated with dilute salt solution. The insoluble portion was removed by filtration and the clear filtrate dialyzed. The small precipitate separated by dialysis was washed and dried, weighed 4.8 grams and formed preparation **6.**

The filtrate from the first precipitation of the substance of preparation **6,** caused by saturating its solution with sodium chloride, as described above, was diluted with water and saturated with ammonium sulphate. The precipitate which resulted was dissolved in water and its solution precipitated by saturating with sodium chloride. Although this substance had previously been soluble in saturated brine, after precipitation with ammonium sulphate it was found to be nearly all insoluble therein, so that almost complete precipitation resulted on again

saturating with sodium chloride. The precipitate so produced was filtered out, dissolved in dilute salt solution and reprecipitated by dialysis. We thus secured 7.6 grams of preparation **7**.

By saturating another aqueous extract of germ meal with sodium chloride a very large quantity of proteid was separated, which was filtered out, exhausted with dilute salt solution and washed thoroughly with water and alcohol. Dried over sulphuric acid, the preparation, **8**, weighed 17 grams.

The filtrate and saline washings from preparation **8** were united and again saturated with salt, and yielded a small precipitate which, dissolved in brine and precipitated by dialysis, gave preparation **9**, weighing 2.8 grams. As the salt-saturated solution from which this preparation had separated contained so little protein, it appears that nearly all the proteid precipitated from the aqueous extract by saturating with salt had been converted into the insoluble substance forming preparation **8**.

The filtrate, from the salt saturation-precipitate produced in the aqueous extract, was dialyzed in water for several days and the still clear solution then dialyzed in alcohol for 24 hours. The proteid, thereby precipitated in a coagulated state, was filtered out, washed with water and then with alcohol, yielding 12.4 grams of preparation **10**.

Another aqueous extract was saturated with sodium chloride and the precipitate, treated in the same way as preparation **8**, yielded 18.0 grams of preparation **11**.

The saline washings of the last preparation were dialyzed free from chlorides and gave a precipitate weighing 2.86 grams when washed and dried, which formed preparation **12**, having the properties of a globulin, dissolving readily on adding salt and being precipitated from such solution by water.

The filtrate from the final precipitation of **12**, when heated in a boiling water bath, gave a coagulum which formed preparation **13**, weighing 1.64 grams.

The salt-saturated filtrate from the first precipitation of **11**, as already described, was heated to boiling and the coagulum produced was filtered out, giving preparation **14**, weighing 5.47 grams.

Since analysis showed that most of the preparations already described contained phosphorus, some even in large amount,

we made an attempt to separate the phosphorus from our extract, in order to determine, if possible, the relation of the preparations free from phosphorus to those which contained much phosphorus.

Two thousand grams of meal were treated with 6 liters of distilled water and the extract (four liters) was squeezed out as completely as possible in a press.

As a preliminary experiment, 100 cc. of this clear, filtered extract were made faintly alkaline to phenolphthalein, with about 40 cc. of $N/_{10}$ KOH solution. To insure a sufficient quantity, 20 cc. more of alkali were added and thereupon a little calcium chloride, which gave a precipitate that seemed to partly dissolve on adding sodium chloride. The undissolved part, when washed with dilute salt solution, water and alcohol and dried, weighed 1.7 p. c. of the meal, contained about 55 p. c. of organic matter and left 45 p. c. of ash, consisting of tricalcium phosphate.

To 2,000 cc. of the original extract were then added 1,350 cc. of a solution containing alkali equivalent to 1,560 cc. $N/_{10}$ solution, with sodium chloride enough to form 6.5 p. c. of the total liquid. To this, a solution of calcium chloride was added, as long as a precipitate formed, and after standing over night the solution was decanted from the precipitate and filtered clear on a pulp filter. Of the clear filtrate, 2,200 cc. were made as neutral as possible to *litmus,* by adding 180 cc. of $N/_{10}$ HCl solution. Of the thus neutralized solution, 1,000 cc. when gradually heated in a water bath, became turbid at 52° and a considerable coagulum separated at 53°. After the temperature had been slowly raised to 65° and kept at this point for some time, the coagulum was filtered out, washed and dried as usual, giving preparation **15**, weighing 6.4 grams. Another portion of this extract, filtered from the calcium chloride precipitate, was saturated with ammonium sulphate while still slightly alkaline to litmus, the resulting precipitate filtered out, dissolved in water, its solution filtered clear and dialyzed. A slight precipitate formed on dialysis, which was removed by filtering, the solution was heated in a boiling water bath and the proteid thus coagulated was filtered out, washed, dried and weighed as usual, giving 3.07 grams of preparation **17.**

To determine what effect the removal of the phosphorized

substance thrown out by calcium chloride had upon precipitation with salt, we made neutral to litmus a liter of the filtrate from the calcium chloride precipitate and then saturated with sodium chloride. The large precipitate which formed was washed by decantation with water, in which it gradually dissolved, until only an insignificant quantity remained. The similarly obtained precipitate from the simple aqueous extract we have shown on page 315, to be nearly all insoluble in water.

To separate globulin from the aqueous extract, 1,200 cc. of clear filtered extract were obtained from 200 grams of the germ meal treated with 2,000 cc. of water. 1,000 cc. of this extract were dialyzed in running water for six days, and the large precipitate resulting filtered out, washed with water and alcohol and dried over sulphuric acid, giving preparation **18**, weighing 9.17 grams.

These preparations, thus variously obtained from the aqueous extract, were dried to constant weight at 110° and analyzed with the following results, most of the figures given in the table being the average of closely agreeing duplicate determinations:

TABLE I.—COMPOSITION OF PREPARATIONS OF PROTEID FROM THE WATER EXTRACT OF THE WHEAT EMBRYO.

	1.	2.	3.	4.	5.	6.	7.	8.	9.
Carbon	51.13	50.52	50.17	52.39	51.77	52.13	52.73	43.59	52.28
Hydrogen	6.85	6.81	7.01	6.83	6.81	7.04	7.11	5.77	6.97
Nitrogen	16.28	16.47	16.66	16.20	16.11	16.48	16.00	15.16	16.38
Sulphur	1.18	1.17	1.00	1.32	1.30	1.49	1.53	0.90	1.39
Phosphorus	0.72	0.97	0.91	trace	0.17	0.06	none	3.38	0.07
Ash	2.73	2.90	3.03	0.35	1.39	0.43	0.39	13.04	0.44
P_2O_5 in Ash	1.88	2.09	1.91	trace	0.47	trace	none	6.73	trace

	10.	11.	12.	13.	14.	15.	16.	17.	18.
Carbon	51.21	46.67	51.87	----	51.95	51.65	----	52.02	49.59
Hydrogen	6.85	6.19	6.89	----	6.86	6.66	----	7.00	6.68
Nitrogen	16.18	15.89	16.65	16.31	16.08	16.02	16.09	16.45	16.34
Sulphur	1.10	0.93	1.19	1.35	1.60	1.13	1.12	1.24	0.91
Phosphorus	0.46	2.53	trace	trace	trace	trace	trace	none	1.85
Ash	2.19	8.17	0.38	0.45	0.32	1.09	2.83	0.56	2.50
P_2O_5 in Ash	1.11	5.71	trace	trace	trace	trace	trace	none	1.79

Assuming that those of the foregoing preparations which contain phosphorus are compounds of protein with the nucleic

acid, which was separated from the aqueous extract of wheat germs and the composition of which is given on page 309 of this paper, and also assuming that all the phosphorus of these preparations is a part of the nucleic acid, we have calculated the composition of these preparations free from nucleic acid. The analyses were further calculated ash-free by subtracting the P_2O_5 contained in the ash from the total ash, which seems permissible since the ash consisted almost wholly of metaphosphates of potassium and sodium, strongly indicating that the P_2O_5 was derived from the nucleic acid. These calculations gave the following results:

TABLE II.—COMPOSITION OF LEUCOSIN CONTAINED IN THE PREPARATIONS FROM WATER EXTRACTS OF THE WHEAT EMBRYO.

	1.	2.	3.	4.	5.	6.	7.	8.	9.
C	52.93	52.75	52.41	52.57	52.57	52.47	52.93	53.23	52.64
H	7.12	7.16	7.38	6.85	6.91	7.08	7.13	7.09	7.02
N	16.45	16.68	16.94	16.26	16.27	16.55	16.06	16.30	16.46
S	1.29	1.32	1.13	1.32	1.34	1.50	1.53	1.60	1.41
O	22.21	22.09	22.14	23.00	22.91	22.40	22.35	21.78	22.47
	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
	10.	**11.**	**12.**	**13.**	**14.**	**15.**	**16.**	**17.**	**18.**
C	52.63	52.44	52.06	----	52.11	52.16	----	52.30	53.45
H	7.06	7.10	6.92	----	6.88	6.73	----	7.04	7.30
N	16.40	16.26	16.71	16.38	16.13	16.20	16.56	16.54	16.57
S	1.17	1.34	1.19	1.35	1.60	1.14	1.15	1.24	1.16
O	22.74	22.86	23.12	----	23.28	23.77	----	22.88	21.52
	100.00	100.00	100.00		100.00	100.00		100.00	100.00

Of these preparations **1, 2, 3, 4, 13, 14, 15, 16** and **17** were obtained by coagulation with heat, **5** and **10** by coagulation with alcohol, **8** and **11** by saturation with sodium chloride, **6, 7, 9** and **12** by dialyzing salt solutions in water, and **18** by direct dialysis of the aqueous extract. Since some of these preparations formed the whole of the precipitable proteid contained in the extract, while others represented fractions, it is evident that all contain one and the same protein substance mostly combined with various proportions of nucleic acid.

Eliminating the nucleic acid, it thus appears that the composition of the protein part of those preparations which contain phosphorus is the same as that of the phosphorus-free proteid

preparations, although the former contain from very little up to more than 37 p. c. of nucleic acid.

Most of these preparations might, in accordance with custom, be called nucleoproteids, while **8** and **11** are, both in properties and composition, very much like nuclein. It is thus evident that these nucleoproteids and nucleins are nucleic acid compounds of protein which, owing to the high molecular weight of the nucleic acid, are more readily recognized as compounds than are those with acids of low molecular weight.

It is to be noted that these preparations show very diverse properties; some being like albumin; some like globulin; some being precipitated by saturation with salt, while others are not. As we have shown, these different properties are the result of changes caused by varying the conditions under which the proteid exists in the extract, and depend chiefly on the degree of acidity of the extract, whereby the numbers and kinds of acid molecules that combine with the protein molecule are altered.

Whatever may be the true cause of these changes, it is evident from the results here described, that the distinctions heretofore made between globulin and albumin, myosin and vitellin, etc., have very little value as a basis for classifying protein substances. This explains the difference between O'Brien's classification of leucosin as a myosin-like globulin, to which reference was made at the beginning of this paper, and our designation of it as an albumin, because of the ready solubility in water and coagulability by heat of the preparations which we had made.

Thus, preparation **18**, weighing 9.17 grams, was insoluble in water and in salt solution and was not a precipitate of globulin, since in the filtrate from which it had separated on dialysis, only 0.87 gram of coagulable albumin were found instead of 9.5 grams as usually found by direct coagulation of the aqueous extracts; moreover the analysis shows it to be a compound of leucosin with 20 p. c. of nucleic acid.

On the preceding pages, it was shown that a small part of the precipitate, produced by saturating the aqueous extract with sodium chloride, is soluble in dilute salt solution and can be precipitated from this solution by dialysis, as a globulin-like substance, readily soluble again in salt solution. The precipitates thus obtained contain little or no nucleic acid, and have very nearly the same elementary composition as leucosin, of which

they are evidently compounds with a small proportion of some body of low molecular weight.

It is plain from these facts that O'Brien's myosin contains the same protein substance as my leucosin.

O'Brien's "*albumin*" coagulating at 75°–80° is unquestionably more of this same leucosin as shown by preparation **3**, which formed about 25 p. c. of the total coagulable proteid. It has been the writer's experience that complete coagulation, especially in a solution quite free from salts, can be effected, if at all, only by heating the solution much above the lower coagulation temperature of the proteid to be separated.

From the whole seed we obtained leucosin with the same composition and general properties as from the embryo, but our preparations from the whole seed were free from phosphorus. This was probably because the proportion of nucleic acid to protein matter was smaller in the whole seed than in the embryo so that on extracting with water the nucleic acid did not form soluble compounds with the leucosin, but remained undissolved in combination with protein. In the following table is given the average of analyses of leucosin from the cereals.

TABLE III.—COMPOSITION OF LEUCOSIN PREPARED FROM VARIOUS CEREALS.

	Wheat Embryo.	Wheat Kernel.	Rye Kernel.	Barley Kernel.	Barley Malt.	Maize* Kernel.
C.....	52.65	53.02	52.97	52.81	53.07	52.72
H	7.04	6.84	6.79	6.78	6.72	7.05
N	16.43	16.80	16.66	16.62	16.71	16.82
S	1.32	1.28	1.35	1.47	23.50	1.32
O	22.56	22.06	22.23	22.32		22.05
	100.00	100.00	100.00	100.00	100.00	100.00

In an earlier paper on the Chemical Nature of Diastase (Jour. Am. Chem. Soc. XVIII, 542; Report Conn. Agr. Exp. Sta., 1895, p. 239) we pointed out that diastatic action appeared to be always associated with leucosin. Since our extracts

* This proteid was described by Chittenden and Osborne, Am. Chem. Jour. XIII, 327, as a myosin-like globulin, and was later, Jour. Am. Chem. Soc. XIX, 525, designated maysin by the writer. Since we now find that leucosin may form compounds having the properties of globulin, it is probable that maysin and leucosin contain one and the same protein substance.

of wheat embryo were so rich in leucosin, we determined the diastatic power of the germ meal by extracting with four times its weight of water and found that, under the conditions of Lintner's test, 0.10 cc. of the extract so made, when added to 10 cc. of a 2 p. c. solution of soluble starch, formed within one hour, at 20°, enough sugar to reduce 5 cc. of Fehling's solution. The 0.10 cc. of extract corresponds to 25 milligrams of the germs, from which it is seen that this meal possesses high diastatic power, though it is inferior in this respect to active malt.

Sodium Chloride Extract.

Wheat germ meal treated with 10 p. c. sodium chloride brine forms a dense jelly-like mass from which it is nearly impossible to separate the solution.

With 3 p. c. brine a manageable extract can be made by using from six to ten times as much solvent as meal. Thus, 100 grams of the meal treated with 600 cc. of 3 p. c. salt solution yielded in fifteen hours 400 cc. of clear filtrate. As has just been shown, the aqueous extract on dialysis, in consequence of a change which affects leucosin, deposits a large amount of proteid, chiefly in the coagulated form. In order to obtain preparations of the proteid substance soluble in salt solutions, but insoluble in water, which should be free from this coagulable albumin, we treated 2,000 grams of germ meal with 20 liters of 3 p. c. salt solution heated to 70°, whereby the leucosin was coagulated and the salt-soluble globulin brought into solution. The extract, neutral to litmus, was filtered clear, at once saturated with ammonium sulphate and the proteids thus precipitated collected on a filter, dissolved in water and the clear solution dialyzed in running water.

Proteid matter separated on dialysis in spheroids which, like legumin, conglutin and amandin, united to a plastic mass on the bottom of the dialyzer.

This precipitate was dissolved in brine, filtered absolutely clear, dialyzed for 48 hours, the large precipitate which separated allowed to settle, and the solution, which was nearly free from protein, decanted.

A portion of the precipitate was washed first with water, which rendered it opaque and dense, then with dilute and finally

with absolute alcohol and dried over sulphuric acid. This weighed 5.22 grams, and is preparation **19**. The rest of the precipitate was completely dissolved in 125 cc. of 10 p. c. salt solution. To this, water was added until its volume was 425 cc., thus making a salt solution of nearly 3 p. c. From this diluted solution a gummy deposit separated from which the fluid was soon completely decanted. The latter was further diluted with 325 cc. of water and the precipitate which resulted allowed to settle to a viscid transparent deposit. From this precipitate the solution was again decanted and dialyzed for 48 hours, but not more than a trace of globulin was deposited. The two precipitates produced by dilution were thoroughly washed with water and alcohol, dried over sulphuric acid and formed preparations **20** and **21**, weighing respectively 11.4 grams and 8.15 grams. A part of each of these preparations was set aside for analysis and the rest, dissolved together in 10 p. c. salt solution, allowed to stand over night at 4°. The solution was then decanted from a slight sediment, filtered clear and heated to 80° in order to coagulate any leucosin which might be present, and after two hours filtered from a very small coagulum which had gradually formed.

This filtrate was dialyzed in water for four days and the globulin which separated was washed with water and with alcohol and dried over sulphuric acid, giving preparation **22**.

The solution filtered from the first dialysis precipitates which yielded preparations **19**, **20** and **21**, was further dialyzed; a little globulin, which separated, was filtered out and the filtrate dialyzed into alcohol for four days. A precipitate was produced which, when washed with absolute alcohol and dried, weighed 25.0 grams. This substance consisted of proteid which will be described later, on p. 328. Another series of fractional precipitations of this globulin-like proteid was made by extracting four kilograms of the oil-free germ meal with 27 liters 3 p. c. brine, heated to 67° at the time it was applied to the meal. The mixture was thoroughly stirred and thrown on filters. A clear filtrate of about 12 liters was finally obtained, which was saturated with ammonium sulphate. The precipitate produced was dissolved in water and its solution dialyzed for forty-eight hours, whereupon a large quantity of spheroids separated which

on settling united to a coherent mass. This precipitate was washed by decantation with water, dissolved in brine and its solution made faintly alkaline to litmus by cautiously adding $N/_{10}$ KOH solution. In order to separate phosphoric acid, a little calcium chloride solution was then added to this very slightly alkaline liquid and the latter, though apparently free from any precipitate of calcium phosphate, was filtered, whereby a little suspended matter was removed. The solution was made exactly neutral to litmus by adding 56 cc. $N/_{10}$ HCl and dialyzed for 18 hours. A gummy precipitate, A, adhering to the bottom of the dialyzer, then separated, from which the solution, B, was decanted almost completely.

The precipitate, A, was dissolved in about 200 cc. of 5 p. c. brine and the liquid was poured into 800 cc. of water. The resulting flocculent precipitate settled rapidly to a coherent deposit from which the solution was decanted. The deposit was repeatedly washed by decantation with water, which caused it to lose its gummy character and become opaque, white and granular. After dehydrating with absolute alcohol and drying over sulphuric acid it weighed 15.5 grams and was marked preparation **23.** The solution marked B was further dialyzed for 48 hours, when a second precipitate formed, which, like **23,** completely dissolved in brine, to a solution perfectly neutral to litmus. This precipitate was washed by decantation with water, but the finer part settled so slowly that it was necessary to decant it together with the water. The sediment after exhausting with absolute alcohol and drying, weighed 23.5 grams, and formed preparation **24.** On long standing, the decanted washings deposited the finely divided matter, which was then collected on a filter, dissolved in brine and its solution precipitated by water, giving 15.4 grams of preparation **25.**

To determine the quantity of globulin contained in our oil-free germ meal, we treated 200 grams of the meal with 2,000 cc. of 3 p. c. salt solution heated to 65° and filtered the extract perfectly clear. Of this, 1,000 cc. were dialyzed until free from chlorides, when the precipitate of spheroids was filtered out, washed with water and with alcohol and dried over sulphuric acid. This preparation, **26,** formed 5.05 p. c. of the oil-free meal.

To obtain a quantity of this globulin for digestion with pepsin, a quantity of germ meal was extracted with 3 p. c. salt

solution heated to 70°, the extract was filtered clear and saturated with ammonium sulphate. The precipitate produced was dissolved in water and the resulting gummy and somewhat turbid solution filtered clear. The filtrate was dialyzed until the solution gave no turbidity on pouring into distilled water. The proteid, which had then separated in spheroids, was filtered out, washed by decantation with water and with alcohol and dried over sulphuric acid, giving 27.3 grams of preparation **27.**

A part of the extract from which **27** had been prepared was mixed with an equal volume of $N/_{10}$ KOH, about twice the quantity necessary to neutralize the extract to phenolphthalein. The solution was then dialyzed in distilled water frequently renewed and in this way a considerable quantity of phosphorus was separated in the alkaline dialysate. When all, or nearly all, which it was possible to separate in this way, had been removed, the solution in the dialyzer was neutralized with $N/_{10}$ HCl until it no longer reacted alkaline to litmus. This caused a turbidity. The acid was then further added until an acid reaction with litmus was obtained, producing a precipitate from which, after settling, the solution was decanted. The precipitate was then dissolved in brine, its solution filtered clear and dialyzed, whereby a substance was precipitated in spheroids, which was filtered out, washed with water and alcohol and formed preparation **28,** weighing 3.0 grams.

These preparations had the following composition:

TABLE IV.—COMPOSITION OF PREPARATIONS EXTRACTED BY SODIUM CHLORIDE SOLUTIONS FROM THE WHEAT EMBRYO.

	19	20	21	22	23	24	25	26	27	28
Carbon	----	----	48.77	50.03	50.23	48.17	49.39	48.75	49.79	48.67
Hydrogen	----	----	6.44	7.04	6.89	6.54	6.78	6.52	6.76	6.56
Nitrogen	18.14	18.21	18.12	18.39	18.23	18.06	17.95	18.16	18.01	17.97
Sulphur	0.49	0.56	0.51	0.60	0.53	0.55	0.48	0.63	0.61	0.61
Phosphorus	1.15	1.03	1.35	0.76	0.56	1.41	1.17	1.41	1.11	1.55
Ash	2.29	1.86	2.25	1.30	1.22	3.85	2.60	2.66	1.11	2.94
P_2O_5 in ash	1.66	1.34	1.68	0.84	0.80	2.00	1.82	2.00	0.68	2.30

These analyses, when calculated free from nucleic acid and ash, as was done for the albumin preparations, in the manner described on page 318, gave the following results:

TABLE V.—COMPOSITION OF THE GLOBULIN CONTAINED IN THE PREPARATIONS EXTRACTED FROM THE WHEAT EMBRYO BY SODIUM CHLORIDE SOLUTION.

	19	20	21	22	23
Carbon	----	----	51.37	51.58	51.40
Hydrogen	----	----	6.83	7.31	7.08
Nitrogen	18.59	18.59	18.62	18.70	18.45
Sulphur	0.57	0.63	0.60	0.66	0.57
Oxygen	----	----	22.58	21.75	22.50
			100.00	100.00	100.00
	24	**25**	**26**	**27**	**28**
Carbon	51.56	51.86	51.40	51.98	51.70
Hydrogen	7.07	7.19	6.94	7.12	7.05
Nitrogen	18.85	18.41	18.71	18.37	18.53
Sulphur	0.67	0.55	0.75	0.70	0.75
Oxygen	21.85	21.99	22.20	21.83	21.97
	100.00	100.00	100.00	100.00	100.00

These figures plainly show that our globulin preparations are mixtures of nucleates of one and the same protein substance and contain from 5 to 15 p. c. of nucleic acid. The preparations contain the same protein as the globulin which one of us has previously described as occurring in the kernel of wheat, rye, barley and maize. In the entire kernel, so little of this globulin is present that it is difficult to prepare it pure therefrom. For this reason, we think, the analyses given below do not agree as closely as they might otherwise be expected to. From the whole seed this globulin is obtained entirely free from phosphorus, which we attribute to the much greater proportion of proteid matter to nucleic acid, in the seed, compared with that existing in the wheat embryo.

TABLE VI.—COMPOSITION OF THE GLOBULIN CONTAINED IN VARIOUS CEREALS.

	Wheat Embryo.	Wheat* Kernel.	Rye† Kernel.	Maize‡ Kernel.	Barley§ Kernel.
Carbon	51.57	51.03	51.19	51.99	50.88
Hydrogen	7.07	6.85	6.74	6.81	6.65
Nitrogen	18.60	18.39	18.19	18.02	18.10
Sulphur	0.65	0.65	23.88	0.66	24.37
Oxygen	22.11	23.08		22.52	
	100.00	100.00	100.00	100.00	100.00

* Am. Chem. Jour., XV, 392.

† Jour. Am. Chem. Soc., XVII, 429, also Report of Conn. Expt. Station for 1894, p. 147.

‡ Am. Chem. Jour., XIII, 327, 385 and XV, 20.

§ Jour. Am. Chem. Soc., XVII, 539; also, Report Conn. Expt. Station for 1894, p. 165.

Having determined the composition of this globulin-like proteid and also that of the albumin, it became clear that several preparations obtained from the aqueous extract were mixtures of these two substances, thus showing the globulin to be present to some extent in the aqueous extract.

As noted, on page 314, when 2,000 cc. of an aqueous extract of about 650 grams of the meal were dialyzed in running water for four days, a dense turbidity was formed which could not be removed by filtration. This, however, on adding a little hydrochloric acid, was converted into a precipitate, which was readily dissolved by adding sodium chloride sufficient to make a 3 p. c. solution, and was precipitated from this solution by dialysis. We thus obtained 9.0 grams of preparation **29**, which, dried at 110°, had the following composition:

COMPOSITION OF PREPARATION **29.**

	I.	II.	Av.	Corrected for Ash & Nucleic Acid.	Calculated for 60 per cent globulin, 40 " " leucosin.
Carbon	48.30	47.92	48.11	51.70	51.95
Hydrogen	6.49	6.41	6.45	7.07	7.07
Nitrogen	17.40	17.24	17.32	17.74	17.74
Sulphur	0.83	0.85	0.84	1.08	0.91
Phosphorus	1.91		1.91	----	
Oxygen	----			22.51	22.53
Ash	3.95				
P_2O_5 in Ash	2.95			100.00	100.00

This analysis corresponds pretty nearly with that of a mixture of 60 p. c. of the globulin with 40 p. c. of leucosin, except that the amount of sulphur found was somewhat greater than that calculated.

After heating another portion of the same aqueous extract to 65° for some time and filtering off the coagulum, the filtrate was dialyzed for five days into alcohol and the precipitate thereby produced filtered out and exhausted with water. The residue of proteid matter coagulated by alcohol, weighing 6.7 grams and marked preparation **30**, was then dried at 110° and analyzed with the following results:

COMPOSITION OF PREPARATION **30.**

		Corrected for Ash and Nucleic Acid.	Calc. for. Globulin 40 p. c. Leucosin 60 p. c.
Carbon	49.49	51.80	52.13
Hydrogen	6.81	7.14	7.03
Nitrogen	16.87	17.32	17.30
Sulphur	0.93	1.14	1.05
Phosphorus	0.89	----	
Oxygen		22.60	22.49
Ash	4.00		
P_2O_5 in Ash	2.01	100.00	100.00

This analysis corresponds quite nearly with that of a mixture of 40 p. c. of the globulin with 60 p. c. of the albumin.

The Proteose of the Wheat Embryo.

In making the preparations already described considerable quantities of crude proteose were obtained from both the aqueous and sodium chloride extracts. After the leucosin and the globulin had been separated as completely as possible, the solutions containing the proteoses, as well as the unseparated residues of other proteids, were dialyzed into alcohol and the precipitates produced, washed and dried over sulphuric acid.

A mixture, weighing 15.4 grams, was made by uniting several such preparations that had been obtained from aqueous extracts from which most of the other proteids had been separated, without heat, by saturating with sodium chloride and dialysis into alcohol. The mixture contained much matter made insoluble in water by the final treatment with alcohol. This was filtered out, washed thoroughly with water and with alcohol and when dried weighed 4.18 grams, and was marked preparation **31**. The filtrate from this was saturated with ammonium sulphate, the precipitate redissolved and again precipitated in the same way. The solution of the second precipitate was dialyzed in cold distilled water, until free from sulphate, and then for several days in alcohol. The precipitate thus produced was dissolved in water, a little insoluble matter filtered out, and its clear solution saturated with sodium chloride, which produced a small precipitate. This was filtered out, dissolved, and its solution dialyzed in water. The salt-saturated filtrate was likewise dialyzed and when both solutions were free from chlorine the dialyzers were transferred to alcohol and the proteose thereby precipitated. The proteose separating on saturation with salt, gave 0.6 gram of preparation **32**, that from the salt-saturated solution, 0.97 gram of preparation **33**. This small yield of proteose indicates great impurity of the original crude product and shows that the proportion of proteose to other proteids is very small.

Another crude product was obtained by dialyzing into alcohol an aqueous extract, after separating leucosin which had

been coagulated by heat. This, weighing 35 grams, was dissolved in water and the insoluble matter filtered out, washed and dried, giving preparation **34**, weighing 7.26 grams.

The filtered solution was saturated with ammonium sulphate, the precipitate dissolved in water, and the clear solution dialyzed in distilled water until free from sulphates, and then in alcohol. The substance thus separated was again dissolved in water, and its solution saturated with salt; the precipitate thus produced was dissolved in water and its solution, as well as the salt-saturated filtrate, were dialyzed in water. When free from chlorine, these solutions were dialyzed in alcohol and yielded, respectively, preparations **35**, weighing 4.0 grams and **36**, weighing 1.84 grams.

Another preparation of crude proteose was obtained by extracting the meal with 3 p. c. sodium chloride solution heated to 70°, dialyzing the extract in water, coagulating the leucosin by heat and precipitating the proteose by dialysis in alcohol. A mixture of such preparations, weighing 31.6 grams, was treated with water, the insoluble matter filtered out, washed and dried, giving 5.16 grams of preparation **37**.

The filtered solution was saturated with ammonium sulphate, the precipitate dissolved in water, the solution dialyzed in distilled water till free from sulphate, and then in alcohol. The separated proteose was redissolved in water and its solution saturated with sodium chloride. The precipitate which resulted was filtered out, dissolved in water and its solution, as well as the salt-saturated filtrate, were dialyzed in water till free from chlorine, and finally in alcohol.

The products thus obtained, formed, respectively, preparations **38**, weighing 0.75 gram and **39**, weighing 1.35 grams. One other proteose preparation was made from the aqueous extract previously described on page 316 from which the phosphorus was largely separated by making it slightly alkaline and adding calcium chloride. After heating the extract to boiling and filtering out the coagulum, the filtrate was dialyzed into alcohol, the resulting precipitate dehydrated with absolute alcohol, dried over sulphuric acid, redissolved in water and precipitated by saturating with ammonium sulphate. The gummy precipitate, having the general appearance and proper-

ties of similar precipitates of the proteoses obtained by the action of pepsin, was dissolved in water, dialyzed free from sulphates and then precipitated by dialysis in alcohol, giving 2 grams of preparation **40**.

These preparations were dried at 110° and analyzed with the following results:

COMPOSITION OF ALCOHOL-COAGULA AND OF PROTEOSE PREPARED FROM THE WHEAT EMBRYO.

TABLE VII.

	Residues of other proteids coagulated by alcohol.			Proteose precipitated by sodium chloride.			Proteose soluble in saturated NaCl sol.			
	31	**34**	**37**	**32**	**35**	**38**	**33**	**36**	**39**	**40**
C	52.36	49.44	51.93		49.94		48.46	48.70	48.44	48.99
H	6.98	6.85	6.87		6.80		6.70	6.73	6.71	6.85
N	16.01	16.00	16.30	16.79	17.08	16.26	16.91	16.76	16.16	16.89
S	1.85	4.08	1.30		1.24		27.93	27.81	28.69	1.10
O	22.80	23.63	23.60		24.94					26.17
	100.00	100.00	100.00		100.00		100.00	100.00	100.00	100.00
Ash	0.81	14.13	0.95	----	0.30	0.77	1.13	1.00	0.74	1.27

From these analyses it is seen that the matter insoluble in water, forming preparations **31**, **34** and **37**, consists of coagulated proteid apparently mostly derived from leucosin. The high proportion of sulphur in **31** and **34** is due to calcium sulphate, precipitated by alcohol from the aqueous extract.

The remaining preparations have the low percentage of carbon, characteristic of proteoses made by pepsin digestion.

Whether the proteose precipitated by saturating its solution with salt is a different protein substance from that soluble in saturated salt solution or whether difference in solubility is due to the presence of different acid compounds of one and the same protein substance, is not demonstrated, but the agreement shown by these analyses, considering the difficulty of making quite pure preparations, indicates that the latter is the case.

The Proportions of the Various Proteid Substances of the Wheat Embyro.

Twenty grams of fresh germ meal, from which the ether-soluble constituents had *not* been separated, were treated with 500 cc. of water and after shaking for some time, the extract was filtered clear. Two portions, of 100 cc. each, were treated with a few drops of very dilute hydrochloric acid and heated in a boiling water bath. The coagulum which separated was collected on a filter and its nitrogen determined. To the filtrate from one coagulum, tannin was added and nitrogen was determined both in the precipitate and in the filtrate. Another lot of 20 grams was treated in the same way and nitrogen determined in the heat-coagulum formed in each of two portions of 100 cc. The amount of nitrogen corresponding to one gram of germ meal was found in the four coagula to be, .0163 gram; .0156 gram; .0159 gram and .0162 gram; in the tannin precipitate .0062 gram and in the solution filtered from the latter .0062 gram.

Twenty grams of germ meal were extracted with 500 cc. of 3 p. c. sodium chloride solution heated to 70°, whereby the leucosin was coagulated and the globulin and proteose dissolved. Of the clear filtered extract, 100 cc. yielded with tannin a precipitate containing .0166 gram N per gram of meal extracted.

Two portions of the meal, each of one gram, were exhausted with 3 p. c. sodium chloride solution heated to 70°, and nitrogen determined in the residues. The .0331 and .0309 gram of nitrogen found in the residues were from the leucosin and insoluble nitrogenous bodies, so that the nitrogen belonging to the latter equalled .0171 gram and .0149 gram. From the average of these figures we find the following amounts of the different forms of nitrogen in one gram of the wheat germ meal.

	Nitrogen Grams.	
Insoluble in water and salt solution	0.0160	
Insoluble in water but soluble in salt solution	0.0100	= globulin nitrogen.
Soluble in water and coagulable by heat	0.0160	= albumin "
Soluble in water, uncoagulable by heat, precipitable by tannin	0.0050	= proteose "
Not precipitable by tannin	0.0060	= non-proteid "
Total	0.0530	
Found by direct nitrogen determination	0.0531	

We have shown that the coagulated leucosin preparations contain about 10 p. c. of nucleic acid, the globulin about 15 p. c., while those of the proteose contain none. Deducting these quantities from the nitrogen given in the table, we find 9.5 p. c. of the embryo to be leucosin, 4.84 p. c. to be globulin, and 3.03 p. c to be proteose.

The bodies which are represented by the insoluble nitrogen, we have been unable to separate from the embryo. The residue after extraction with hot salt solution contained .0076 gram of phosphorus. Since there was in this residue about .1000 gram of coagulated leucosin, in which we have usually found about 1 p. c. of phosphorus, we have .0061 gram of phosphorus remaining over. In view of the large proportion of nucleic acid found in the extracts of the embryo, it is not improbable that this phosphorus mostly belongs to nucleic acid, in which case there would be about 6.75 p. c. of nucleic acid containing .0108 gram of nitrogen, which leaves only .0052 of nitrogen for proteid matter in the insoluble residue. It seems probable therefore that this insoluble nitrogen largely belongs to compounds of the proteid with relatively much nucleic acid.

Digestion of the Phosphorus-containing Proteids with Pepsin-hydrochloric Acid.

Leucosin nucleate. Ten grams of the coagulated albumin, preparation **2**, were suspended in 400 cc. of water and dissolved by adding 100 cc. of $N/_{10}$ KOH solution. To the nearly clear solution which resulted, an equal volume of 0.4 p. c. HCl was added, together with some pepsin, and the mixture digested at 37°. In a short time the solution became perfectly clear, but later deposited a large coherent precipitate, which gradually contracted, but at the same time retained the form of the lower part of the beaker. From this the clear solution was decanted, the precipitate thoroughly washed by decantation, suspended in water and dissolved by adding 28 cc. of $N/_{10}$ KOH solution, an amount of alkali just sufficient to dissolve all the substance and at the same time make the solution neutral to litmus. When to this solution decinormal acid was gradually added, no precipitate appeared until nearly one-half the quantity of acid required for complete neutralization

had been added, but with 28 cc., the solution was neutralized and also completely precipitated, the addition of 2 cc. more acid giving no turbidity in the solution filtered from the precipitate.

This precipitate was washed with water and with alcohol and dried over sulphuric acid, forming preparation **41**, weighing 1.54 grams.

To precipitate this substance a quantity of acid was added exceeding that of the alkali employed for solution by just 2 cc. The filtrate from the precipitate, however, required not 2 cc. of alkali, but 8.5 cc. for neutralization to phenolphthalein, showing 6.5 cc. of alkali to have been neutralized by the acid of the nuclein originally dissolved. The neutralized filtrate left on evaporation 0.3975 gram of substance, the aqueous solution of which was precipitated by hydrochloric or nitric acid, but *not* by ammonium molybdate solution until after boiling with acid for some little time, when yellow phosphomolybdate was precipitated. These facts indicate the presence in this filtrate of a nucleic acid.

More nuclein was made from the same preparation, **2**, by suspending thirty grams in 0.2 p. c. HCl, containing pepsin, which, even at 20°, caused within two hours complete solution of the coagulated proteid. The solution was digested at 37° for 48 hours, during which time much nuclein separated, having the appearance and properties of the preparation just described.

After decanting the clear solution and thoroughly washing the residual nuclein, the latter was suspended in water and dissolved in 72 cc. $N/_{10}$ KOH. The solution thus obtained was made neutral to litmus by adding 11 cc. of $N/_{10}$ HCl, but no precipitate appeared till 1.5 cc. more of acid were added. To the solution 72 cc. $N/_{10}$ HCl were added, giving a precipitate which, when washed and dried, made preparation **42** and weighed 3.4 grams. The filtrate from this precipitate, as in the former case, was strongly acid, requiring 12 cc. of $N/_{10}$ KOH to neutralize it to phenolphthalein. Two other preparations of nuclein were made from 8.493 grams of **8**, and 9.804 grams of **11**, both being substances precipitated from the aqueous extract by saturating with sodium chloride. Each portion was suspended in about 300 cc. of 0.2 p. c. HCl, containing 0.1 gram of pepsin and, with frequent stirring, digested at 40° for 24 hours. Throughout

the digestion a large part of the substance remained undissolved. An equal volume of 0.2 p. c. HCl, containing 0.1 gram of pepsin, was again added to each and the digestion continued for 24 hours longer. The insoluble matter which remained was not coherent like the two former nuclein products, but consisted of a white, very finely divided substance which was easily filtered out and washed. After dehydrating with absolute alcohol these preparations were dried over sulphuric acid; from **8**, 4.04 grams of preparation **43** were obtained and from **11**, 4.16 grams of **44**.

Globulin nucleate. Fifteen grams of a mixture of nearly equal parts of the globulin preparations **23** and **24** were next suspended in 0.2 p. c. HCl, containing 0.2 grams of pepsin, which, within a short time, almost completely dissolved the proteid matter. From this solution, on further digestion, the nuclein separated, forming a coherent deposit. After 72 hours digestion, the clear solution was decanted, the deposit dissolved in a little ammonia and its solution filtered perfectly clear from a very slight gelatinous residue. The resulting solution was then treated with acetic acid, added in excess of the amount necessary to neutralize the solution to litmus. Since, even on standing, the precipitate so produced separated imperfectly, an equal volume of alcohol was added. The substance, which then separated well, was filtered out, washed with dilute and with absolute alcohol and dried over sulphuric acid, giving 2.38 grams of preparation **45**, or about 16 p. c. of the original substance.

The filtrate from the acetic acid precipitate gave a further slight precipitate on adding HCl, which had properties characteristic of nucleic acid.

Still another preparation of nuclein was made from the globulin by suspending 10 grams of **27** in water and adding 50 cc. of $N/_{10}$ KOH. This solution was neutralized and an equal volume of 0.4 p. c. HCl at once added, producing a turbid solution, which, however, contained no visible particles. To this, pepsin was added and the mixture digested for 40 hours, during which time a coherent deposit of nuclein formed on the bottom of the beaker. From this the clear solution was decanted. The deposit was then thoroughly washed with water and dissolved in 43 cc. of $N/_{10}$ KOH. To this clear solution

43 cc. of $N/_{10}$ HCl were added, causing a gummy precipitate which could not be filtered until 15 cc. more acid had been added, when the precipitate rapidly settled as a coherent deposit, from which the solution was soon decanted. This solution required for neutralization to litmus 16 cc. $N/_{10}$ KOH, and to phenolphthalein 18 cc. The precipitate when washed and dried gave 2.2 grams of preparation **46.**

These six preparations were all dried at 110° and analyzed with the following results:

TABLE VIII.—COMPOSITION OF NUCLEIN FROM THE PROTEIDS OF THE WHEAT EMBRYO.

	41	42	43	44	45	46
Carbon	44.87	44.35	42.68	43.35	39.42	41.92
Hydrogen ..	5.82	5.77	5.45	5.47	5.03	5.25
Nitrogen ...	16.04	16.64	16.12	16.01	16.05	17.00
Sulphur	0.97	1.03	0.65	0.85	0.53	0.46
Phosphorus	4.58	5.07	5.32	4.88	5.27	5.63
Ash	0.60	0.78	1.72	1.72	17.42	1.17
P_2O_5 in Ash	0.29	0.55	1.24	0.94	10.56	0.69

If we subtract from the total ash the amount of P_2O_5 found in it, we shall have a determination of the bases contained in the ash of these preparations.

We have calculated these analyses of nuclein free from the bases of the ash and from nucleic acid, in the way previously described, with the following results:

TABLE IX.—COMPOSITION OF PROTEIN MATTER CONTAINED IN THE NUCLEIN.

	41	42	43	44	45	46
Carbon	53.65	54.77	51.80	52.36	52.30	51.64
Hydrogen ..	7.23	7.46	6.85	6.73	6.91	6.60
Nitrogen ...	16.68	17.56	16.31	16.31	19.31	18.93
Sulphur	1.98	2.37	1.61	1.89	1.53	1.25
Oxygen	20.46	17.84	23.43	22.71	19.95	21.58
	100.00	100.00	100.00	100.00	100.00	100.00

The composition of the proteid matter in **43** and **44** is very nearly that of leucosin except as regards sulphur, the amount of which is decidedly greater. On the other hand, **41** and **42**, which also were derived from preparations whose protein matter was leucosin, differ in composition very decidedly from

that substance. This is probably because on pepsin digestion the substance of preparations **43** and **44** remained throughout undissolved, whereas **41** and **42** separated on pepsin digestion from nearly clear solutions and therefore doubtless their protein matter had been to some degree altered by the pepsin before separating as an insoluble compound with nucleic acid. The two nucleins, **45** and **46**, from the globulin which also had separated from solution, show similar differences in composition when compared with the unaltered globulin, carbon and nitrogen being higher and sulphur very much higher than in the globulin. The greatly increased proportion of sulphur would indicate that sulphur in some acid form had split from the proteid molecules undergoing hydrolysis and had become a part of the insoluble nuclein, as did the nucleic acid.

Conclusion.

The embryo of the wheat kernel contains:

1. A *nucleic acid* in considerable quantity. This acid is insoluble in water, forms soluble as well as insoluble compounds with proteid substances, and on hydrolysis yields guanin, adenin, phosphoric acid and other products not yet identified. It has the following composition:

NUCLEIC ACID.

Carbon	36.48
Hydrogen	4.48
Nitrogen	16.17
Phosphorus	8.96
Oxygen	33.91
	100.00

This acid is not identical with any nucleic acid heretofore described. On hydrolysis it does not yield any form of sugar. From guanylic acid recently described by Bang it also differs distinctly, in that its potash salt is extremely soluble in cold water and the ratio of P to N, being 1 to 4 instead of 1 to 5.

2. *Leucosin,* an albumin, (yield about 10 p. c. of the embryo) formerly found by the writer in small quantity in the whole kernel of wheat, rye and barley, and abundantly in malt. Leucosin begins to separate as a flocculent coagulum when the very

slightly acid aqueous extract of the wheat kernel or wheat embryo is heated to 52°. Even after long heating at 65°, the leucosin is only partly separated, and about one-third more coagulum of the same elementary composition is obtained on raising the temperature from 65° to 100°.

By saturating extracts of the kernel or of the embryo with sodium chloride, the leucosin is largely precipitated, from the former as a substance readily soluble again in water, from the latter as an insoluble compound containing about 30 p. c. of nucleic acid. From the latter precipitate, dilute salt solution extracts a small amount of nearly phosphorus-free proteid, which behaves like a globulin, being precipitated by dilution or by dialysis, but having essentially the same ultimate composition as leucosin.

By dialyzing the aqueous extract in water, nearly all the leucosin contained in it is precipitated, not like a globulin, but as an insoluble compound containing about 20 p. c. of nucleic acid. The following figures give the average of accordant analyses, calculated nucleic acid free, of 18 different preparations representing complete as well as fractional precipitations under the above and other conditions. These figures agree closely with the composition of the leucosin of wheat, rye, barley and malt.

LEUCOSIN.

Carbon	52.65
Hydrogen	7.04
Nitrogen	16.43
Sulphur	1.32
Oxygen	22.56
	100.00

3. A globulin, precipitated in spheroids by dialysis and by dilution as a coherent deposit. The yield is about 5 p. c. of the embryo. The solution of this globulin in 10 p. c. sodium chloride brine becomes turbid on heating to about 87°, and at 90°, on continued heating, a considerable flocculent coagulum separates.

Our preparations of this globulin contained from 6 to 17 p. c. of nucleic acid, most of them from 12 to 15 p. c. From this the proteid could not be separated by fractional precipitation.

Analyses of 10 different preparations of this globulin gave very closely agreeing figures when calculated free from nucleic acid, the average of which is as follows:

GLOBULIN.

Carbon	51.57
Hydrogen	7.07
Nitrogen	18.60
Sulphur	0.65
Oxygen	22.11
	100.00

In composition and properties this globulin agrees with that found by the writer in the kernels of wheat, rye and barley. So far as we have been able to observe, it differs from edestin, the crystalline globulin obtained from seeds of hemp, flax and squash only in containing two-thirds as much sulphur.

4. *Proteose,* precipitated by saturating the aqueous extract, freed from globulin and albumin, with salt. One preparation, **35,** was phosphorus free, and had the following composition:

PROTEOSE.

Carbon	49.94
Hydrogen	6.80
Nitrogen	17.08
Sulphur	1.24
Oxygen	24.94
	100.00

5. *Proteose,* soluble in the salt-saturated solution filtered from the foregoing proteose and obtained free from phosphorus by precipitating with alcohol its solution freed from salt by dialysis. The average of analyses of four preparations of this proteose is the following:

PROTEOSE.

Carbon	48.65
Hydrogen	6.75
Nitrogen	16.68
Sulphur	1.10
Oxygen	26.82
	100.00

These proteoses together form about 3 p. c. of the embryo.

6. About one-third of the total nitrogen of the embryo is not extracted by water and salt solutions and appears to belong to insoluble compounds. This nitrogen is accompanied by phosphorus corresponding to about 6.75 p. c. of nucleic acid, which would contain two-thirds of this insoluble nitrogen. It seems probable, therefore, that this insoluble nitrogen belongs largely to insoluble compounds of nucleic acid and protein.

7. These phosphorus-containing preparations of globulin and leucosin, when digested with pepsin-hydrochloric acid, yield nuclein in proportion to the phosphorus which they contain. Calculated free from nucleic acid, the analyses of these nucleins show the protein constituent to have nearly the same composition as the proteid from which they were derived, the most marked difference being a greater proportion of sulphur in the former.

8. The proteids of the embryo differ from those of the dormant endosperm, of this as well as of other seeds, in the facility with which they undergo changes. These changes are the result of a redistribution of acids among the protein and other basic molecules, so that compounds form in the extracts of the embryo which contain various proportions of nucleic acid according to the changing conditions.

The writer has shown that the globulin, edestin, forms crystalline compounds with one and with two molecules of acid and also compounds with a greater number of acid molecules. There is reason to believe that all other native protein substances form similar compounds; in other words, that proteins are distinctly polyacid bases and that the acid characters which proteids display are due to acids united to their protein molecules, probably in the same manner as in the salts of the purin bases.

These nucleic acid compounds of the protein constituents of the wheat embryo appear to be compounds of this order. According to this view, no special distinction can be made between nucleins and nucleoproteids, the former being simply compounds containing a greater number of molecules of nucleic acid united to one molecule of protein.

That the wheat embryo in fact contained the same nucleic acid *compounds* as we have obtained from the extracts, is highly